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### RESEARCH ARTICLE

#### THE EFFICACY OF PLATELET RICH PLASMA IN TREATMENT OF ANDROGENETIC ALOPECIA

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#### Abstract

Dermoscopy is a non-invasive diagnostic technique for the observation of pigmented skin lesions, permitting the recognition of morphologic structures not visible by the naked eye. The technique consists of placing mineral oil, alcohol or even water on the skin lesion that is subsequently inspected using a hand-held lens, a hand-held dermatoscope, a stereomicroscope, a camera, or a digital imaging system. The magnifications of these various instruments range from 6x even up to 100x. The fluid placed on the lesion eliminates surface reflection and renders the cornified layer translucent, thus allowing a better visualization of pigmented structures within the epidermis, the dermoepidermal junction and the superficial dermis. Male-pattern hair loss (MPHL), also known as androgenic alopecia and male pattern baldness, is hair loss that occurs due to an underlying susceptibility of hair follicles to shrinkage due to the influence of androgenic hormones. Male-pattern hair loss is the most common cause of hair loss and will affect up to 70% of men and 40% of women at some point in their lifetimes. Men typically present with progressive hair loss at the temples and vertex balding, whereas women typically present with diffuse hair loss over the top of their scalps. Platelet-rich plasma is defined as a volume of the plasma fraction of autologous blood with an above baseline platelet concentration usually more than 1,000,000 platelets/ $\mu$ L. PRP's regenerative potential depends on the levels of released GFs. Alpha granules of platelets contain GFs, which upon activation, are responsible for the initiation and maintenance of the healing response. PRP is known to carry more than 20 GFs and other protein molecules, such as adhesion molecules, chemokines, which interact to promote inflammation, cell proliferation, differentiation, and regeneration. In this study, the aim was to objectively assess the proposed therapeutic effect of PRP in treatment of AGA through measuring hair density using dermoscopic evaluation, hair pull test, gross pictures and patients' satisfaction scale. The study included 30 patients of different grades of androgenetic alopecia, our patients were 15 males and 15 females ranging from grade I to III by Ludwig classification for FPHL and from grade 3 to grade 6 for Norwood and Hamilton classification for male androgenetic alopecia of a total 6 sessions, 4 successive ones with 3 weeks apart of a total 12 weeks and 2 separate sessions,

24wk and the last evaluation was done 1 year later to the 1st session. The results were classified depending on the lasting effect of the PRP into short term results lasting up to 4 months from the start of the sessions which gave statistically positive values regarding the hair pull test results and hair follicles counted by dermoscope and the long term results starting from the 5th month up to 1 year duration from the start of the sessions which showed decline in both numbers of hair pull test and Which indicates the need for retreatment or addition of another line of medical treatment e.g., minoxidil or finasteride. Also PRP can be considered a humble tool in the treatment of AGA, as it is sufficient alone without the medical treatment.

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### Introduction:-

Androgenetic alopecia or male pattern baldness is a very common type of hair loss observed in both males and females (*Khatu et al., 2014*).

Platelet rich plasma is an autologous preparation of platelets in concentrated plasma. Although the optimal PRP platelet concentration is unclear, the current methods by which PRP is prepared involve reported 300% to 700% enrichment with platelet concentrations consequently increasing to more than 1,000,000 platelets/ L. PRP has attracted attention in several medical fields because of its ability to promote wound healing. Generally, platelets were previously thought only to contribute to homeostasis, but they are now known to initiate wound healing by secreting various growth factors and cytokines (*Li et al., 2012*).

PRP is an innovative therapy and has been used since 1987 to help promote healing in orthopedic surgery, dental surgery and dermatology. There have been reports supporting the use of PRP in the treatment of hair loss (*Chaudhari et al., 2012*).

In this process referred to as “activation” platelets alpha granules become activated and release numerous proteins including platelet derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), insulin like growth factor (IGF), epidermal growth factor (EGF) and interleukin (IL)1,3,4. PRP has also attracted attention in plastic surgery and dermatology because of its potential use during facial plastic surgery and aesthetic skin rejuvenating effects. PRP enhances the proliferation of human dermal fibroblasts and human adipose derived stem cells, whereas recent clinical reports describe the use of PRP as a scaffold for injectable soft tissue augmentation and treatment for acne scars and nasolabial folds. Another recent study evaluated the effects of treatment with platelet plasma growth factors during male pattern baldness surgery. The authors observed a significant improvement in hair density and stimulation of growth when follicular units were pretreated with platelet plasma growth factors before their implantation. They hypothesized that growth factors released from platelets may act on stem cells in the bulge area of the follicles, stimulating the development of new follicles and promoting neovascularization (*Li et al., 2012*).

