



Identification of Platelet Rich Plasma (PRP) Hair losses Prevent: A Study in Birdem General Hospital, Dhaka, Bangladesh

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Abstract

Introduction: Newer method of treatment of alopecia is Platelet Rich Plasma (PRP). Clinicians have begun to inject PRP. PRP is defined as a volume of the plasma fraction of autologous blood with a base line platelet concentration (usually more than 1000000 platelet/ μ L) ¹. Androgenetic alopecia may be a quite common clinical condition encountered by the dermatologists in their daily observe.

Objectives: To assess the Clinical Profile of PRP Prevent Birdem General Hospital, Dhaka, Bangladesh.

Settings and design: Observational and interventional study.

Methods: The present study was Birdem General Hospital, Dhaka, Bangladesh from 1st July 2018 to December 2018. A complete of thirty cases of clinically diagnosed male patients with androgenetic alopecia within the age bracket of 15-50 years deep-rooted the topic material for gift study. Elaborated history and clinical examination of cases was done. All the routine investigations were dispensed. Statistical analysis used: information analysis was done by victimization applied math package for social sciences (SPSS) and "paired t test".

Results: A complete of thirty patients was studied. Sizable amount belonged to the age bracket 26-30(36.7%) years and 21-25 years (33.3) severally. Majority are from category a pair of to five, Hamilton Norwood classification of Aga. The case history was positive in nineteen cases (63.3%). Frontal, membrane bone and bone areas were concerned in most variety of patients i.e. in twenty patients (66.7%). The patients according clinical improvement within the kind of hair count, hair thickness, hair density with statistically vital p-value (p-0.00) when a median of 2-3 sessions.

Conclusion: In our study of thirty patients, twenty patients showed improvement of varied degrees starting from +1 to +7 in line with Jaeschke scale with vital p-value (p-0.00) when treatment with platelet blood living substance thrombolytic protoplasm living substance made plasma (PRP) primarily based upon that we have a tendency to conclude that platelet made plasma (PRP) is efficacious within the treatment of androgenetic alopecia.

Keywords: MPHL, androgenetic alopecia; platelet-rich plasma (PRP), efficacy, growth factors

1. Introduction

Hair is important for physical attractiveness and recognition. It is symbol of youth and potency. Hair loss which causes psychological stress. It is associated with poorer self-esteem and body image with greater depression, introversion and neuroticism. Physician should record details. Of hair loss started for how many days and progression. Seasonal association especially winter and autumn. Usually family history positive. In case of female in chronic hair loss due to iron deficiency, infection, diet, chemotherapeutic drug, smoking, weight loss. Patient need complete gynecological history. It includes menarche, menopause, regular irregular bleeding, excessive hair growth, systemic hormonal contraceptive and polycystic ovary syndrome ^{18, 91}. Androgenetic Alopecia, also known as androgenetic alopecia or male pattern baldness, is a common disorder that affects men and women. In case of male prevalence is 70%. Forty female develop diffuse thinning of hair. Androgenetic alopecia (AGA) is an illness characterized by a progressive hair thinning and miniaturization, diminishing both the length and the diameter, transforming it in fuzz so that with time it

completely atrophies and is characterised by the loss of hair from the scalp in a defined pattern. Determining factors appear to be genetic predisposition coupled with the presence of sufficient circulating androgens. The prevalence of this condition is high (up to 50% of white males are affected by 45 years of age ¹¹⁻³¹). AGA affects 80% of men and 50% of women, presenting two different patterns: a central and more diffuse in women (FAGA) and slightly different in men, in which the main characteristic is the recession of the frontoparietal line (MAGA) ^{2, 31}. MAGA usually starts around the 25 years of age in men, affecting about 50% of the entire male population at 45 years of age and around 70% of men will eventually develop MAGA sometime in their lives ^{2, 4, 51}. AGA is an androgenetic dependent illness, modulated by an active testosterone metabolite, called dihydrotestosterone (DHT) ^{2-4, 6-81}. Although the tissue distribution does vary, both enzyme 5 α -reductase types are found in the scalp follicles, which specifically concentrate in the dermal papilla ^{2, 91}. To pursue its effect, androgens bind to the human androgen receptor (AR), a member of the steroid-thyroid hormone receptor superfamily ⁹¹. Both testosterone and DHT can bind to the

AR domain, which act as a transcription factor, regulating the expression of androgen-sensitive genes [10]. The concentration of DHT, AR and 5 α -reductase has been demonstrated to be higher in the balding scalp [11-14]. Moreover, evidence has shown that AGA has a polygenic mode of inheritance, after finding that 81.5% of balding sons had fathers with type 3 or more in the Hamilton-Norwood scale [15], exceeding the autosomal dominant expectation of 50%. However, it has been difficult to encounter a gene related to AGA, since none were found in the Y chromosome neither the aromatase gene nor the 5 α -reductase [16, 17]. Moreover, it's been incontestible that patients littered with title of respect, particularly those in early ages, understand themselves older than they're, adding stress and extensive preoccupation to their lives title of respect actually becomes a psico-social drawback for the patient changing into bald at a young age, AGA truly becomes a psico-social problem for the patient becoming bald at a young age, who feels unattractive and less socially successful [1, 18-22]. At the present time, there are only two approved FDA treatments to deal with MAGA; oral Finasteride, which selectively inhibits the 5 α -reductase enzyme and reduces the concentration of DHT in the scalp follicle around 70%, inhibiting or even reducing the miniaturization of the hair follicle [23-27], and topic Minoxidil, which action mechanism remains unclear [3, 27-30]. Even though both treatments are effective, they are not exempt of complications; the first and more importantly, they are both chronic treatments [3, 4]. For this reason, Finasteride over time can produce: depression, gynecomastia, hepatic enzymes elevation, cholesterol elevation, sexual dysfunction, impotence, decreased libido and mood

