"A THREE ARM OPEN LABEL RANDOMIZED CONTROLLED TRIAL COMPARING MINOXIDIL 5% SOLUTION AND ORAL BIOTIN VERSUS MINOXIDIL 5% + FINASTERIDE 0.1% AND ORAL PLACEBO VERSUS MINOXIDIL 5% AND ORAL PLACEBO IN THE MANAGEMENT OF MALE ANDROGENIC ALOPECIA"

Thesis

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfillment of the requirement for the degree of

DOCTOR OF MEDICINE (MD)

(DERMATOLOGY, VENEREOLOGY AND LEPROLOGY)

JULY, 2020 **Dr. YAMINI SIHAG**

AIIMS, JODHPUR

All India Institute of Medical Sciences, Jodhpur

CERTIFICATE

This is to certify that the thesis titled **"A three arm open label randomized controlled trial comparing minoxidil 5% solution and oral biotin versus minoxidil 5% + finasteride 0.1% and oral placebo versus minoxidil 5% and oral placebo in the management of male Androgenic Alopecia"** is the bonafide work of **Dr. Yamini Sihag**, in the Department of Dermatology, Venereology and Leprology, All India Institute of Medical Sciences, Jodhpur.

Dr. Abhishek Bhardwaj
Additional Professor and Head
Department of Dermatology, Venereology & Leprology
All India Institute of Medical Sciences, Jodhpur

All India Institute of Medical Sciences, Jodhpur

CERTIFICATE

This is to certify that the thesis titled **"A three arm open label randomized controlled trial comparing minoxidil 5% solution and oral biotin versus minoxidil 5% + finasteride 0.1% and oral placebo versus minoxidil 5% and oral placebo in the management of male Androgenic Alopecia"** is the bonafide work of **Dr. Yamini Sihag,** carried out under our guidance and supervision, in the Department of Dermatology, Venereology and Leprology, All India Institute of Medical Sciences, Jodhpur.

Supervisor

Dr. Saurabh Singh
Additional Professor
Department of Dermatology, Venereology & Leprology

Co-Supervisors

Dr. Abhishek Bhardwaj
Additional Professor and Head
Department of Dermatology,
Venereology & Leprology

Dr. Anil Budania
Associate Professor
Department of Dermatology,
Venereology & Leprology

Dr. Anupama Bains
Associate Professor
Department of Dermatology,
Venereology & Leprology

Dr. Suman Patra
Assistant Professor
Department of Dermatology,
Venereology and Leprology

Dr. Shrimanjunath Sankanagoudar
Associate Professor
Department of Biochemistry

ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR

DECLARATION

I hereby declare that the work reported in the thesis **"A three arm open label randomized controlled trial comparing minoxidil 5% solution and oral biotin versus minoxidil 5% + finasteride 0.1% and oral placebo versus minoxidil 5% and oral placebo in the management of male Androgenic Alopecia"** embodies the result of original work carried out by the undersigned in the Department of Dermatology, Venereology and Leprology, All India Institute of Medical Sciences, Jodhpur.

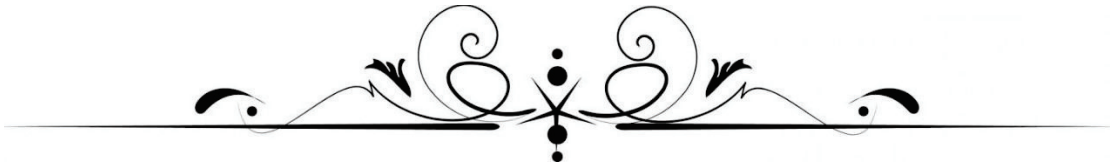
I further state that no part of this thesis has been submitted either in part or in full for any other degree of All India Institute of Medical Sciences, Jodhpur or any other institution.

Dr. Yamini Sihag

Junior Resident,

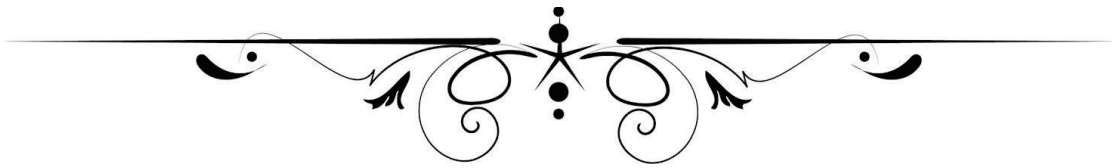
Department of Dermatology, Venereology and Leprology

All India Institute of Medical Sciences, Jodhpur



DEDICATED TO MY PARENTS,

TEACHERS AND PATIENTS



ACKNOWLEDGMENT

First and foremost, I would like to thank Almighty for giving me the strength, knowledge, and ability to undertake this research and to persevere and complete it satisfactorily. Without his blessings, this research would not have been possible.

