Journal of Stem Cells
Volume 11, Number 4

ISSN: 1556-8539

Efficacy of Activated 3X Platelet-Rich Plasma in the Treatment of Androgenic Alopecia

Rani James, Rosy Chetry, Vignesh Subramanian, Abhishek Ashtekar, Nandagiri Srikruthi, Sankar Ramachandran, Prasad S Koka, and Kaushik Deb*
Merisis Therapeutics, DiponEd BioIntelligence, Bangalore, Karnataka, India

Abstract

Background: Platelet-rich plasma (PRP) has shown remarkable beneficial effects without any major adverse reactions in the treatment of androgenic alopecia .The growth factors in activated autologous PRP induces the proliferation of dermal papilla cells.
Objectives: To investigate the clinical efficacy of Platelet Rich Plasma prepared using Merisis One Step Gel Separation Technology in treatment of androgenic alopecia.
Methods: Five patients were given autologous PRP injections on the affected area of alopecia over a period of three months at interval of two - three weeks and results were assessed.
Results: Three months after the treatment, the patients presented clinical improvement in the hair counts, hair thickness, hair root strength and overall alopecia.
Conclusion: PRP appears to be a cheap, effective and promising therapy for androgenic alopecia with no major adverse effects.

Introduction

PRP is an autologous concentration of platelets in concentrated plasma. It is considered to be a rich source of autologous growth factors (GFs), which appear to enhance angiogenesis, extracellular matrix remodeling, and cellular effects as cell proliferation and differentiation. Since then, it has been investigated and used in numerous fields of medicine, such as orthopedics, oral and maxillofacial surgery, plastic surgery and also dermatology. Activated PRP appeared to contribute to the formation of hair epithelium and the differentiation of stem cells into hair follicle cells, through an upregulation of β-catenin, strongly expressed in the bulge region of the human anagen hair follicle, and to prolong anagen phase of hair cycle, through an increase in expression of fibroblast growth factor [1]. Recently there have been a few peer-reviewed studies investigating the

* Corresponding Author: Kaushik Deb, DiponEd BioIntelligence LLP, #60/A, 2nd Floor, Karnataka Bank Building, Jigani Road, Bomasandra Industrial Area, Bangalore, Karnataka – 560099, India. Phones: +91 99005 20141 (Cell). Landline: +91 (0)80 2783 5022. Email: deb@diponed.com

clinical results of PRP applications on Androgenetic alopecia [2-4]. The objectives of this study are to determine the effectiveness of PRP preparation and its possible mechanism of action in promoting hair growth.

Androgenetic alopecia (AGA), a hereditary and androgen-dependent progressive thinning of the scalp hair in a defined pattern, is a common dermatological disorder affecting men more than women, with significant negative impact on their social and psychological well-being. It commonly begins by 20 years of age and affects nearly 50% of men by the age of 50 years [5].

Treatment options for androgenic alopecia are very limited. Except scalp surgery, which is a surgical treatment option, drug therapies include usage of finasteride and minoxidil. Minoxidil appears to prolong anagen phase and promote survival of dermal papilla cells and increase in hair follicle size. However, there are several reported side effects such as headache and increase in facial hairs for minoxidil. Finasteride also interferes with genital development in a male fetus and is contraindicated in pregnant women and those likely to become pregnant. Compared to minoxidil and finasteride, PRP therapy is safe, cheap, and non-allergic and it appears to be a useful adjuvant in the management of Alopecia [6].

Concerns about the efficacy and safety during the requisite long-term treatment of AGA with oral finasteride and topical minoxidil therapy prompted the use of a newer modality of platelet-rich plasma (PRP) which has shown beneficial effects [6]. As a concentrated source of autologous platelets, PRP contains several different growth factors and other cytokines that can stimulate angiogenesis, extracellular matrix remodeling, and cell proliferation and differentiation [7]. Growth factors play a fundamental role in the life-long cyclic transformation of the hair follicle from anagen (active hair shaft production) to catagen (apoptosis-driven regression) to telogen (resting phase with the involution of hair follicle) [8]. Main growth factors stored in α-granules of platelets are platelet-derived growth factor (PDGF), transforming growth factor-b (TGF-b) and vascular endothelial growth factor (VEGF) with their isoforms. Binding GFs (PDGF, TGF-β, and VEGF) activate the proliferative phase of the hair, giving rise to the future follicular unit [9].

