

A COMPARATIVE STUDY OF THE EFFICACY OF PLATELET RICH PLASMA, 10% MINOXIDIL AND MICRONEEDLING IN PATIENTS OF ANDROGENETIC ALOPECIA***Gauri Vats, Durgesh Sonare, Dilip Kachhawa, Aditi Agrawal, Vinod Jain and Rohit Kataria**

Room No.35, PG Hospitel, MDM Hospital Campus, Jophpur.

***Corresponding Author: Gauri Vats**

Room No.35, PG Hospitel, MDM Hospital Campus, Jophpur.

Article Received on 29/04/2017

Article Revised on 20/05/2017

Article Accepted on 09/06/2017

INTRODUCTION

Androgenetic alopecia (AGA) is a non scarring alopecia that affects both males and females.^[1] Its prevalence increases with age and affects 50% of men and women by 50 years of age.^[2] By the age of 20, over 90% of males demonstrate some degree of hair loss which is usually progressive and patterned.^[3] In contrast, in females the alopecia presents as diffuse loss of hair largely affecting the frontal and vertex areas.

Androgenetic alopecia involves the action of androgens which are needed for regulation of hair growth in both sexes.^[4] The hormone specifically involved is the dihydrotestosterone (DHT) which leads to change in local metabolism leading to conversion of susceptible terminal hairs into vellus hairs.^[1] Additionally Transforming Growth Factor–Beta (TGF-B) an inhibitory factor secreted by hair follicles, microneedlings proliferation and cellular differentiation.

Treatment modalities proposed for promoting hair growth in AGA abound and important ones include topical minoxidil, topical amexil, oral and topical 5-alpha reductase inhibitors (finasteride and dutasteride).

Minoxidil is a vasodilator which was initially used as an oral drug to treat high blood pressure, however was found to cause hypertrichosis.^[1,2]

Minoxidil increases microcirculation by vasodilation and stimulates the mitosis in epithelial cells. Minoxidil 2% solution has been available as an Food and Drug Administration (FDA) approved topical treatment for hair loss in both men and women in United States. Strengths ranging from 2% to 12.5% are now available worldwide but 2% and 5% have been approved by FDA.^[5] Side effects may include pruritus, contact dermatitis and exaggeration of seborrheic dermatitis.

Finasteride was approved by FDA in 1997 for the treatment of AGA in men.^[2] Finasteride is a type II 5 alpha-reductase inhibitor. 5 alpha reductase is an enzyme that converts testosterone to DHT.^[6] Difference between Type1 and Type2 finasteride decreases the serum concentration of DHT decreases by 65% within 24 hrs.^[6]

Finasteride is well absorbed in the gastrointestinal tract, metabolized in the liver and excreted in urine. Approximately 1 in 50 males(2%) reported infrequent adverse effects in men including loss of libido, erectile and ejaculatory dysfunction, gynecomastia(0.04%), and isolated reports of depression have also been documented.^[2]

They are not usually effective for advanced AGA and complete baldness or bitemporal recession without visible hair does not respond to the treatment. Even in those who respond, there may be disappointment at the limited extent of improvement.

Biotin has also been tried but in vitro studies show no influence on cultured human follicular keratinocytes.^[7]

Hair transplant is considered a more definitive treatment. The first hair transplant surgery was performed in 1952 in New York.^[8]

Hair transplant is a surgical technique that moves individual hair follicles from a non balding area of scalp to the bald area of scalp. This hair transplant procedure is follicular unit transplantation (FUT) and was introduced in 1995.^[8] In this, stereo microscopic graft dissection is used to transplant hair in its naturally occurring groups. Harvesting follicular unit grafts by removing them directly from the donor area is known as follicular unit extraction (FUE). It has proven itself to be useful when strip harvesting is not indicated.