The PRP treatment is indicated in cases of male AGA, Hamilton Norwood grades AGA as the possibility of stimulating the growth of new hairs is significantly smaller in cases of feminine AGA; the treatment is indicated with Ludwig grades III.

The earlier the treatment is performed, the more likely is a significant stimulation of hair regrowth (*Higgins and Christiano, 2014*).

### Aim of work

The aim is to assess the efficacy of platelet rich plasma in the treatment of androgenetic alopecia.

## Patients and Methods:-

The study was conducted at Alsalam teaching hospital, dermatology department from May 2020 to May 2021, and included 30 volunteers; half of them (15) were males and the other half (15) were females.

### Inclusion criteria

- 1-Patients with androgenetic alopecia with grade 1-4 of Norwood and Hamilton and grade I-III of Ludwig classification.
- 2-All participants were  $\geq 18$  years.
- 3-Patients who had not received any topical or systematic treatment for their hair loss during the last 6 months.

### Exclusion criteria

1. Patients with present or a history of immune suppression (malignancy, chemotherapy and steroid therapy).
2. Dermatological diseases affecting the scalp
3. Autoimmune disorders, hematologic disorders, 3-platelet dysfunction syndrome and on anticoagulation therapy.
4. Patients with a tendency for keloids.
5. Patients taking aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) discontinued their use 7 days before treatment.

### Diagnosis

Diagnosis of AGA was made in all patients based on a detailed medical history (any drugs causing hair loss), clinical examination and laboratory tests. Laboratory tests included:

### CBC

Serum iron, serum ferritin, TIBC (Total Iron-Binding capacity)  
Folic acid  
T3, T4, TSH, fT3, fT4, anti-TPO

### VDRL

For women, female hormone profile (DHEAS, testosterone, prolactin, follicle stimulating and leutinising hormone).

Laboratory tests were assessed in order to exclude other hair loss causes, such as anemia, poor nutrition, thyroid dysfunction, syphilis and polycystic ovary syndrome.

### PRP preparation

It was done according to the recommendation of (*Perez et al. 2014*).

PRP was prepared using a double-spin method. Ten cc of whole blood was withdrawn from the patient and collected in disposable sterile test-tubes containing 1c.c. acid citrate dextrose (ACD) solution anticoagulant with rate 1:10 (anticoagulant: blood).

The citrated blood was centrifuged at 1000 rpm for 10 minutes (Table-Top Refrigerated Centrifuge 2800: Roter, RS-240, Kubota Corp., China)

The upper part was gently extracted and then centrifuged again at 2000 rpm for another 10 minutes.

Calcium chloride 10% was then added to PRP as an activator (1:9) and then incubated at room temperature for 10 minutes. Through special micromole pipettes plasma was promptly withdrawn with a sterile disposable syringe.

Before the PRP applications, all patients were informed about the process and its potent adverse effects and they signed a consent form. They did not have their hair washed two days prior to the treatment. Local anesthesia was applied to the scalp. None of the patients received any other treatment for hair loss during PRP treatment or previous 6 months.

PRP (0,05-0,1 ml/cm<sup>2</sup>) was injected, with a 27-G needle, into the androgen-related areas (frontal, parietal, occipital) of the scalp in men and into the problematic areas in women, using built-in insulin 1 ml syringes. Nappage

technique was performed in a depth of 1.5-2.5 mm [Figure 17]. Our protocol proposed three treatment sessions with an interval of 3 weeks. At 6 months from the beginning of the treatment, a booster session was also performed and the last evaluation was conducted after 1 year.



**Fig.(1):-** Injecting activated PRP.

### Evaluation

In our study, we evaluated hair loss, hair density (hair/cm<sup>2</sup>). Evaluation methods included hair pull test, dermoscopic photomicrographs, macroscopic photographs.