disorder [31]. On the other hand, the harmful effects of Minoxidil include: contact dermatitis, facial hyper-trichosis and transitory hair loss [3]. An enormous economic and human effort is being done trying to find new strategies and treatments for AGA, and a promising one is the Platelet-rich plasma (PRP) [3, 32]. PRP consists of an autologous preparation of concentrated plasma with a threefold to eightfold increase in platelet number, ~1.500.000/ μ L, compared to normal plasma [33-35], which is extensively used to promote soft tissue healing. PRP started to be used in the 70s for its healing properties for cutaneous ulcers [32]. Moreover, it has been utilized in other specialties like traumatology, plastic surgery and maxillofacial surgery. Numerous studies have been conducted lately to assess the efficacy of PRP as a hair prevention treatment option, but the question whether is effective for AGA or not remains unsolved. Thus, a systematic review in June 2018 has been conducted in order to gather all the evidence by searching through PubMed and Cochrane databases, using the keywords "Alopecia AND PRP". This search resulted with a total of 15 articles, which we excluded 9 review articles, 3 studies using PRP to treat other hair diseases, 3 expert opinions, 2 articles were off topic, 1 abstract, and 1 article was conducted only with animals, resulting in a total of 14 studies. We found the prevent procese of 6 article. We focused on the most recent studies, especially those that included placebo-controlled trial. These 14 studies were carefully evaluated, focusing on the PRP protocol used and the results obtained in hair prevention, all summarized in three tables grouped by: study outcomes, PRP protocol, and PRP application. The first 10 article's data were plotted to graphically show their hair prevention results.

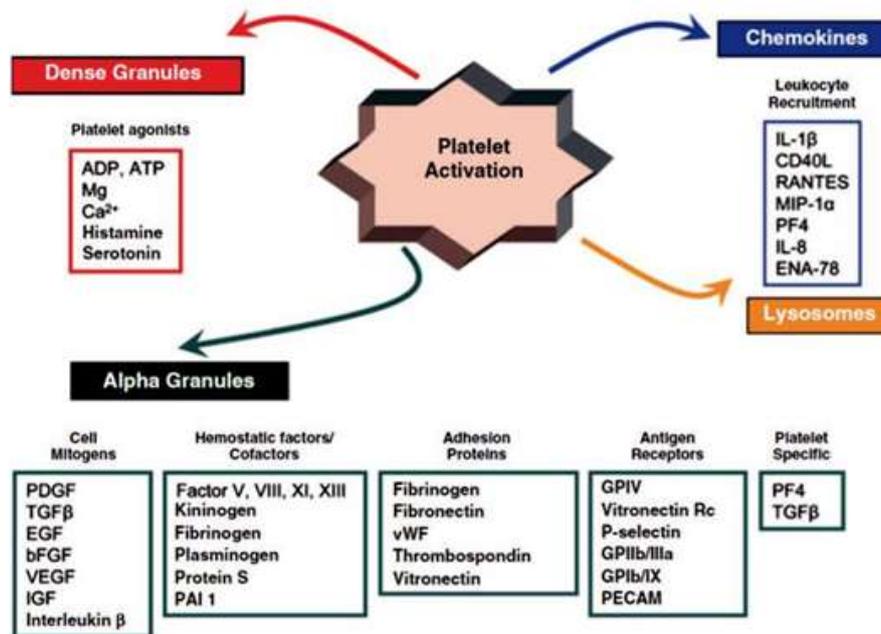


Fig 1: Platelet components [44].

2. Objectives

General objective

- a) To assess the Clinical Profile of PRP Prevent Birdem General Hospital, Dhaka, Bangladesh.

Specific objectives

- a) To follow the patients minimum up to 15-50 years after

enrolment in the study.

- b) To analyze the Hair growth and rewards.

3. Methods

The present study was Birdem General Hospital, Dhaka, Bangladesh from 1st July 2018 to December 2018. An observational and interventional study the efficacy of

platelet rich plasma (PRP) in the treatment of androgenetic alopecia in male patients and female patients. In this study PRP was given prevention as a monotherapy without any concomitant treatment. A total of 30 cases of clinically diagnosed male patients with androgenetic alopecia constituted the subject material for present study. Detailed history and clinical examination of cases was done. All the routine investigations were carried out. Prior approval for the study and the protocol was obtained from the ethical committee. The cases were selected on the basis of the following inclusion and exclusion criteria:

Inclusion criteria

1. Patients PRP prevention who are willing for the procedure.
2. Male patients in age group 15-50 years.
3. Patients with androgenetic alopecia of all classes as per Hamilton Norwood classification.

Exclusion criteria

1. Patients with alopecia other than androgenetic alopecia, such as alopecia areata, alopecia to talis, telogen

- effluvium, anagen effluvium, acquired cicatricial alopecia etc.
2. Patients with history of bleeding disorders.
3. Patients on anti-coagulant medications (aspirin, warfarin, heparin).
4. Patients with active infection at the local site.
5. Patients with keloidal tendency.
6. Patients with history of psoriasis or lichen planus because of risk of Koebner’s phenomenon.

Procedure

1. Collection of the patient’s blood sample.
2. Preparation of Platelet Rich Plasma by ultracentrifugation of the blood sample (manual double spin method).
3. Activation of the procedure site (scalp) by micro needling technique using a derma-roller with needle length 1.5 mm.
4. Application of extracted plasma on the activated site and massaging, to allow it to percolate through the epidermis.