Surgical therapy is expensive and has its own risks. It should rarely be considered before the age of 30 because unless the hair loss has stabilized with loss of temporal points, the results of early surgery may be jeopardised by further hair fall.^[1] Hair thinning (sudden, unexplained

loss of more than 50-100 hairs daily) and shock loss (loss of either existing or transplanted hair after hair transplant procedure) are common side effects that are usually temporary. Other complications include infection, scarring, poor density.

Hair implantation is a controversial technique in which either artificial fibres or processed human hairs are implanted into the scalp. Only patients with an insufficient donor area should be considered for this surgery. However the associated side effects such as allergic reaction, foreign body granulomatous reactions, cicatricial alopecia and risk of development of malignancy have affected its use.^[9]

Conventional therapies for AGA may not be always effective, require long term compliance and may be associated with unacceptable side effects. Wigs and hair piece cover the entire area of hair loss with no chemical side effects and are useful when patients desire greater density that can be achieved with medications and surgery alone. Laser light of 650-900nm wavelength at 5mW has been suggested as an effective option for AGA.^[10]

It has been suggested that Botulinum Toxin relaxes scalp muscles thus reducing pressure on the perforating vasculature and improving blood flow and oxygen concentration, which helps to improve AGA as low oxygen environment favour the conversion of testosterone to DHT.^[11]

Platelet rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma^[12] and is an exciting non surgical therapeutic option for hair growth and stimulation.^[13]

PRP in medicine was first used in 1987 as an autologous transfusion component after an open heart operation to avoid homologous blood product transfusion.^[14]

Subsequently, PRP has been tried for various indications in the fields of sports, medicine, dentistry, cardiology, plastic surgery and orthopaedics.^[14]

PRP contains platelets many times greater than occurs normally in blood. Platelets contain an abundance of growth factors and cytokines many of which are implicated in hair growth including Platelet Derived Growth Factor (PDGF), Transforming Growth Factor Beta (TGF- β), Vascular Endothelial Growth Factor (VEGF), Epidermal Growth Factor (EGF), Fibroblast Growth Factor-2 (FGF-2), Insulin like Growth Factor (IGF), Keratinocyte Growth Factor (KGF), Connective Tissue Growth Factor (CTGF), Interleukin-1 (IL-1), IL-3, IL-4 and IL-8.^[12]

Growth factors promote tissue repair, angiogenesis and collagen production and also encourages normalization of hair follicular unit. As PRP is created from patients

own blood, it is considered as relatively low risk treatment.^[15]

Considering the early clinical evidence and basic science that supports the application of PRP in hair restoration surgery, we feel it reasonable to evaluate PRP for treatment of androgenetic alopecia.

Recently microneedling induced hair growth in mice has been reported and study by Rachita durat et al showed that microneedling is a safe and a promising tool in hair stimulation both for male and female Androgenetic alopecia. Microneedling works by stimulation of stem cells and inducing activation of growth factors.

In the present study, we are comparing the effect of intralesional injection of autologous Platelet Rich Plasma (PRP) with the topical application of 10% Minoxidil and microneedling in groups of 30 patients each, on hair growth in androgenetic alopecia.

AIM AND OBJECTIVES

1. To evaluate the efficacy of PRP versus topical 10% minoxidil and microneedling in the treatment of androgenic alopecia.
2. To study the safety profile of these modalities in the treatment of androgenetic alopecia.
3. To assess patient satisfaction in the above three modalities.

MATERIAL AND METHOD

Study design : Prospective study
 Study site : Department of Skin, STD and Leprosy
 Study duration : 1 year (2015-2016)
 Number of cases : 90 (30 cases of PRP, 30 cases of minoxidil group and 30 microneedling Group).

Material required

Trichoscan
 10% minoxidil
 Dermaroller 1.5 mm
 Acid citrate dextrose (anticoagulant) tube
 Sterile syringe

Method

Inclusion Criteria

1. Presenting with patterned hair loss.
2. Not taking any treatment for last 6 months.
3. Patients of age group 18-54 yrs.
4. Patients who will give written informed consent.