PRP preparation process is rapid and requires minimal specialized equipment or instrumentation. PRP can be applied to a patient within hours of a treatment decision. These features make PRP extremely attractive for clinical use in a variety of settings, including not only hospitals and outpatient clinics, but also in field applications or other areas with limited medical facilities and resources [10, 11, 12]. Varied methods of preparation of PRP have been reported in the literature. These include commercial kits and manual methods using laboratory centrifuge [13, 14]. The clinical effectiveness of each of these methods remains unclear and depends on several variables like centrifugation rate and time [7]. The objective is to investigate growth factor concentration in the Merisis one step gel separation kit and to study its clinical efficacy in treatment of androgenic alopecia.

Materials and Methods

Platelet-rich plasma therapy was given to males in the age group of 30-50 years having mild to moderate AGA. After taking adequate consent, they were given PRP therapy over a period of 3 months and their condition was assessed at regular intervals. The subjects who were systemically healthy, not presently under any medications were included in the study. An informed consent was obtained from all the participants included in the study. The study was in accordance with the ethical standards of the responsible committee and was approved by the Institutional Ethics Committee.

PRP is obtained from the patient's own blood and is injected subcutaneously into the area of alopecia. Area of the scalp is cleansed with spirit and povidone-iodine. With the help of insulin syringe PRP is injected over affected area by nappage technique (multiple small injections in a linear pattern 1-cm apart) under proper aseptic precaution in the minor operation theatre.

The effectiveness of the medication will be measured by changes in hair diameter and its growth, determined by physical exam and digital photography. Patients will be followed up for another 3 months after the initiation of treatment. To evaluate overall hair growth, hair volume, hair quality, and fullness,

global pictures are taken in every session from the front, vertex, lateral, and back view.

Blood Sample Collection

Peripheral blood samples were collected from five subjects under aseptic conditions. Venous blood (20ml) were drawn in BD vacutainer tubes containing EDTA from the study subjects. Baseline RBC and Platelets counts for all the subjects were assessed using Abbott CELL-DYN 3700 System. Growth factor concentration of PRP fraction was determined by Enzyme Linked Immunosorbent Assay (ELISA).

PRP Preparation

PRP was prepared via one step gel separator technology developed by Merisis therapeutics (Figure 1). The samples were centrifuged using the laboratory centrifuge (REMI R-8C) at 3500 rpm for 25 minutes. Following centrifugation, the tube contains 3 layers: the upper layer of platelet rich plasma, middle layer of separating gel and bottom layer of RBC. Granulocytes and erythrocytes have a higher density and therefore sediment through the gel layer during centrifugation. The PRP was removed with a pipette avoiding disturbance to the underlying gel.

Figure 1. Merisis One Step Gel Separation Technology.

Hair Growth Measurement

A photograph using digital camera enables an objective measurement of hair growth on a selected area.

Estimation of Growth Factors-ELISA

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for growth factors has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any growth factors present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for growth factors is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of growth factors bound in the initial step. The color development is stopped and the intensity of the color is measured. Optical density (OD) was measured at 450 nm using a microtitre plate reader (TECAN ELISA READER) and growth concentrations were determined.

Results

Platelets counts were measured before PRP injection in two samples. We recovered 80% of total initial platelets and the procedure resulted in a 3-fold increase in platelet concentration. Platelets were highly purified, because only <0.1% from the initial red blood cells and leukocytes were present in the final PRP preparation. The values are shown in Table 1-4 and Figures 7 & 8.

High concentrations of endothelial growth factor, epidermal growth factor, and fibroblast growth factor were secreted, together with insulin like growth factor, transforming growth factor and other cytokines [Table 5].

Hair growth was seen in all the recruited patients by the end of 3 months [Figures 2-6]. Our study resulted in a consistent PRP preparation method that yielded a cytokine and growth factor pool from different donors with high reproducibility.