The procedure has been illustrated as a simplified flow chart below

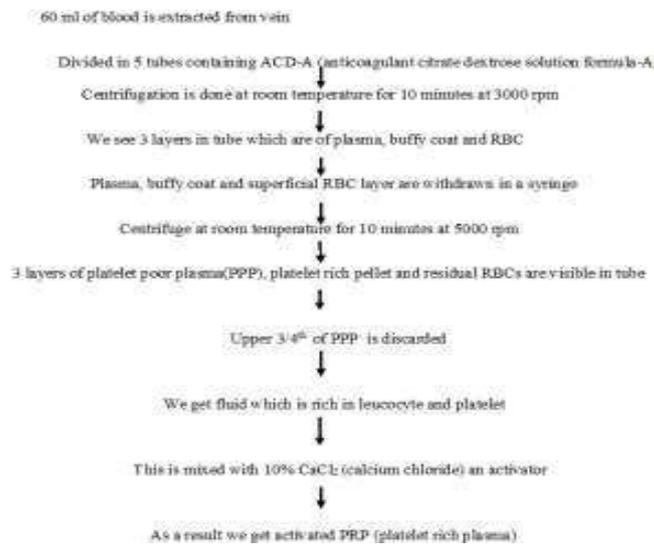


Fig 2

The molecular mechanism of action of PRP can be explained with the help of the following:

1. There is increased bcl-2 levels which being anti-apoptotic prolongs the survival of dermal papilla cells.
2. Expression of FGF-7 in dermal papilla cells leading to prolongation of anagen phase of hair cycle.
3. Upregulation of transcriptional activity of β -catenin leading to differentiation of stem cells into hair follicle cells.
4. Ncreased VEGF and PDGF which being pro-angiogenic increases the perifollicular vascular plexus.

There occurs activation of AKT and ERK signalling pathways which prolongs the survival of dermal papilla cells. A total of 6 such sittings will be given to each patient at interval of 3 weeks each, over a total period of 3 months. Photographs will be taken before and after each sitting with the help of a camera (Canon A800 power shot digital camera). In this way photographs will be taken periodically

every 3 weeks for the first 3 months, and then monthly after all the sittings have been completed for another 3 months. In our study we adopted a macro photography protocol. We preferred this to the spot and ultra spot images obtained with software-assisted methods, because techniques such as the trichoscan, although very popular, have been criticized over the last decade. In fact, though wishing on such pictures would have stressed the virtually microscopic details of the cyst units rising on the scalp, this method would have incomprehensible the “wider shot” pictures that will give a plan of the “scalp framed as a full,” that we have a tendency to rate because the most relevant aesthetic parameter for a practical analysis of any reasonably clinical improvement. The baseline severity of the condition was estimated by the independent evaluator (principal investigator) using a 15-point scale proposed by Jaeschke and colleagues. As a result of our study was ethically approved as Associate in nursing data-based study, our analysis was essentially unnatural to “before/after” observations. The clinical change between the

first assessment and the end of the follow-up was rated by the evaluator using the same 15-point scale used for assessing the baseline severity. The evaluator had to answer the following question: Overall, has there been any change in this patient's condition since the first visit? Please indicate if there has been any change by choosing one of the following options. The answers ranged from -7, corresponding to "A very great deal worse," to +7, corresponding to "A very great deal better," and with 0 corresponding to "About the same." The evaluations were made by an independent observer who was blinded to pre and post-information. We report here the whole Jaeschke scale:

19. -7: A very great deal worse.
20. -6: A great deal worse.
21. -5: A good deal worse.
22. -4: Moderately worse.
23. -3: Somewhat worse
24. -2: A little worse.
25. -1: A little worse, hardly any worse at all.
26. 0: About the same.
27. +1: A little better, hardly any better at all.
28. +2: A little better.
29. +3: Somewhat better.

In Androgenic Alopecia

Most common Miniaturization of hair is hall mark. In progression of the disease miniaturization continues and muscle completely loss attachment to the secondary follicles. When entire follicular unit miniaturization then blandness occurs. PRP shows potential benefit as adjuvant to minoxidil and finasteride. Singhal *et al.* found good hair growth 65%. New hair growth 6 patient out of ten.

Alopecia Areata

Singh *et al.* found that out of 50 patients one had relapse.

Telogen effluvium

PRP telogen effluvium has limited study. Improve diet and over 6 months if improved.

Cicatricial alopecia

Lichen planopilaris, frontal fibrosing alopecia, folliculitis decalvans, cutaneous discoid lupus erythematosus etc. an adequate evaluation including detailed history and management is essential for appropriate patient care and successful treatment of alopecia. Microneedling with platelet rich plasma effective for treating cicatricial alopecia, recalcitrant alopecia and traction alopecia. PRP injections improves cutaneous ischemic condition, increase vascular structure around HFs. New follicle through FUE punch grafting and PRP improve hair density in scotting alopecia^[11-16]. PRP can be classified into four main families depending on their cell content and fibrin architecture:

- a) Pure PRP (P-PRP) here leucocyte poor platelet rich plasma preparation;
- b) Leucocyte and platelet rich plasma (L-PRP) here leukocytes with formation of low density fibrin network on activation;
- c) P-PRP (Pure Platelet Rich Fibrin): Leucocyte poor platelet rich fibrin. Highly dense fibrin network;
- d) L-PRF (Leucocyte and PRF): Leucocyte with highly dense fibrin network.

Contra indications

- A) Absolute contra indication: Platelet Dysfunction Syndrome, critical thrombocytopenia, Hemodynamic Instability, Septicemia, Local infection at the site, patient unwilling to sign consent;
- B) Relative contra indication: Consistent use of NSAIDs within 48 hours of procedure, corticosteroid injection at treatment site within 1 month, systemic use of corticosteroids within 2 weeks, Tobacco consumption, recent fever/illness, cancer especially haematopietic or bone, hemoglobin <10g/dl, platelet count $10^5/uL$.

Complications

It is usually safe procedure. Minor complication may develop like pain in injected area, headache, heaviness of head, swelling, redness, infection, allergic reaction. Urticarial rash, temporary skin discoloration, bruising etc.^[17-19].