Hair density (hair/cm<sup>2</sup>) was performed by dermoscope (dermlite4) and number of hair was counted manually at magnification power of 20x. Phototrichogram, a more objective evaluation method was not performed as it needs to be performed on a shaven part of the patient's scalp which is not accepted by most patients, particularly women. Nevertheless, an adequate means of measuring hair growth over time in a reproducible, economical and non-invasive manner is not available and all the above methods give a relatively fair assessment of the results after treatment.

All patients were evaluated at six time points: T1, beginning of study; T2 at 3 weeks; T3 at 6 weeks; T4 at 3 months; T5 at 6 months and T6 at 1 year.

In order to check the same area at all time points, we used 'V' (Kang's point), as proposed by Lee et al. 'V' is the point of intersection between the midsagittal line and the coronal line connecting the tips of the tragus. By using a plastic headband and a tapeline, 'V' can be measured conveniently because the headband presents the coronal line connecting the roots of the ear tragus, and the tapeline easily shows the midsagittal line. We measured 'V', which is located roughly 1 to 1.3 cm in front of the anterior margin of the headband and record the distance from the headband to the midpoint of the line connecting the lower margins of the eyebrows for reproducibility (Lee E H. et al., 2011).



**Fig. (2):-** Defining V point using a headband and a tapeline (Lee et al., 2011).

**Results:-**

The present study included 30 patients with androgenetic alopecia of grade III to grade V by Norwood & Hamilton classification of male androgenetic alopecia and from grade I to grade III of Ludwig classification of female androgenetic alopecia.

The study group is composed of 15 male and 15 females of different age group ranging from 19-50 years old with the mean age in males 27.87 and 34.27 in females.

**Table 1:-** Demographic distribution regarding the age and sex.

Allpatients	Mean±SD	Range
Age	31.07±8.41	19-50
Sexn(%)		
Male	15(50.0)	
Female	15(50.0)	

**Table 2:-** Comparison of hair pull test in the successive sessions.

Hairpulltest	Mean±SD	P 1	P 2	P3	P4	P5
T 1	9.37±1.94					
T 2	6.87±1.85	0.001**				
T 3	4.97±1.94	0.001**	0.001**			
T 4	3.57±1.79	0.001**	0.001**	0.001**		
T 5	4.97±1.68	0.001**	0.001**	0.879	0.001**	
T 6	6.42±1.53	0.001**	0.02*	0.001**	0.001**	0.001**

The results show that there is a significant statistical difference between each session of hair pull test and the successive session.

Hairpulltest	Mean±SD	Pairedt test	P1
AtT1	9.37±1.94		
AtT2	6.87±1.85	12.38	0.001**
AtT3	4.97±1.94	12.54	0.001**
AtT4	3.57±1.79	15.19	0.001**
AtT5	4.97±1.68	13.05	0.001**
AtT6	6.42±1.53	8.25	0.001**

Dermoscopicresults	Mean±SD	P1	P2	P3	P4	P5
FU1	114.2±16.77					
FU2	124.67±17.43	0.001**				
FU3	135.1±17.88	0.001**	0.001**			
FU4	142.97±18.11	0.001**	0.001**	0.001**		
FU5	142.0±15.03	0.001**	0.001**	0.016*	0.001**	
FU6	133.0±15.03	0.001**	0.019*	0.002**	0.001**	0.001**

**Table 3:-** Mean number of follicular units detected by dermoscope at every session.

Grades of alopecia	No	Age mean±SD	F	P value	%
III	1	32.0±0	0.944	0.498	6.7
IIIA	4	25.25±2.75			26.7
III Vertex	5	27.20±5.45			33.3
IV	3	33.3±9.02			20.0
IVA	1	24.0±-			6.7
VA	1	25.0±-			6.7

**Table 4:-** Age and number distribution among different grades of alopecia in male patients study group.

Hairpull		Range		Mean±SD	S.	t. test	p.value	
1	Male	6	–	12	9.00 ±	1.93	1.076	0.308
	Female	6	–	12	9.73 ±	1.94		
2	Male	3	–	10	6.47 ±	2.07	1.420	0.243
	Female	5	–	10	7.27 ±	1.58		
3	Male	2	–	8	4.73 ±	2.02	0.426	0.519
	Female	3	–	9	5.20 ±	1.90		
4	Male	1	–	8	3.40 ±	1.96	0.252	0.619
	Female	1	–	8	3.73 ±	1.67		
5	Male	2	–	8	4.80 ±	1.97	0.294	0.592
	Female	3	–	7	5.14 ±	1.35		
6	Male	4	–	9	6.31 ±	1.65	0.139	0.713
	Female	4	–	9	6.55 ±	1.44		