Exclusion Criteria

1. Patients with alopecia other than androgenetic alopecia.
2. Patients with history of bleeding disorders or on anti-coagulant medications (aspirin, warfarin, heparin).
3. Patients with active infection at the local site.
4. Patients with keloidal tendency.

Selected patients were thoroughly examined by trichoscan for

1. Hair thickness.
2. Hair density.

Patients were randomly divided into 3 equal groups.

Group 1: Platelet Rich Plasma.

Group 2: 10% Minoxidil.

Group 3: Microneedling.

Methodology of PRP preparation

After obtaining informed consent 8.5 ml blood sample was aspirated using 18G needle and collected in ACD vacutainer tube. The first centrifugation or "soft spin" was carried out at 1200 rpm for 8 minutes, and the separated buffy coat with PPP was collected with the help of a pipette in another test tube. This tube underwent a second centrifugation, a faster "hard spin at 2400 rpm for 4 minutes. The upper layer containing PPP was discarded and the lower layer of PRP was taken for platelet count.

Administration of Various Therapies

- One hour prior to the administration of PRP OR MICRONEEDLING, local anaesthetic cream was applied over the area of the scalp to be treated and cleaned with spirit and betadine.
- With the help of an insulin syringe PRP was injected by intradermal technique (multiple small injections in a linear pattern 1mm apart) under proper aseptic precaution in minor operation theatre in the androgen-related areas of scalp. Similarly microneedling was done in the allocated group of patients with the help of a dermaroller 1.5mm. Treatment sessions were performed with a 3 weekly interval. For each patient 6 such sittings were done
- Patients allocated to the minoxidil group were told to apply 10% minoxidil 1ml twice a day and followed up similarly.
- At each visit, hair count was checked over the prefixed square area. The patients were further evaluated at the end of 6 sittings.

OBSERVATIONS

In the present study comparing topical application of 10% minoxidil lotion with intralesional injection of PRP and microneedling, 90 cases of male pattern alopecia, who attended the Department of Dermatology between Aug 2015 and July 2016 were included. 30 cases of AGA each were included in all the above three groups.

Table 1: Age distribution in different groups.

Group	Age (in years)		p value
	No. of cases	Mean±SD	
PRP	30	36.03±9.91	0.51
Minoxidil	30	33.56±9.18	
Microneedling	30	35.9±8.53	

In the present study conducted, average age of patients in the PRP group was 36.03 yrs, Minoxidil group was 33.56 yrs and the microneedling group was 35.9 yrs. There was no significant difference between the mean age of all the three groups, signifying the groups to be comparable.

Table 2: Hair grading in different groups according to norwood hamilton grade.

Grading	PRP	Minoxidil	Microneedling	Total
Grade III	13 (43.33)	10 (33.33)	8 (26.67)	31
Grade IV	7 (23.33)	12 (40)	10 (33.33)	29
Grade V	6 (20)	4 (13.33)	8 (26.67)	18
Grade VI	4 (13.33)	4 (13.33)	4 (13.33)	12

In the present study conducted on 90 patients, 14.3% of patients were present in each of the Grade II and III of Norwood Hamilton Grade. Grade IV, V and VI had 23%, 13% and 4% and 2.9% patients respectively. Statistically comparable number of patients were present in each group.

Table 3: Mean hair thickness in 1st and last visit in different groups.

	Mean±SD hair thickness (micron)		
	PRP	Minoxidil	Microneedling
First visit	22.17±1.00	21.19±0.82	22.02±1.02
Last visit	24.43±1.21	29.95±1.22	23.79±1.54
p value	<0.0001	<0.0001	<0.0001

In the study conducted, the mean hair thickness of PRP group in the 1st and last visit was 22.17 and 24.43, Minoxidil group was 21.19 and 29.95 and the microneedling group was 22.02 and 23.79 respectively. Mean hair thickness after last visit didn't showed any significant difference in any of the groups statistically or clinically. The mean hair thickness increased by 2.9 micron in PRP group, 2.6 micron in minoxidil group and 2.7 micron in microneedling group. Though the increase in thickness was more in PRP group, this was not statistically significant.