Procedure

Under aseptic condition 20cc fresh blood is taken by venipuncture. Container containing blood should have sodium citrate. Blood containing tubes are rotated in centrifuge machine at 1500 revolutions per minute for at least 6 minutes. First centrifugation is known as "soft spin". Blood separated into three layers. Target layer is intermediate layer PRP (5% of total volume). At bottom RBC layer (55% of total volume), topmost acellular plasma layer known platelet poor plasma (PPP, 40% of total volume). This intermediate layer called. 'Buffy coat'. Now Buffy Coat with PPP is collected with Finn Pipette into another test tube. Soon after, second centrifugation have done. Which is longer and faster known 'hard spin'. At least 2500 revolution per minute for 15 minutes. Platelet settle at the bottom of the tube. Upper layer containing PPP is discarded lower layer of PRP now loaded in an insulin syringe. Insulin syringe containing calcium chloride which act as an activation. The ratio is 1:9. Anesthetic clean have to apply on bald area. It is better to apply one hour before PRP. Scalp area is cleaned with spirit or providing-iodine. PRP is given by insulin syringe on affected area by nappage technique (several small injections in given in a linear pattern one one-cm apart). 2-3 cc injected in one setting. Repeat every two neck at least four setting^[20].

Growth factors in PRP

Platelet derived growth factor (PDGF), Transforming Growth Factor B (IGFB), Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF). Insulin like growth factor (IGF) interleukin-I^[2,3].

Machanism of action

Growth factor released from platelets act on stem cells in the bulge area of the follicles, stimulate development of new follicle, tissue angiogenesis, and growth of new organic structure, promote cell proliferation, differentiation and regeneration. PRP initiate and prolong anagen phase of the hair growth cycle. PRP increased expression of Type 1 collagen, mRNA-I and MRNA in the human dermal fibroblast and promote the proliferation of human adipose derived stem cells. Endothelial cells and keratinocyte produce PDGF (Platelet derived growth factor) which is responsible for cell growth and proliferation. In study it

shows that PDGF is responsible for maintain anagen phase of hair cycle, epidermis follicle interaction and hair canal formation, VEGF is major mediator of hair follicle growth, revascularization thus promot hair growth. The IGF-I develop catagen like status. Basic fibroblast growth factor (bFGF) helps in elongation of hair shaft. PRP activate increase level of anti-apoptotic protein Bcl-2 thereby preventing apoptosis. PRP act on rejuvenation keratinocyte (PDGF), stem cell factor (SCF), dermal papilla (IGF and fibroblast growth factor, vasculature (VEGF and PDGF) and neural cell (nerve growth factor) [4, 5, 6, 7].

4. Results

A total of 30 male patients of androgenetic alopecia were studied. The age ranged from 15-50 years. Majority of cases were in 26-30 years age group(36.7%), followed by 21-25 years(33.3%), 15-20 & 31-35 years(10% each), 36-40 years(6.7%) and >40years(3.3%). The family history was positive in 19 cases (63.3%). Frontal, parietal and occipital areas were involved in maximum number of patients i.e. in 20 patients (66.7%). Majority of patients ranged from class 2 to 5 of Hamilton Norwood classification. The patients reported clinical benefit in the form of hair count, hair thickness, and hair density after an average of 2-3 sessions. The improvement was maximum in class 2-5 and minimum in class 6-7. The latest study published by Alves and Grimalt in 2017 brought us an innovative approach on the AGA’s treatment, by combining PRP with minoxidil or finasteride. In this study, they recruited 30 patients with AGA, 11 males and 13 females, and treat all of them with PRP on one side of the head and saline solution on the other half-side, in combination of finasteride or minoxidil solution as ongoing treatment. After randomization, thirteen patients were treated with 1mL 5% minoxidil solution twice daily and 12 patients were medicated with 1 mg/day of oral finasteride, for a total of 30 patients. Results were assessed by TrichoScan analysis at 3 and 6 months after the last treatment dosage, resulting in a statistically significant of mean hair count (hairs/0.65 cm²), hair density (1/cm²), and terminal hair density (1/cm²) in the PRP associated with medication group versus placebo after 6 months (*p* < 0.05; month 6 vs baseline, data not shown). The combination of PRP and 5% minoxidil group showed higher hair restoration than PRP and finasteride, resulting in an increase of the total hair count, total mean hair density, anagen and telogen percentages, and mean anagen/telogen ration (*p* < 0.05: PRP + minoxidil vs PRP + finasteride). Biologically speaking, it makes sense to obtain a synergic effect by combining two therapies that potentiate both angiogenesis and hair buldge proliferation by two different mechanisms. Minoxidil and PRP together have shown higher restoration hair than the combination with Finasteride, opening new doors to AGA treatment in the future. However, we must take into account that the study was conducted with low grade alopecia in both men and women subjects recruited, and most of them were non-smokers, two aspects that may potentiate the therapeutic effects seen in this trial. As reported later on this review by Gentile *et al.*, the effect of PRP therapy seems to decay over time, so future longer follow-up must be accomplished to see the most adequate therapy frequency. Gentile *et al.* also in 2017 made a very interesting approach to assess whether is important to activate the concentrated platelet plasma by comparing CPuntT Preparation System, a non-activated (A-PRP) system, to Arthrex Angel System or

Regen Blood Cell Therapy, both being activated PRP (AA-PRP) systems. Proteins released from platelets were quantified before and after its activation with calcium, data shown in the following table. Table 3 and figure 1. Pretends to proof the non-similarity of secret-ed proteins with the C-PuntT system over the other two that needed previous activation. The amount of VEGF and PDGF-BB in the activated systems is significantly higher over the C-PuntT system, and equal to the rest of parameters. IGF-1 was not measured with the C-PuntT system that is why it is not represented. This study shows an improvement in hair density with the C-PuntT system of +64 ±3 hairs/cm² after 3 months of treatment compared to hair loss.