Figure 2. Before After 12 weeks

Figure 3. Before After 12 weeks

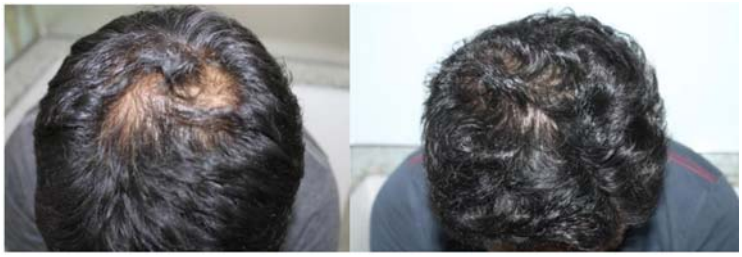

Figure 4. Before After 12 weeks

Figure 5. Before After 12 weeks

Figure 6. Before After 12 weeks

Table 1. Quantification of RBC and Platelets in Whole Blood (sample 1)

Parameter	Count in Whole Blood (µl)		Limit	
WBC	10.48	10^3/µl	4.00	11.00
NEU	6.38	10^3/µl	2.00	7.50
LYM	2.39	10^3/µl	1.00	5.00
MON	0.64	10^3/µl	0.10	1.00
EO	0.95	10^3/µl	0.00	0.40
BAS	0.12	10^3/µl	0.00	0.20
NEU%	60.9	%	40.0	75.0
LYM%	22.8	%	20.0	50.0
MON%	6.1	%	2.0	10.0
EO%	9.1	%	0.0	7.0
BAS%	1.1	%	0.0	2.0
RBC	5.53	10^6/µl	4.0	5.00
HGB	16.5	g/dL	14.0	16.0
HCT	49.1	%	39.0	48.0
MCV	88.8	Fl	70.0	90.0
MCH	29.8	pg	27.0	32.0
MCHC	33.5	g/dL	30.0	35.0
RDWsd	34.4	Fl	46.0	59.0
RDWcv	13.3	%	11.0	14.0
PLT	**190**	**10^3/µl**	**150**	**400**
PCT	0.13	%		
MPV	6.8	fL	8.0	15.0
PDWsd	17.6	fL		
PDWcv	37.5	%		
PLCR	29.95	%		
PLCC	57	10^3/µl		

Table 2. Quantification of RBC and Platelets in PRP Extracted Using Merisis One Step Gel Separation Technology (Sample 1)

Parameter	Count in Platelet Rich Plasma (µl)		Limit
WBC	10.20	10^3/ µl	4.00-11.00
NEU	1.66 -	10^3/ µl	2.00-7.50
LYM	6.09 +	10^3/ µl	1.00-5.00
MON	2.21++	10^3/ µl	0.10-1.00
EO	0.08	10^3/ µl	0.00-0.40
BAS	0.15	10^3/ µl	0.00-0.20
NEU%	16.3--	%	40.00-75.0
LYM%	59.7+	%	20.0-50.0
MON%	21.7++	%	2.0=10.0
EO%	0.8	%	0.0-7.0
BAS%	1.5	%	0.0-2.0
RBC	0.07	10^6/ µl	4.00-5.00
HGB	0.2*	g/dL	14.0-16.0
HCT	----**	%	39.0-48.0
MCV	---**	fL	70.0-90.0
MCH	0.0	pg	27.0-32.0
MCHC	---**	g/dL	30.0-35.0
RDWsd	0.0--	fl	46.0-59.0
RDWcv	---**	%	11.0-14.0
PLT	**631**	**10^3/ µl**	**150-400**
PCT	0.38	%	-
MPV	6.1	fl	8.0-15-0
PDWsd	18.1	fl	-
PDWcv	37.3	%	-
PLCR	26.70	%	-
PLCC	169	10^3/ µl	-

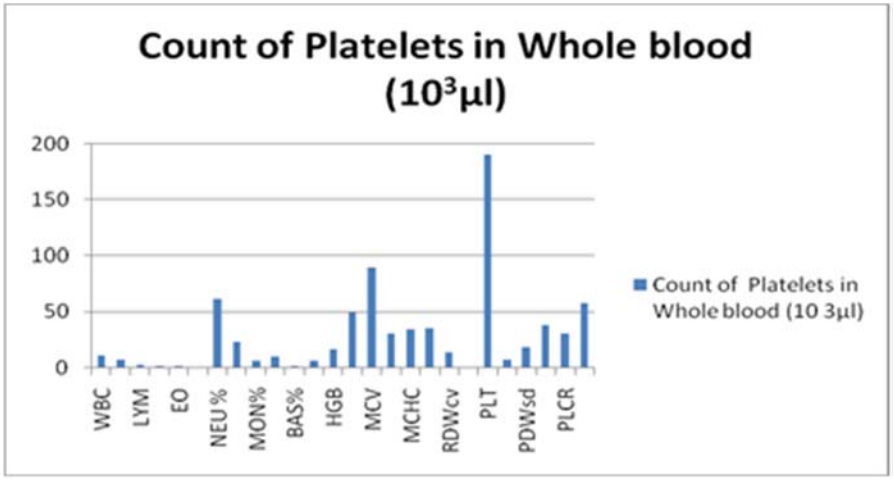

Figure 7(a). Quantification of Platelets in Whole Blood (Sample 1).