Table 4: Distribution of hair density in 1st and last visit according to different groups.

	Mean±SD hair thickness (micron)		
	PRP	Minoxidil	Microneedling
First visit	101.36±1.14	101.36±1.11	101.6±0.95
Last visit	111.12±4.89	104.23±3.51	108.35±5.67
p value	<0.0001	<0.0001	<0.0001

In our present study, the mean hair density in the PRP group at the 1st and the last visit was 100.9 and 121.8, Minoxidil group was 98.4 and 106.4 and the microneedling group was 104.2 and 114.97 respectively. Patients of PRP group showed statistically significant increase in their mean hair density. Though the mean hair density

increased in the other 2 groups as well, this was not statistically significant.

Table 5: Table showing incidence of family history in all the three groups.

Family history	Microneedling	Minoxidil	PRP	Total
“No” Count (% within group)	14 40%	15 42.9%	10 28.6%	39 37.1%
“Yes”	21 60%	20 57.1%	25 71.4%	66 62.9%
Total	35	35	35	105

In the present study, 62.9% of the patients had a family history for Androgenetic Alopecia while 37.1% did not. Statistically, this was not found to be significant.

Table 6: Table showing diet of the three groups.

Diet	Microneedling	Minoxidil	PRP	Total
“Non-veg”	5 28.6%	10 42.9%	11 45.7%	26 39%
“veg”	25 71.4%	20 57.1%	19 54.3%	64 61%
Total	35	35	35	105

In our study, 61% patients were vegetarian and 39% patients were non vegetarian with no significant difference in the incidence of Male Pattern Alopecia.

Table 7: Table showing smoking habit in all the three groups.

Smoking	Microneedling	Minoxidil	PRP	Total
No	23 80%	18 65.7%	22 77.1%	63 74.3%
yes	7 20%	12 34.3%	8 22.9%	27 25.7%
Total	35	35	35	105

In the current study, 25.7% patients were smokers while 74.3% patients were non smokers. Smoking was not found to have a significant effect on development of Androgenetic Alopecia.

Table 8: Table showing alcohol habit in the three groups.

Alcohol	Microneedling	Minoxidil	PRP	Total
No	24 82.9%	18 65.7%	22 77.1%	64 75.2%
yes	6 17.1%	12 34.3%	8 22.9%	26 24.8%
Total	30	30	30	90

In the current study, 24.8% patients were alcoholic and 75.2% were non alcoholic. Alcohol was not found to

contribute significantly towards developing male pattern alopecia.

Table 9: Table showing habit of tobacco in all the three groups.

Tobacco	Microneedling	Minoxidil	PRP	Total
No	27 91.4%	24 82.9%	26 88.6%	77 87.6%
yes	3 8.6%	6 17.1%	4 11.4%	13 12.4%
Total	30	30	30	90

Post procedural

In the present study conducted on 90 patients, 12.4% patients were tobacco chewers and the rest 87.6% patients were non tobacco chewers. Tobacco chewing was not found to contribute significantly towards development of male pattern alopecia.

Table 10: Table showing post procedure perception in all the three groups after 1 month.

Post procedural perception	Microneedling	Minoxidil	PRP	Total
Hairfall stopped	0 (0%)	6 (17.1%)	2 (5.7%)	8 (7.6%)
Reduced hairfall	12(34.3%)	25(71.4%)	5(14.3%)	42(40%)
No change in hairfall	15 (48.6%)	4 (11.4%)	24 (68.6%)	45 (42.8%)
Increased hairfall	6 (17.1%)	0 (0%)	4 (11.4%)	10 (9.5%)
Total	30	30	30	90

In the present study of 90 patients, 7.6% patients perceived stoppage of hair fall after 1 month of therapy with maximum benefit seen among PRP patients (17.1%). 40% patients perceived reduced hair fall with maximum benefit seen in the PRP group (71.4%).