Table 1: Distribution of cases according to Hamilton Norwood classification (n30).

Hamilton Norwood classification (Class)	Our study (No of patients)
I.	2
II.	4
III.	9
IV.	6
V.	4
VI.	3
VII.	2
Total	30

Table 2: Distribution of cases according to site involved (n=30).

Site	No of patients	Percentage (%)
Frontal, parietal	5	16.7
Frontal, parietal, temporal	1	3.3
Frontal, parietal, temporal, occipital	3	10
Frontal, parietal, Occipital	20	66.7
Parietal, occipital	1	3.3
Total	30	100

Appropriate statistical tool (“paired t test”) was applied and the result was obtained as follows:

Table 3: Paired Samples Statistics before and after (n=30).

Paired Samples Statistics						
		Mean	N	Std. Deviation	t value	p value
Pair 2	Before treatment score	-3.4667	30	1.56983	7.25	0.000
	After treatment score	1.3000	30	3.79791		

Table 4: Trichoscan analysis assessed by Alves and Grimalt (n=30).

	Placebo		PRP Treatment	
	Mean	SD	Mean	SD
Hair Count (hairs/0.65 cm ²)				
Finasteride (n = 11)	0.9	16.3	0.6	10.8
5% Minoxidil (n = 13)	3.7	14.5	9.8	26.9
<i>p</i> -value	0.400		0.011	
Hair Density (1/cm ²)				
Finasteride (n = 11)	1.4	25.1	1.8	16.7
5% Minoxidil (n = 13)	5.1	23.9	12.3	34.2
<i>p</i> -value	0.283		0.010	
Terminal hair density (1/cm ²)				
Finasteride (n = 11)	-1.9	28.3	3.9	15.2
5% Minoxidil (n = 13)	5.6	21.9	3.2	38.4
<i>p</i> -value	0.138		0.175	
In bold <i>p</i> -value < 0.05.				

The scalp histopathological examination under the microscope was conducted after two weeks of treatment with CPunT system, which showed an increase of epidermal thickness, number of follicles, ki67+ expression, and capillary density assessed by CD31 expression over placebo. They conducted an interesting study to approach whether platelets needed to be activated, but many questions remain unanswered. The scalp histopathological evaluation was only assessed in the A-PRP treatment group, leaving the AA-PRP group microscopic effects out of the picture. Also, the A-PRP was assessed at 3 months after treatment and the AA-PRP after 6 months, showing an inconsistent analysis between the treatment groups. Finally, the number of patients included in both groups was 3 times higher for the A-PRP group, leaving only 3 patients for each AA-PRP treatment group, and placebo data was not shown in the AA-PRP group. The conclusion statement from Gentile *et al.* was that PRP does not need to be activated because a greater increase in hair count and total hair density is found in the A-PRP group using the C-PunT system over the AA-PRP. Although that statement is true when comparing CPunT system to Regen, it is not suitable when looking at the group treated with the Arthrex system, as we can see in the following graph. Anitua *et al.* developed a pilot study in which the use of PRP resulted positive for hair follicle regeneration. To do so, they took 50 patients with AGA, 32 males and 18 females, and treat them with five injections of PRP at months 1, 2, 3, 4 and 7, previously activated by the addition of platelet rich growth factor (PRGF). Compared to baseline, all outcome measures demonstrated a positive result after 5 sessions of PRP injections, assessed at 12 months when compared to baseline. The growth factors released after centrifugation were quantified by ELISA from the supernatant, and the results are described in table 2 and the PDGF, TGF-1, and VEGF are graphed in figure 4 in order to visually compare to other articles' results. Some of the results from Anitua *et al.* are depicted in figure 5, where hair density, hair diameter and terminal/vellus hair are represented. We can see a statistically significant improvement ($p < 0.05$) in hair density, hair diameter and the ratio of terminal/vellus hair after 5 sessions of activated PRP at 12 months follow-up. Histopathological examination also showed a statistically significant ($p < 0.05$) increase in epidermal thickness, basal keratinocytes proliferation (ki67+), perifollicular neoangiogenesis, and terminal/ vellus hair ratio together with a decrease of the inflammatory perivascular infiltrate and an increase of bulge stem cell population after PRP treatment. On top of these objective positive results, 13 out of 19 patients referred to be satisfied with the results obtained. Gupta *et al.* conducted an open-labeled, not placebo-controlled pilot study with 30 male patients aged 20 to 50 years old. Each participant received 6 PRP treatments, administered 15 days apart, but their PRP treatment administration approach was unique from the other studies. After the scalp was activated by micro needling, PRP solution was massaged on the scalp. Hair density and diameter were measured at the vertex, 10cm from the glabella, by using Tricho Scan, analysis, photographs analyzed by a blinded observer and self-assessment questionnaires. The results obtained showed an improvement of hair density and hair diameter detailed below, a $30.2 \pm 12\%$ average improvement in the photograph analysis, and a mean percentage improvement

of 30 ± 13.1 on the basis of self-assessment evaluation. Interestingly enough, treatment response showed a higher efficacy in different clinical scenarios analyzed. Those with a lower grade of alopecia, subjects with shorter duration of disease before undergoing PRP therapy, and those subjects without a family history of alopecia benefit more from PRP therapy than the rest. The following tables summarize the results. Alves and Grimalt conducted a randomized, placebo-controlled, double-blinded, half-headed clinical trial with 25 subjects. Subjects were grouped according to the half-head treated side; 3 mL of PRP on the right half of the head and 3mL of saline placebo on the left side. Results were assessed after three and six months, resulting in an increase of the mean anagen hair, mean telogen hair, hair density, and terminal hair density in PRP treated areas when compared with base-line. Mean total hair density at 3 and 6 months was the only result found to be statistically significant in the PRP treatment group when compared to placebo ($p < 0.05$).