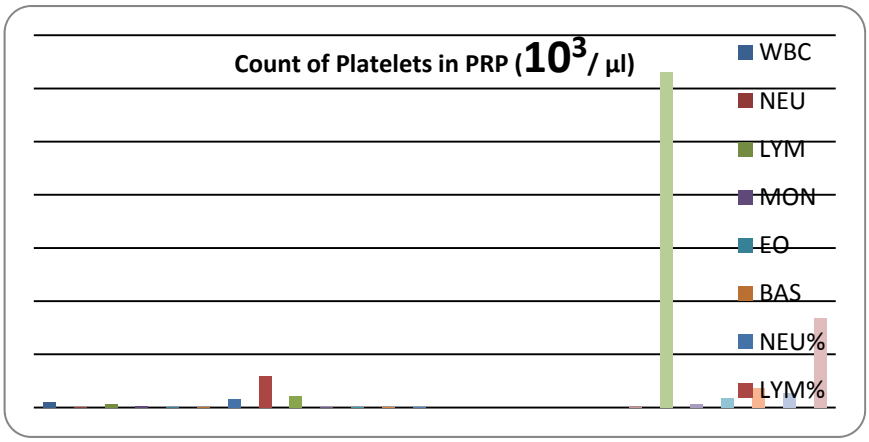

Figure 7(b). Quantification of Platelets in PRP (Sample 1).

Table 3. Quantification of RBC and Platelets in Whole Blood (Sample 2)

Parameter	Count in Platelets in Whole Blood (µl)		Limit	
WBC	8.28	10^3/µl	4.00	11.00
NEU	5.34	10^3/µl	2.00	7.50
LYM	1.70	10^3/µl	1.00	5.00
MON	0.59	10^3/µl	0.10	1.00
EO	0.51	10^3/µl	0.00	0.40
BAS	0.14	10^3/µl	0.00	0.20
NEU%	64.5	%	40.0	75.0
LYM%	20.5	%	20.0	50.0
MON%	7.1	%	2.0	10.0
EO%	6.2	%	0.0	7.0
BAS%	1.7	%	0.0	2.0
RBC	5.45	10^6/µl	4.0	5.00
HGB	16.3	g/dL	14.0	16.0
HCT	49.3	%	39.0	48.0
MCV	90.5	Fl	70.0	90.0
MCH	29.9	pg	27.0	32.0
MCHC	33.0	g/dL	30.0	35.0
RDWsd	39.5	Fl	46.0	59.0
RDWcv	14.1	%	11.0	14.0
PLT	**215**	**10^3/µl**	**150**	**400**
PCT	0.13	%		
MPV	5.9	fL	8.0	15.0
PDWsd	15.8	fL		
PDWcv	37.3	%		
PLCR	24.38	%		
PLCC	52	10^3/µl		

Table 4. Quantification of RBC and Platelets in PRP Extracted using Merisis One Step Gel Separation Technology (Sample 2)

Parameter	Count in Platelet Rich Plasma(µl)		Limit
WBC	1.42	10^3/ µl	4.00-11.00
NEU	0.31	10^3/ µl	2.00-7.50
LYM	0.64	10^3/ µl	1.00-5.00
MON	0.40	10^3/ µl	0.10-1.00
EO	0.07	10^3/ µl	0.00-0.40
BAS	0.01	10^3/ µl	0.00-0.20
NEU%	21.6	%	40.00-75.0
LYM%	44.8	%	20.0-50.0
MON%	28.33 **	%	2.0=10.0
EO%	4.7	%	0.0-7.0
BAS%	0.6	%	0.0-2.0
RBC	0.02	10^6/ µl	4.00-5.00
HGB	0.0	g/dL	14.0-16.0
HCT	----**	%	39.0-48.0
MCV	---**	fL	70.0-90.0
MCH	0.0	pg	27.0-32.0
MCHC	---**	g/dL	30.0-35.0
RDWsd	0.0--	fl	46.0-59.0
RDWcv	---**	%	11.0-14.0
PLT	**606**	**10^3/ µl**	**150-400**
PCT	0.28	%	-
MPV	4.5	fl	8.0-15-0
PDWsd	14.8	fl	-
PDWcv	38.0	%	-
PLCR	18.63	%	-
PLCC	113	10^3/ µl	-