42.8% of the patients perceived no change in the hair fall. 9.5% patients perceived increased hair fall with most patients in the minoxidil group (17.1%).

Table 11: Table showing post procedure perception in all the three groups after last visit.

Post procedural perception after last visit	Micro needling	Minoxidil	PRP	Total
Hairfall stopped	2 5.7%	6 17.1%	2 5.7%	10 9.5%
Reduced hairfall	21 60%	24 68.6%	5 14.3%	50 47.6%
No change in hairfall	10 28.5%	4 11.4%	24 68.6%	38 36.1%
Increased hairfall	2 5.7%	1 2.9%	4 11.4%	7 16.6%
Total	30	30	30	90

In the current study on 90 patients, 9.5% patients perceived stoppage of hair fall after 3 months of therapy with maximum benefits in PRP group (17.1%). 47.6% patients perceived reduced hair fall with maximum benefit in PRP group (68.6%) and minimum in microneedling group (14.3%). 36.1% of total patients perceived no change in the hair fall. 6.6% patients perceived increased hair fall with 11.4% in microneedling group and 2.9% in the PRP group.

DISCUSSION

The present study on the effect of Intralesional injection of Autologous Platelet Rich Plasma (PRP) compared with Topical application of 10% minoxidil and Microneedling in Male Pattern Baldness was conducted in the Department of Dermatology, STD and Leprology.

In the present study, average age of patients who received Platelet Rich Plasma (PRP) was 25.8 yrs. The average age of patients who received minoxidil was 26.8 yrs and the average age of microneedling group was 26.8 yrs.

In the present study, there were 14.3% of patients who presented with each of the Grade II and III of Norwood Hamilton Grade. 27.6% patients were falling in, 21.9% patients were falling in Grade IV, 12.4% patients were falling in Grade V, 3.8% were in Grade VI while 2.9% patients were in Grade VII (Table 2).

Trancik et al in 2001 conducted a study on 496 adolescents in the age group of 15-17 yrs, and found that 77 (15.5%) subjects were rated as having stage 2 or greater hair loss on the Hamilton-Norwood grading scale. Although number of patients in our study were less still all patients having AGA stage 2 or higher grade were in the age group of 18-40 yrs.

In the present study, overall 62.9% of the patients had a positive family history for androgenetic alopecia whereas 37.1% patients had negative family history. Out of the above stated 62.9% patients, 37.8% belonged to PRP group, 30.3% in minoxidil group and 31.8% in the microneedling group (Table 5).

Trancik et al in 2001 concluded that a family history of AGA was present either on the father's side or the mother's side, or on both sides.

Nyholt DR et al in 2003 concluded that the major contributing factor to AGA is heredity which accounts for 80% of the variants. However, the genetic aspect lacks specificity because an individual carrying a risk associated will not suffer from AGA until he or she reaches a certain age.

Chumlea WC et al in 2004 concluded that men whose father had hair loss were 2.5 times as likely to have had some level of hair loss compared to men whose father had no hair loss.

Savant N et al in 2010 also concluded that family history definitely play a role and appears to be associated with age of onset of hair loss and patients with positive family history seem to present at young age.

In the present study, overall 61% patients were vegetarian and the rest 39% patients were non-vegetarians. Out of 61% vegetarians, 29.6% patients were in the PRP group, 31.2% in Minoxidil group and 39% in microneedling group. The difference was not found to be significant signifying that a predilection for a specific diet (ie veg or non veg) does not play a significant role in hair loss or growth (Table 6).