Table 5: Growth factors and cytokines concentrations (n=30).

Protein	Collection System	A-PRP	Ca+2 AA-PRP
	Regen (AA-PRP)	1.2 ± 0.3	4.0 ± 2
PDGF-BB (ng/mL)	Athrex (AA-PRP)	1.1 ± 0.6	3.0 ± 1
	C-PunT (A-PRP)	1.8 ± 0.4	-
TGF-β1 (ng/mL)	Regen	11 ± 2	15 ± 3
	Athrex	12 ± 1	13 ± 0
IGF-1 (ng/mL)	Regen	130 ± 20	140 ± 20
	Athrex	150 ± 40	150 ± 60
VEGF (pg/mL)	Regen	61 ± 20	210 ± 40
	Athrex	61 ± 20	260 ± 70
	C-PunT	100 ± 20	-
FGF (pg/mL)	C-PunT	280 ± 60	-

Table 6: Growth factors and cytokines concentrations (n=30).

	Mean	SD
PDGF (ng/mL)	33	± 10
TGFB-1 (ng/mL)	21	± 12
VEGF (pg/mL)	218	± 127
EGF (ng/mL)	826	± 221
TSP-1 (μg/mL)	268	± 58
Ang-1 (μg/mL)	392	± 122

Ang-1: angiotensin-1; EGF: epidermal growth factor; PDGF: platelet-Derived growth factor; PRGF: plasma rich in growth factor; TGFb1: Transforming growth factor b1.

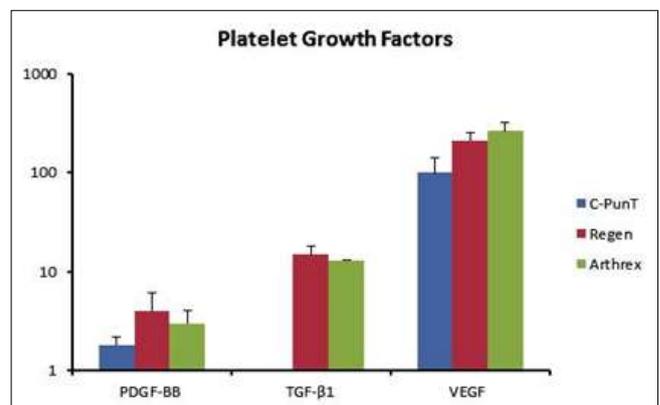


Fig 1: Platelet growth factors (Log scale 10). (*p-value>0.05)

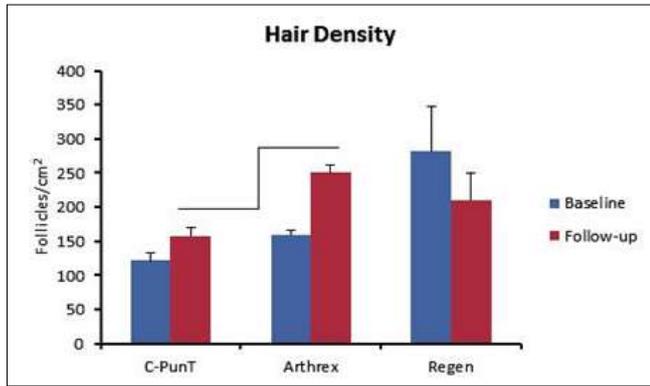


Fig 2: Hair density (hairs/cm²). (***)p-value <0.001

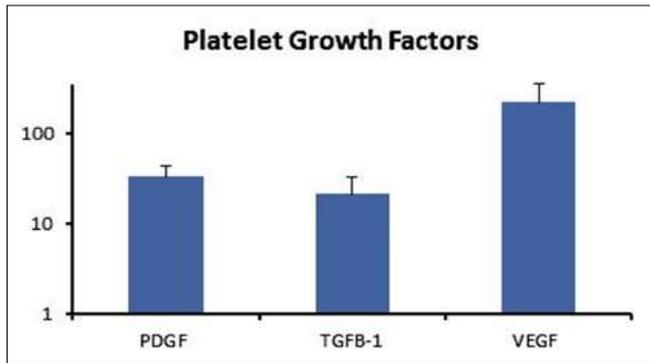


Fig 3: Platelet growth factors (Log Scale ^[1])

Looking at the demographic characteristics of patients, treatment with PRP showed a statistically significant correlation between hair density and patients aged ≤ 45 years old, start hair loss ≥ 25 years old, a positive family history, and more than 25 years of AGA’s appearance, when compared to placebo. This study also defends a positive correlation in PRP treated areas between anagen hairs (%) and patients aged more than 45 years old and beginning of AGA ≥ 25 years old, at 6 months. Another similar randomized, placebo-controlled, double-blinded, half-headed clinical trial was done by Gentile *et al.* with 23 male patients. PRP was injected in two out of four areas with apparent hair loss, and saline solution was injected into the other two remaining areas. The study found a statistically significant increase in many out-come measures; mean hair count, hair density and terminal hair density, after 3 months of PRP treatment compared to placebo. Specifically, a mean increase of 45.9 hairs/cm² in the treated area compared to 3.8 hairs/cm² in the placebo treated area after 3 months ($p < 0.0001$). The terminal hair density also improved significantly by 40.1 hairs/cm² with PRP over 5.6 hairs/cm² in the control area of the scalp ($p < 0.003$). Microscopic evaluation after 2 weeks from the last PRP treatment translated into a significant increase in the number of follicles, epidermal and hair follicular bulge cells, an increase of ki67+ basal keratinocytes, and an increase in small blood vessels around hair follicles in the treated skin area compared to baseline ($p < 0.05$).