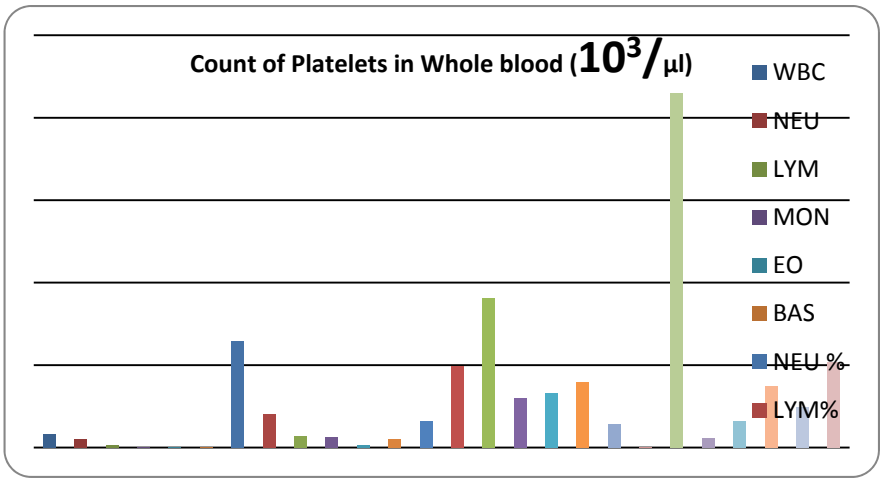

Figure 8(a). Quantification of Platelets in Whole Blood (Sample 2).

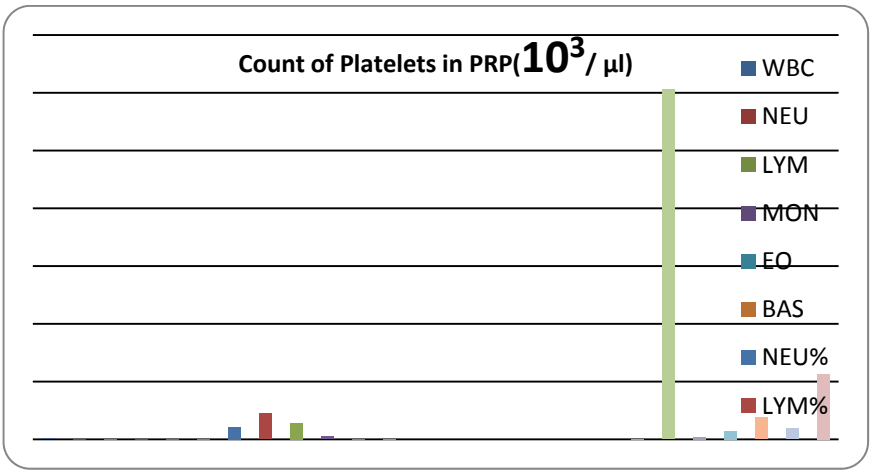

Figure 8(b). Quantification of Platelets in PRP (sample 2).

Table 5. Quantification of Cytokine and Growth Factor Concentration in Whole Blood and PRP Extracted Using Merisis One Step Gel Separation Technology

Cytokine and growth factor concentrates	Whole blood	PRP
PDGFA	3.3 ± 0.9 ng/ml	17 ± 8 ng/ml
VEGF	155 ± 110 pg/ml	955 ± 1030 pg/ml
TGFβ	35 ± 8 ng/ml	120 ± 42 ng/ml
IGF1	105.30 ± 48.44 ng/ml	110.30 ± 45.44 ng/ml
EGF	129 ± 61 pg/ml	470 ± 320 pg/ml
FGF-2	5 ± 50 pg/ml	226 ± 95 pg/ml

Discussion

Hair growth was seen in all the patients by the end of 3 months. The results of this study showed a significant increase in the mean hair count for the treatment area after three months. In this study, a mean 6,31000 platelets/µl in the PRP preparation could effectively stimulate follicular and perifollicular

angiogenesis, which is suggested to be one of the major factors in active hair growth. Platelet concentration in PRP was almost 3 times that of blood. Platelet concentration 3-7 times the mean levels is generally regarded as the therapeutically effective concentration of PRP [15].