**Fig.(3):-** Photographs and Dermoscope of patient number 1.

### **Discussion:-**

Androgenetic alopecia (AGA) is a hereditary, androgen-dependent dermatological disorder more common in men. It is occasionally seen in women. It commonly begins by the age of 20 and nearly 50% of men are affected by the age of 50 (*Trink A. et al., 2013*).

AGA is a progressive thinning of the scalp hair in a defined pattern causing significant lowering of the self-esteem and psychological well-being of the patient. It is a growing concern for the dermatologists around the world. Some genetic factors also have been implicated in its etiology. It is an androgen-dependent disorder modulated via the testosterone metabolite dihydrotestosterone (DHT) and the hair follicle-related androgen receptor (AR) (*Rallis E. et al., 2014*).

The treatment modalities are limited, mainly minoxidil, 5-alpha- reductase inhibitors, and hair transplantation. These have numerous side effects ranging from hypertrichosis, possible birth defects if given to women of childbearing age, decreased libido, and the possibility of prolonged impotence (*Kang BK et al., 2013*).

Growing list of studies and research findings have pointed out the role of platelet derived growth factors as biologically active source in many key regenerative steps as angiogenesis, healing of resistant wounds in medical field as sport medicine, plastic and aesthetic surgery. In dermatology, its role in enhancing the formation of keratin, regeneration of hair matrix and increased cell proliferation had been studied but not fully elaborated (*Schiavone et al., 2014*).

Vascular endothelial growth factor (VEGF) expressed in PRP was found to stimulate hair growth. In contrast, other growth factors were found to have dual effect as both stimulatory and inhibitory on hair growth; for example epidermal growth factor (EGF) at a certain level stimulates mitosis of epithelial cells and fibroblasts, increases the ratio of anagen and inhibits the entry into the catagen phase. On the other hand, at higher levels or doses, it induces regression of the follicle, as if it serves as a biological switch for entering and leaving the anagen phase (*Amgar and Bouhanna, 2013*).

Nerve growth factor (NGF) as well, was found to stimulate hair growth and slow down apoptosis at the level of outer root sheath when expressed with a certain receptor (NGF TrKA), while its expression with another receptor (NGF jp 75 NTR) promotes apoptosis and regression of growth of hair follicle. This is probably the best explanation for the increased hair loss correlated with stress. It is assumed that over expression of NGF with the inhibitory receptors is the key that promotes neuroimmune communication leading to increased perifollicular inflammation, apoptosis in hair follicle and premature catagen development (*Rogers, 2012*). Platelet rich plasma (PRP) injection as one of the treatments of AGA has been the subject of investigations in few studies, but none had an objective method to evaluate the efficacy of treatment or the best method of preparation of PRP that insured the intended stimulatory interaction and adjusted doses of all factors in vivo (*Shiavone et al., 2014*). Dermoscopy is a non-invasive, in vivo technique that has been recently utilized for the diagnosis and management of hair and scalp disorders. Many studies on dermoscopy of hair and scalp disorders have been published in the last few years and the term trichoscopy has specifically been coined to describe this novel application of the technique. Trichoscopy is very useful for in vivo diagnosis of scalp and hair disorders and can greatly improve clinical management. Both handheld dermoscope and videodermoscope can be utilized, the former however providing the possibility of a fast storage of images for future comparison and follow-up studies. with the introduction of dermoscope&folioscope ,the dermoscopic patterns observed in normal scalp, in inflammatory and infectious scalp disorders, hair shaft alterations, and the dermoscopic features described in non-scarring and scarring alopecia could be easily evaluated (*Silverberg NB et al., 2009*). In this study, the aim was to objectively assess the proposed therapeutic effect of PRP in treatment of AGA through measuring hair density using dermoscopic evaluation and gross pictures and patients' satisfaction scale. The study included 30 patients of different grades of androgenetic alopecia, 15 males and 15 females ranging from grade I to III by Ludwig classification for FPHL and from grade 3 to grade 6 for Norwood and Hamilton classification for male androgenetic alopecia for a total of 5 sessions, 4 successive ones with 3 weeks apart for a total of 12 weeks and 2 separate sessions, 24wk and the last evaluation was done 1 year later to the 1st session.