Tables 7, 8, 9 and 10: Tricho Scan analysis and subgroups analysis by alopecia grade, duration of alopecia, and family history assessed by Gupta

Hair Diameter (mm)	PRP Treatment	
	Mean	SD
Baseline	0.055	0.015
3 months	0.072	0.017
6 month	0.075	0.019
% Change	39.85	17.21
Hair Density (1/10 mm²)	Mean	SD
Baseline	6.13	1.72
3 months	7.67	1.88
6 month	8.43	2.06
% Change	39.85	16.54
6 Months Increase (Mean ± SD)		
Androgenetic Alopecia Grade	Hair Density (1/10 mm²)	Hair Diameter (mm)
3	3 ± 0	0.025 ± 0.007
4	3.5 ± 0.71	0.025 ± 0.007
5	2.33 ± 0.58	0.03 ± 0.01
6	1.73 ± 0.47	0.015 ± 0.007
p-value	0.044	0.019
6 Months Increase (Mean ± SD)		
Duration of Alopecia	Hair Density (1/10 mm²)	Hair Diameter (mm)
Up to 5 years	2.68 ± 1	0.025 ± 0.007
6-10 years	1.6 ± 0.52	0.025 ± 0.007
> 10 years	2.0 ± 0	0.03 ± 0.01
p-value	0.048	0.009
6 Months Increase (Mean ± SD)		
Family History	Hair Density (1/10 mm²)	Hair Diameter (mm)
Present	2.15 ± 0.97	0.019 ± 0.007
Absent	3.25 ± 0.5	0.03 ± 0.008
p-value	0.011	0.027

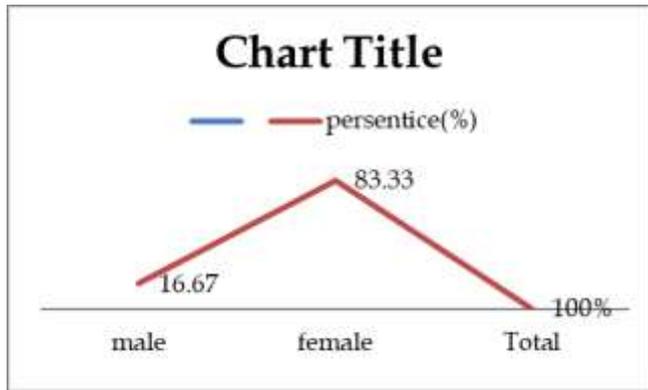


Fig 4: prevent of PRP.

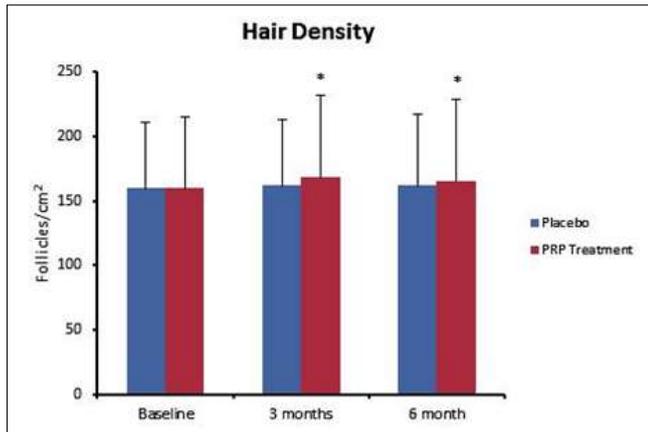


Fig 5: Results at 12 months follow-up. (*p-value <0.05).

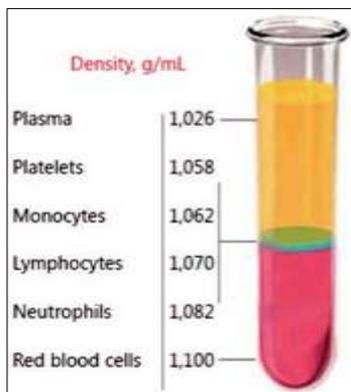


Fig 6: Results at 12 months follow-up. (*p-value < 0.05) [35].

The other older studies conducted by Schiavone *et al.*, Takikawa *et al.*, Khatu *et al.*, and Gkini *et al.* are summarized in Tables 1 to 3 [45, 47]. PRP treatment has been consistently shown to be effective at the microscopic level, by measuring an increase in the epidermal thick-ness, basal keratinocytes proliferation (ki67+), and perifollicular neo-angiogenesis after 2 to 4 weeks after treatment. The question of how long this effect may last have not been checked by any of the studies analyzed, understanding the obvious inconvenience of the biopsy of the hair scalp for the patient. In order to approach the real effect of the PRP therapy compared to the biological effect from the wound itself made by the needle (recently proven to stimulate epidermal and hair follicle stem cells differentiation), we merged all the published data to date. We are aware of the differences encountered in the stud-ies analyzed, and thus, we have only used hair density data from Trichoscan

analysis and placebo-controlled trials, data shown in the following table. Despite the differences from all the studies analyzed, PRP has shown a statistically significant difference when compared to placebo at 3 months, showing an increase of 30 hair follicles/cm². Thus, we can conclude that PRP seems a very promising therapy for AGA, and will bring patients better results once we have it standardized and maybe combined with other approved therapies, such as 5% minoxidil. Future studies will need to be consistent with the PRP protocol used, use half-head system as placebo, include more patients, ana-lyzed data with Trichoscan, gather the subjective benefit after treat-ment, and undergo a longer follow-up in order to assess the real efficacy of PRP and approach the best posology for this promising therapy.