It is my proud privilege to express my deep sense of gratitude and thanks to my supervisor ***Dr. Saurabh Singh****, Associate Professor, Department of Dermatology, Venereology and Leprology, AIIMS, Jodhpur who provided me an opportunity to work under his supervision. His guidance was paramount in providing a well-rounded experience and knowledge. He has always been a source of encouragement and inspiration. I wish to express my deep gratitude for devoting his valuable time out of his busy schedule and being concerned for the completion of this project. I shall remain grateful to him forever.*

I express my sincere gratitude to the Head of the department and co-guide ***Dr. Abhishek Bhardwaj*** *for guiding me, inspiring me and moulding me into the person I am today. I also express my sincere gratitude to* ***Dr. Anil Budania*** *(Associate Professor),* ***Dr. Anupama Bains*** *(Associate professor) and* ***Dr. Suman Patra*** *(Assistant Professor) for their constant support, encouragement, guidance and help throughout my work.*

I thank my co-guide ***Dr. Shrimanjunath Sankanagoudar*** *for facilitating storage of samples and assessment of biochemical parameters and sharing his expertise regarding the same.*

I am also obligated to Dr. Akhil Dhanesh Goel for helping with the statistical analysis.

Most importantly I would like to thank my parents, Mr. Jai Singh Sihag and Mrs. Pushpa for their constant support and my loving brother Yateendra. Their support, encouragement and unconditional love were undeniably the bedrock upon which my life has been built.

I am also extremely thankful to my batchmates Dr. Shilpi, Dr. Priyanka, Dr. Thoyyib with whom I worked closely. They were a constant source of support during the entire journey. I

would like to thank my immediate senior Dr. Karthick, Dr. Akriti, for their help and companionship during this period. I would like to sincerely thank Dr. Bandhala Rajan, Dr. Ravi Mudugal, Dr. Varun Rajagopal, Dr. Utkrist Lahoria, Dr. Afroz Alam, Dr. Sahiba Rafi, Dr. Neelam for their invaluable suggestions from the start to the end of the study. I would like to acknowledge the immense help and assistance that I have received during this period from my amazing juniors Dr. Shubham, Dr. Nazneen, Dr. Kriti, Dr. Subhajit, Dr. Benetta, Dr. Monisha, Dr. Nagbhushan, Dr. Pranjal, Dr. Prajakta, Dr. Gaurav, Dr. Akhilesh, Dr. Niharika, Dr. Shubham, Dr. Sabyasachi, Dr. Sowmya. I am grateful to them for their love and support.

Last but not least I feel grateful to my friends for the emotional support offered by them during this period. In particular, I wish to thank Arvindar, Sangwan, Anup, Tanya for their constant support.

I thank the minor OT nursing officer Mr. Deepchand, ward lady Mrs. Ruby, clerk Mrs. Saroj and OPD staff Mr. Rameez Raja, Mr. Praveen, Dr. Prabhkar for their assistance during recruitment and sample collection.

Dr. Yamini Sihag

INDEX

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AGA	Androgenetic alopecia	KC	Keratinocyte
FDA	Food and drug administration	DKK1	Dikkopf-related protein 1
PRP	Platelet rich plasma	IL-6	Interleukin 6
FNS	Finasteride	Wnt	Wnt signalling pathway
DHT	Dihydrotestosterone	CXXC5	CXXC Finger Protein 5
HS	Hair shaft	GSK3	Glycogen-synthase kinase-3
IRS	Inner root sheath	PGD2	Prostaglandin 2
ORS	Outer root sheath	SOD	Superoxide dismutase
DP	Dermal papilla	UV	Ultraviolet
MPHL	Male pattern hair loss	APM	Arrector pili muscle
PHL	Pattern hair loss	BASP	Basic and specific
CAD	Coronary artery disease	HSTH	Hair shaft thickness heterogeneity
AR	Androgen receptor	BPPS	Brown peripilar sign
EDA2R	Ectodysplasin 2 receptor	WPPS	White peripilar sign
5R	5 Reductase	MAGA	Male androgenetic alopecia
AR-DHT	Androgen receptor-dihydrotestosterone	SHCP	Scalp honeycomb pigmentation
DNA	Deoxyribonucleases	LLLT	Low level light therapy
RNA	Ribonuclease	LED	Light emitting diode
SRD5A1	Steroid 5 alpha-reductase type 1	MSC	Mesenchymal stem cell
SRD5A2	Steroid 5 alpha-reductase 2	FUE	Follicular unit extraction
FUT	Follicular unit transplantation	M+B	Topical minoxidil 5%, 1 ml local application twice a day with oral biotin 5 mg