PRP's regenerative potential depends on the levels of released growth factors. Once injected into the dermal layer, the platelets are activated and growth factors are released .The most important growth factors are VEGF, EGF, IGF, FGF etc. VEGF appears to be a major mediator of HF growth and cycling providing direct evidence that the improved follicle revascularization promotes hair growth. PDGF stimulate the growth of dermal mesenchyme. PDGF signals are involved in both epidermis-follicle interaction and dermal mesenchyme interaction required for hair canal formation and the growth of dermal mesenchyme respectively. EGF stimulates mitosis on epithelial cells and fibroblasts and improves the ratio of anagen. IGF slows down apoptosis. FGF stimulates the proliferation and differentiation of keratinocytes and endothelial cells. High concentrations of growth factors were found in PRP prepared using our kit [16].

Despite the growing interest in regenerative medicine, few trials investigating PRP's efficacy on hair growth have been published. Presently, there are limited published data regarding PRP's potential effect on hair. Ubel et al. [9] in 2005 studied 23 patients of hair transplant after enriching the hair root grafts with PRP and without PRP. Two areas (2.5 cm^2) each were marked on the scalp and planted with 20grafts/cm^2. After one year, the area implanted with the PRP-enriched grafts demonstrated an increase in follicular density of 15.7%. Li et al. [17] performed an *in vivo* study, where mice received subcutaneous injections of PRP, and their results were compared with control mice. Activated PRP increased the proliferation of dermal papilla (DP) cells. Studies conducted by Yong Miao et al. [18] found a significant difference ($p < 0.05$) in the number of newly formed follicles in the area of reconstituted skin (344 ± 27 with 10% PRP vs 288 ± 35 without PRP).

Current strategies for the treatment of pattern hair loss are mainly focused on promoting cellular proliferation and differentiation during the hair growth cycle. Minoxidil promotes the survival of dermal papilla cells by increasing Bcl-2/Bax ratio and by activating ERK and Akt .Oral finasteride also induces the prolongation of anagen hairs, which results in gradual thickening and elongation of the hairs [19]. Anti-apoptotic effects of activated PRP have been suggested as one of the major contributing factors stimulating hair growth. PRP-induced activation of anti-apoptotic regulators, such as the Bcl-2 protein and Akt signaling, prolongs the survival of dermal papilla cells during the hair cycle [20]. In addition, the upregulation of FGF-7/β-catenin signaling pathways with PRP treatment is suggested to stimulate hair growth by inducing follicular stem cell differentiation as well as prolonging the anagen phase of the hair growth cycle [21].

The ever increasing literature has also seen modifications of PRP to increase its therapeutic results. We are reporting the efficacy of Platelet Rich Plasma separated by Merisis one step gel separation Technology. Virtually eliminating granulocytes and RBC from PRP, which are considered not beneficial in terms of regeneration process and may contribute to a catabolic effect by secreting catabolic mediators and inducing apoptosis [22, 23]. Blood samples were centrifuged according to a single-centrifugation protocol. The double-centrifugation protocol caused alterations in platelet morphology and was more sensitive to small processing errors. Our study resulted in a consistent PRP preparation that yielded high growth factor concentration from different donors with high reproducibility.

Concluding, PRP may serve as a potential treatment for hair loss for AGA, with encouraging results. Considering its excellent safety profile and relatively low cost, PRP hair treatment is a promising treatment option for patients with thinning hair.

References

[1] Li ZJ, Choi HI, Choi DK, Sohn KC, Im M, Seo YJ, et al. Autologous platelet-rich plasma: A potential therapeutic tool for promoting hair growth. *Dermatol Surg* 2012; 38: 1040-1046.

[2] Arora NS, Ramanayake T, Ren YF, Romanos GE. Platelet-rich plasma: A literature review. *Implant Dent* 2009;18: 303-310.

[3] Marx RE. Platelet‐rich plasma: Evidence to support its use. *J Oral Maxillofac Surg* 2004; 62: 489‐496.

[4] Sánchez‐González DJ, Méndez‐Bolaina E, Trejo‐Bahena NI. Platelet‐rich plasma peptides: Key for regeneration. *Int J Pept* 2012; 2012: 1‐12.

[5] Ellis JA, Sinclair R, Harrap SB. Androgenetic alopecia: Pathogenesis and potential for therapy. *Expert Rev Mol Med.* 2002; 4: 1–11.