By this protocol, other limitation of similar previously conducted studies were eliminated as most of previous studies lacked an objective method for assessment and the period of treatment was not extended up to a whole year duration (8-10 weeks in most of studies) unlike this current study besides, the last evaluation of cases was done at the end of the 1 year treatment (*Li et al., 2012*).

Based on the duration of PRP treatments, our results were classified into short term results (from 1 month to 3 months) and long term results (from 4 months up to 1 year). This classification can be attributed to the difference in our results and the duration of our research which was conducted for a whole year, while most of the previous PRP researches only assessed the effect of PRP for a maximum of 4 months duration which did not give a clear idea about the need for a future booster sessions or even the need for repeating the course of treatment.



In the short term results, a significant statistical difference in hair density was found after the 4th session of treatment assessed by the increased number of follicular units by dermoscope conceding with decrease in hair pull test results.

These findings are in agreement with the study of *Kang et al. 2014* who injected CD34+ cell-containing PRP preparation into the scalps of 13 patients with male and female pattern hair loss, and treated 13 patients with interfollicular placental extract as a control. Their results showed significant increase in the mean number of hairs and mean hair thickness in PRP treated patients compared to controls. The procedure revealed that CD34+ cell containing PRP treatment presented a higher degree of improvement than placental extract treatment in hair thickness and overall clinical improvement.

Our findings are also in agreement with the study of *Khatu et al. 2014* who examined eleven male patients with androgenic alopecia injected every 2 weeks for a total of four times with the outcome assessed after 3 months. A significant reduction in hair loss was observed. Hair count increased from average number of 71 hair follicular units/cm<sup>2</sup> to 93 follicular units/cm<sup>2</sup>. After the fourth session, the pull test was negative in 9 patients.

All these previous studies were conducted for maximum of 3 months duration and the last evaluation only weeks after the session, so long term effect of PRP on androgenetic alopecia and its potential to replace the medical treatment or as a sole treatment for AGA needs a long period of assessment of the results with an objective method (dermoscope).

Assessment of the number of the number of pulled hair and the number of the follicular units by hair pull test and dermoscope respectively after the 4th session 6 months and 12 month from the beginning of the treatment, the results showed a decline in the mean results, yet not reaching the base line of the 1st assessment. Only 2 patients (6.6%) showed no response. One of them was on antihypertensive drugs and antipsychotic drugs for OCD (Obsessive compulsive disorder) and our finding agree with that states that lithium causes hair loss in up to 20% of long term users. This may also be a consequence of lithium induced hypothyroidism. Valporic acid and divalporax frequently cause dose dependant hair loss which may affect up to 30% of patients taking high doses. hair loss is commonly observed in patients taking fluoxetine or paroxetine. Also ACE inhibitors and  $\beta$  blockers cause hair loss (*AntonellaTosti*).•Bianca Maria Piraccini

This is in agreement with some authors claiming a questionable role for platelet rich plasma in the treatment of androgenetic alopecia demanding more evidence to verify its efficacy (*Valente Duarte de Sousa and Tosti, 2013*) which suggest that PRP has a mild to modest effect (approximately 10% increase) on increasing hair density in a specific patient population. This split-scalp, placebo-controlled study is one of the few of its kind in the field of PRP research and the authors should be applauded for the design of this study. However, it would be beneficial to have a 1-year follow-up to see if the results are maintained and to better help determine if maintenance injections would be needed to sustain the results, which was already fulfilled in our study.

Our results can be compared to the study of *Gkini et al. 2015* who injected PRP in 20 patients, males and females, with AGA. Three treatment sessions were performed with an interval of 21 days and a booster session at 6 months following the onset of therapy. Three months after the first treatment a significant increase in hair density was noted

)170.70  $\pm$  .37.81P < 0.001). At 6 months and at 1-year, hair density was also significantly increased, 156.25  $\pm$  37.75 (P < 0.001) and 153.70 $\pm$

39.92) P < 0.001) respectively, comparing to that of baseline. Nevertheless, it was lower than that of 3 months. Only one patient presented no change (5%). At T5, one patient (5%) presented a decrease of 1hair/cm<sup>2</sup> in hair density comparing to that of T1, while at T6 30% presented a mean decrease of 2 hairs/cm<sup>2</sup>.