In the current study, 25.7% patients were smokers and the rest 74.3% were non-smokers. 29.6% smokers belonged to the PRP group, 44.4% to minoxidil group and 25.9% to microneedling group. Smoking was not found to have a significant effect on development of androgenetic alopecia in our study (Table 7).

Trueb RM et al in 2002 concluded that smoking affects the development of AGA because the genotoxic compounds in cigarettes may damage the DNA in hair follicles and subsequently cause microvascular poisoning in hair papillae.

Su LH et al in 2007 conducted a community based survey and found an association between smoking and AGA among Asian men.

Lai CH et al in 2013 conducted a study on 354 men and concluded a significant correlation between cigarette smoking and AGA.

In the current study, 24.8% patients were alcoholic with 30.7% in PRP group, 46.1% in minoxidil group and 23.07% in microneedling group. Alcohol was not found to contribute significantly towards developing male pattern alopecia (Table 8).

In the present study, 12.4% patients were tobacco chewers and the rest 87.6% were non tobacco chewers. PRP group had 30.7% tobacco chewers, Minoxidil group had 46.1% and microneedling group had 23.07%. Tobacco chewing was not found to contribute significantly towards development of male pattern alopecia (Table 9).

In our study, the mean hair thickness of PRP group in the 1st and last visit was 22.8 and 25.7, minoxidil group was 20.9 and 23.5 and the microneedling group was 24 and 25.7 respectively. The mean hair thickness increased by 2.9 micron (12.7%) in PRP group, 2.6 micron (12.4%) in minoxidil group and 2.7 micron (11.2%) in microneedling group. Though the increase in thickness was more in PRP group, this was not statistically significant (Table 3).

Greco et al in 2009 had observed an increase of 9.7% in average hair shaft diameter at 4 months and then 6.1% at 8 months with PRP treatment. In accordance with the

above study, there was an increase in hair thickness in our study as well, however, this was not found to be statistically significant.

In our study, the mean hair density in the PRP group at the 1st and the last visit was 100.9 and 121.8, minoxidil group was 98.4 and 106.4 and the microneedling group was 104.2 and 114.9 respectively. Patients of PRP group showed statistically significant increase in their mean hair density. Though the mean hair density increased in the minoxidil group as well, this was not statistically significant (Table 4).

Dr. Uebel in 2005, showed that area treated with PRP demonstrated a yield of 18.7 follicular units/cm² v/s 16.4 follicular units/cm² of the placebo group, an increase in follicular density of 15.1%. Greco et al in 2009 observed an increase in hair density of 18.8% at 3 months and 29% at 9 months in patients treated with PRP group.

Olsen EA et al studied 31 men using 2% or 3% minoxidil for AGA and showed that hair regrowth tended to peak at 1 year, and that non-vellus hair, beyond that seen at baseline, were maintained at 4.5 to 5 years later. Another study with the topical use of 2% and 5% minoxidil demonstrated statistically significant increase in hair weights compared with placebo [Price VH et al, 1999].

Rachita Dhurat et al in 2013 observed an increase in mean hair count of 91.4 in patients who underwent microneedling along with 5% minoxidil application as compared to patients who had applied only 5% minoxidil topically, who showed an increase of 22.7.

In the current study, 9.5% patients perceived cessation of hair fall after 3 months of therapy with maximum benefit in PRP group (60%). 47.6% patients perceived reduced hair fall with maximum benefit in PRP group (48%) and minimum in microneedling group (10%). 36.1% of total patients perceived no change in the hair fall. 6.6% patients perceived increased hair fall with 57.1% in microneedling group and 14.2% in the PRP group, while 28.5% of minoxidil group was affected (Table 11).

Knighton DR et al in 2007 used PRP for the treatment of non-healing wounds and found that topical application of PRP to areas of tissue containing hair follicles showed increased hair growth, where no growth or limited growth was previously observed.

Olsen EA in 2007 in a placebo controlled trial of 5% foam of minoxidil showed a statistically significant increase in hair counts and subjective assessment over placebo during a 16-week period of twice daily usage.