[6] Nitin D Chaudhari, Yugal K Sharma, Kedar Dash, and Palak Deshmukh. Role of Platelet-rich Plasma in the Management of Androgenetic *Alopecia. Int J Trichology*. 2012 Oct-Dec; 4(4): 291–292.

[7] Marx RE. Platelet-rich plasma: Evidence to support its use. *J Oral Maxillofac Surg* 2004; 62: 489-496.

[8] Kaufman KD, Olsen EA, Whiting D, Savin R, DeVillez R, Bergfeld W, et al. Finasteride in the treatment of men with androgenetic alopecia. Finasteride male pattern hair loss study group. *J Am Acad Dermatol*. 1998; 39: 578–589.

[9] Uebel C. A new advance in baldness surgery using platelet-derived growth factor. *Hair Transplant Forum Int'l*. 2005; 15: 77–84.

[10] Torricelli P, Fini M, Filardo G, Tschon M, Pischedda M, Pacorini A, Kon E, Giardino R (2011) Regenerative medicine for the treatment of musculoskeletal overuse injuries in competition horses. *Int Orthop* 35(10):1569–1576.

[11] Okabe K, Yamada Y, Ito K, Kohgo T, Yoshimi R, Ueda M (2009) Injectable soft-tissue augmentation by tissue engineering and regenerative medicine with human mesenchymal stromal cells, platelet-rich plasma and hyaluronic acid scaffolds. *Cytotherapy* 11(3): 307–316.

[12] Wu CC, Chen WH, Zao B, Lai PL, Lin TC, Lo HY, ShiehYH, WuCH,DengWP (2011) Regenerative potentials of platelet-rich plasma enhanced by collagen in retrieving pro-inflammatory cytokineinhibited chondrogenesis. *Biomaterials* 32(25): 5847–5854.

[13] Landesberg R, Roy M, Glickman RS. Quantification of growth factors levels using a simplified method of platelet rich plasma gel preparation. *J Oral Maxillofac Surg*. 2000; 58: 297–300.

[14] Okuda K, Kawase T, Momose M, Murata M, Saito Y, Suzuki H, Wolff LF, Yoshie H. Platelet-rich plasma contains high levels of platelet derived growth factors and transforming growth factor-β and modulates the proliferation of periodontally related cells *in vitro*. *J Periodontol.* 2003; 74: 849-857.

[15] Parul Singhal, Sachin Agarwal, Paramjeet Singh Dhot, Satish K. Sayal. Efficacy of platelet-rich plasma in treatment of androgenic alopecia. *Asian J Transfus Sci.* 2015 Jul-Dec; 9(2): 159–162.

[16] Gilbert Amgar, Pierre Bouhanna.Platelet Rich Plasma, a therapy for hair growth. *Prime Journal* 2013; 20-31.

[17] Li ZJ, Choi HI, Choi DK, Sohn KC, Im M, Seo YJ, et al. Autologous platelet-rich plasma: A potential therapeutic tool for promoting hair growth. *Dermatol Surg.* 2012; 38: 1040–1046.

[18] Yong miao, Ya-Bin Sun, Xi-Jin-Sun, Ben-Jun du, Jin-dou Jiang, zhi-Qi-Hu. Promotional Effect of Platelet rich plasma on hair follicle reconstitution *in vivo*. *Dermatologic Surgery* 10/2013: 1-9.

[19] Tosti A, B. M. Piraccini, Finasteride and the hair cycle, *Journal of the American Academy of Dermatology,* 2000; 42(5): 848–849.

[20] Kwon, H. K. Pyo, Y. J. Oh, et al. Promotive effect of minoxidil combined with all-*trans* retinoic acid (tretinoin) on human hair growth *in vitro*, *Journal of Korean Medical Science* 2007; 22(2): 283–289.

[21] Sohn, G. Shi, S. Jang et al. Pitx2, a *β*-catenin-regulated transcription factor, regulates the differentiation of outer root sheath cells cultured *in vitro*. *Journal of Dermatological Science* 2009; 54(1): 6–11.

[22] Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. *Am J Sports Med.* 2011 Oct; 39(10): 2135-2140.

[23] Fredriksson K, Stridh H, Lundahl J, Rennard SI, Skold CM. Red blood cells inhibit proliferation and stimulate apoptosis in human lung fibroblasts *in vitro*. *Scand J Immunol.* 2004 Jun; 59(6): 559-565.