Also our results can be compared with *Cervelli et al. 2014* they conducted a study on 23 patients with androgenetic alopecia. The patients' scalps were divided into two halves. One side was injected with PRP and the other with placebo. Three treatments were administered to each patient at 30-day intervals. The

endpoints were hair regrowth, hair dystrophy as measured by dermoscopy, burning or itching sensation, and cell proliferation as measured by Ki67 evaluation. Patients were followed for 2 years.

At the end of the 3 treatment cycles, the patients presented clinical improvement in the mean number of hairs, with a mean increase of 33.6 hairs in the target area, and a mean increase in total hair density of 45.9 hairs per cm<sup>2</sup> compared with baseline values. Microscopic evaluation showed the increase of epidermis thickness and of the number of hair follicles 2 weeks after the last PRP treatment compared with baseline value ( $p < .05$ ). There was an increase of Ki67 (+) keratinocytes in the epidermis and of hair follicular bulge cells, and a slight increase of small blood vessels around hair follicles in the treated skin compared with baseline ( $p < .05$ ).

Relapse of androgenic alopecia was not evaluated in all patients until 12 months after the last treatment. After 12 months, 4 patients reported progressive hair loss; this was more evident 16 months after the last treatment. Those four patients were re-treated.

Our explanation for our results can be ascribed to the fact that most of androgenetic alopecia patients are mostly accompanied with chronic telogen effluvium which responds well to PRP, so the short term effect predominate, but for the other element of androgenetic alopecia, it has a mild to moderate response which can be evaluated on the long term effect of it.

Our results had shown that there was no significant relation between the starting age of androgenetic alopecia and the duration of the disease with neither the hair pull test results nor the hair density results.

Our results are in agreement with the results of *El korashy SA. et. al., 2015* who studied 40 patients with FPHL divided into 30 cases and 10 controls and follisopic examination at the 1st visit and 3 months after the end of the study was done. The injections of PRP were done every 2 weeks for a total of 5 times. Meanwhile, the 10 controls were injected saline in the same sessions. Observation of hair regrowth and inquiry about hair fall was recorded before the next injection each session. Follow-up was done 1 and 3 months after the end of the study.

In a study conducted by *Shiavone et al., 2014*, who injected PRP in AGA patients concluded that PRP treatment may induce some degree of clinical advantage for male and female pattern hair baldness and recommended more randomized controlled trials to formally test the procedure of PRP injected, but again they lacked the objective measurement and their results depended on clinical double blind evaluators who examined clinical photos before and after.

Our results disagree with the results of *Gkini et al., 2015* who examined the efficacy of PRP injection in treatment of different types of hair loss including AGA and their results showed an overall patients satisfaction more in patients suffering from AGA for less than 2 years.

Our results showed that no significant statistical difference between males and females was found regarding both hair pull test results and number of follicular units..

In a study conducted by *Amgar and Bouhanna, 2013*, they concluded in their review that PRP application in hair loss treatment is far from being minor although it can't replace the conventional drug therapy or in severe cases the surgical hair graft, and it may delay or provide better results if used as pre-treatment before hair follicle transplantation. Also they suggested that clinicians should use objective methods to evaluate their studies, thus their recommendations were fulfilled in the current study.

The limitation in this study is that the study could have been performed on a wider sample of patients of both sexes with different age groups, disease durations and courses to minimize statistical errors and also to detect if there is a difference in obtained results in relation to these data.

The field of hair restoration and regeneration will continue to grow as newer technologies in hair stem cell injections come to the forefront as well. Rigorous studies will be needed to better help physicians and patients make the right treatment decisions.

**Conclusion:-**

PRP is an excellent tool in non cicatricial hair loss generally, but for AGA specifically it is considered as a moderate tool to gain the short time promoting effect which gives the patient an incentive to be compliant on the medical treatment.

Patients may need retreatment on the long run which cannot be practically applied, so PRP can be considered as transient alternative for minoxidil contraindicated patients with other supportive topical and systemic treatments.

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