Rinaldi et al (2011) found that growth factors from PRP could prevent dermal papilla apoptosis, prolong anagen phase, delay catagen and telogen, eventually reducing

diffuse hair loss and stimulating hair re-growth in androgenetic alopecia, without side effects during the treatment period and after 12 months from the end of treatment.

In the present study, 94.2% patients perceived no side effects from the therapy given with 51.51% in PRP and microneedling group, 48.48% in minoxidil group. Overall 5.7% patients perceived side effects with 25% of PRP group and 75% of minoxidil group showing some adverse effects like mild pain, erythema and edema in PRP and microneedling group and mild inflammation in minoxidil group (table 12). All the above side effects were reversible.

CONCLUSION

PRP is an exciting new non surgical therapeutic option for hair growth and stimulation in patients of androgenetic alopecia. Platelet growth factors are probably capable of regulating the life cycle of hair bulbs, thus ensuring a better growth of hair. As a result a growth of hair and even an increase in its density are obtained. Considering its excellent safety profile, it is a promising treatment option for patients with AGA who cannot afford hair transplant surgery. While PRP is in early stages of scientific research in hair restoration, it is a promising non surgical therapeutic option for those patients with androgenetic alopecia. As of this date, there have been no large clinical studies to prove that PRP can positively affect hair growth on the long term. Overall, PRP seems to be a promising therapeutic modality but the level of evidence as of now, from the available published data is low. This demands further studies to gain more evidence before it is used more extensively.

REFERENCES

1. Wadhwa SL, Knopkar U, Nischal KC. "Hair and scalp disorders" In: Valia RG, Valia AR editors IADVL, Textbook of Dermatology, 3rd edition: Bhalani publishing house, 2008; 887-894.
2. Nicole E Rogers and Marc R "medical treatments for male and female pattern hair loss" American J of Dermatol, 2008.
3. Sinclair RD. "Disorders of Hair In Burns" T, Breathnach S, Cox N, Griffiths C, editors. Rook's Textbook of Dermatology. 8th edition. UK: Blackwell Publishing Ltd, 2010; 66: 16-66.31
4. "Treating female pattern hair loss" Harvard Health Publications, 2009 June.
5. "Is minoxidil actually a wonder drug to treat hair loss? If yes, then how does it work". www.akclinics.com
6. Stephen E. Wolverton "Comprehensive dermatologic drug therapy" 3rd edition, Page no 368, Elsevier Publications.
7. Limat A, Suormala T, Hunziker T "Proliferation and differentiation of cultured human follicular keratinocytes are not influenced by biotin" Arch Dermatol Res 1996; 288: 31-8.

8. A brief history of hair transplant surgery. Bernstein Medical centre for hair restoration. American society for dermatologic surgery.
9. Mysore V. "Synthetic hairs: should they be used?" Indian Journal Dermatol Venereol Leprol, 2006; 72: 5-7
10. Rangwala S, Rashid RM "Alopecia: A review of laser and light therapies" Dermatol Online J., 2012; 18: 3.
11. Feroze Kaliyadan, Ajit Nambiar "Androgenetic alopecia: An update" IJDVL, Year 2013; 79(5).
12. Zheng Jun Li, Hye In Choi, Dae Kyoung Choi et.al "autologous platelet rich plasma: a potential therapeutic tool for promoting hair growth" American society of dermatology surgery, 2012; 1-7.
13. "PRP therapy in hair restoration" Irvine orange country, California, FUE Neograft.
14. M. Ferrari et.al "a new technique for haemodilution, preparation of autologous PRP and intraoperative blood salvage in cardiac surgery". Int J Artificial Organs, 1987 Jan; 10(1): 47-50.
15. Robert Kohen, Russel F Warren "Platelet Rich Plasma (PRP) treatment:can overview" HSS edu. Hospital for special surgery New York.