5. Discussion

Androgenetic alopecia (AGA), common baldness is the result of progressive, patterned hair loss in genetically predisposed individuals exposed to androgens. Whether someone is considered bald or prematurely bald, is a matter of subjective assessment. The process by which common baldness occurs is androgen-mediated conversion of susceptible terminal hairs into vellus hairs. The lack of balding in eunuchs, pseudohermaphrodites and individuals with androgen insensitivity syndrome confirms that androgens are a prerequisite for common baldness. The prevalence and severity of male baldness increases with age. Platelet Rich Plasma is widely used in a number of medical and surgical specialities to enhance tissue repair and healing. Its potentiality to promote hair growth in areas containing hair follicles is known since 1900. The early clinical evidence and basic science supports the application of Platelet Rich Plasma in hair growth. But the awareness regarding its use in hair growth is limited as the procedure is a recent upcoming procedure. Many dermatologists worldwide use PRP with the idea of helping patients affected by a hair loss problem, but the assessment of the real efficacy obtained with this treatment, either for the doctor or the patient, is not an easy task. The idea behind this text is to gauge the various approximations to get PRP with scientific proof, and develop a meticulous study technique that may enable USA to acknowledge is that the real result of the PRP on treating Agha. According to this review, there is not a consensus protocol in any of the studies reviewed. In many occasions, dermatologists apply what the manufacturer from the PRP isolation machinery suggests as the standard protocol. The exact concentration, dose, deepness of the dosage, frequency of injections or the number of PRP sessions needed to be effective in AGA differ significantly and are not always specified. However, some common steps exist between the studies analyzed to obtain PRP, which are detailed below.*Platelet-Rich-Plasma Extraction Common Steps: (1) Peripheral blood is withdrawn from the same patient and transferred it into a tube with 3.8% sodium citrate. (2) The blood obtained is centrifuged at no more than 1.200 rpm. The centrifugation separates the sample into 3 visually recognizable layers; a top yellow layer (plasma), a thin layer in the middle called the buffy coat (white blood cells and platelets), and red blood cells (RBCs) in the bottom layer. (3) The plasma and the top part of the buffy coat (leukocytes rich) is dis-carded and the remaining buffy coat layer and a fraction of the RBCs are transferred into another tube. (4) Some authors undergo a second spin to achieve a more concentrated

solution. (5) The upper $\frac{3}{4}$ of the supernatant is discarded from the tube. (6) The pellet obtained is homogenized with the remaining fluid and the PRP solution is finished. In many cases, an activator such as calcium is added to the PRP in order to "activate" the platelets. (7) The PRP is injected with a 30-G needle into hair depleted areas. In their study hair loss was reduced and at 3 months it reached normal levels. Hair density reached a peak at 3 months. At 6 months and at 1 year, it was significantly increased compared to baseline. Patients were satisfied with a mean result rating of 7.1 on a scale of 1-10. No remarkable adverse effects were noted. The possible limitations of our study are:

- 1 We have adopted a macrophotographic method instead of the trichoscan.
- 2 The follow-up period has to be extended, to verify whether the improvement observed at an interval of 6 months after the intervention may be maintained over time.
- 3 Both measures used to assess the clinical severity and the possible improvement at follow-up, are subjective and so are exposed to some extent to the bias of the investigator.
- 4 Significant pain and headache were the two side effects observed in the patients.

It is important to determine the frequency and the number of sessions not only based on half-life of platelets but also up to patient's background. The role of age, AGA staging and correlation with androgenic activity may be considered. These factors could interfere with PRP efficiency. For example, in a young subject, the regenerative capacity and androgenic activity is equally high. These two factors can thwart variously and alter PRP efficacy. On the other hand AGA staging and disease progress are important. PRP seems to be less effective in late AGA and/or rapidly progressing AGA. Patient compliance is not always very good with minoxidil, because of cosmetic effects and long term use. Finasteride has raised concerns for some patients regarding its sexual side effects (decreased libido, hypofertility). PRP as an autologous and non-chemical treatment is better accepted by patients who wish to avoid these disadvantages. Consequently for non-responders to these therapies, PRP can be an alternative. As the mechanism of action of PRP is different from the two other treatments, we may have an additional positive effect by using PRP in responders to minoxidil and finasteride. In terms of costs, PRP may be more cost effective in long term than conventional therapies.

Unlike the conventional treatments of AGA, our approach does not require long term therapy or high degree of compliance with no potential side effects.

For optimum results select the following responders:

44. Young subjects (less than 40- year-old).
45. Early staging (stage III to V).
46. Recent AGA (less than 4 years of progress).

6. Conclusion

We believe that a randomised, placebo-controlled, double-blinded, half-head study would be the most effective thanks to objectively assess the important effectuality of PRP. To raised clarify the utilization of PRP activation, we tend to propose to endure a two-arm study; during which 1/2 the patients are randomised into a gaggle receiving either

activated PRP with atomic number 20 and isosmotic solution within the spouse of the pinnacle, and therefore the spouse can receive PRP while not being activated and isosmotic solution within the spouse. Solely by comparison the micro-trauma done by the injection of answer, we'll be able to confirm whether or not the therapeutic effects return from the "needle effect" or the PRP itself. All knowledge can have to be compelled to be processed by Trichoscan analysis, pictures and subjective questionnaires before and when treatment. The opposite question that continues to be nonreciprocal is whether or not the PRP activation is critical and that technology and protocol steps we want to try and do to get the most effective quality PRP. An extended follow-up, concerning sixteen months when stopping the treatment, could also be of interest so as to get however long the result of the PRP medical care lasts. From our own expertise, mixed beside recent proof from this systematic review, we tend to propose the subsequent protocol which may amendment within the future if new scientific proof incontestable alternative results.

7. Limitations of the study

This study was conducted in one Centre with small sample size. So study results might not reflect the scenarios of the Birdem General Hospital, Dhaka, Bangladesh.

8. Acknowledgement

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Conflict of interest: The Author no conflict of interest.

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