VEGF	Vascular endothelial growth factor	M+P	Topical minoxidil 5%, 1 ml local application twice a day with oral placebo i.e. Calcium 500 mg plus vitamin D 250 IU
VEGF	Vascular endothelial growth factor	SD	Standard deviation
RCT	Randomized controlled trial	GPA	Global photographic assessment
FMX	Finasteride plus minoxidil	VAS	Visual analogue scale
MNX	Minoxidil	OPD	Outpatient department
ELISA	Enzyme linked immunosorbent assay	AIIMS	All India Institute of Medical Sciences
HRP	Horseradish peroxidase	OTC	Over the counter
OD	optical density	TAHW	Target area hair width
CV	coefficient of variation	TAHC	Target area hair count
ITT	Intension to treat	FAGA	Female androgenetic alopecia
PP	Per protocol		
MF+P	Topical minoxidil 5%, plus finasteride 0.10%, 1 ml local application twice a day With oral placebo i.e. Calcium 500 mg plus vitamin D 250 IU		

SUMMARY

Background

Androgenetic alopecia (AGA) is the most common form of hair loss in men and women with a characteristic pattern. Currently, Food and Drug Administration approved treatment modalities for AGA includes, topical minoxidil (2% solution, 5% solution and 5% foam), oral finasteride 1 mg daily and low-level laser therapy. Use of oral supplements for hair loss is prevalent among which biotin is one of the most commonly prescribed supplements but evidence supporting such practice is limited.

Aims and objectives:

To study and to compare the efficacy and safety of minoxidil 5% solution plus oral biotin versus minoxidil 5 % solution plus oral placebo versus minoxidil 5 % solution with finasteride 0.10% plus oral placebo in hair growth in male with androgenetic alopecia.

Methods:

This was an open-labelled randomised controlled trial, recruiting_male patients aged 18-50 years with androgenetic alopecia of Hamilton Norwood grade I to IV(n=167). After documentation of baseline parameters, patients were randomized into 3 groups: minoxidil 5% (1ml LA BD) + oral placebo (Group A, n=54), minoxidil 5% with finasteride 0.10% (1ml LA BD) + oral placebo (Group B, n=57) and minoxidil 5% (1ml LA BD) + oral biotin 5mg OD (Group C, n=56). Treatment was given for 6 months. Clinical photography based global photographic assessment, target area hair count (TAHC), target area hair width (TAHW) and visual analogue scale (VAS) was calculated at baseline and compared with their values at 24 weeks. Side effects were noted as and when reported.

Results:

Total 113 patients completed the study. The intra-group analysis at the end of 6 months showed a significant increase ($p<0.05$) in TAHC, TAHW and VAS score in all 3 groups. All three groups were comparable in terms of change from baseline in Global photographic assessment, TAHC and TAHW, VAS score at the end of 6 month with a non-significant p value ($p>0.05$). Hair shaft thickness heterogeneity followed by brown peripilar sign were the most common dermoscopic parameter present in recruited patients. In total, lost to follow up were 54 patients. An increased occurrence of dryness and scaling was noted in all three groups. Overall, baseline serum biotin calculated came out to be 17.43±33.02 pg/ml for the entire study group.

Conclusion:

All three groups in our study showed significant improvement from baseline but at the end of 24 weeks all groups were comparable in terms of efficacy parameters ($p>0.05$). This is probably indicative of 6 months being adequate period for achieving significant change in hair parameters on using topical 5% minoxidil therapy for male AGA. However, the efficacy of added drugs (oral biotin, topical finasteride 0.1%, oral calcium + vitamin D3) is as yet unproven to be beneficial and requires larger clinical trials with possibly longer follow up.

Limitations:

Small sample size, difficulties in follows up due to the prevailing COVID-19 situation.

INTRODUCTION

INTRODUCTION

Hair serves many functions, including thermoregulation and protection. In animals and nonhuman primates, hair maintains temperature by retaining heat and prevents cold. Hair can also provide camouflage and serve as a sexual attractant.

Androgenetic alopecia (AGA) is the most common form of hair loss in men and women. AGA is a nonscarring progressive miniaturization of the hair follicle with a characteristic pattern that involves recession of the frontal hair line and loss of hair from the vertex and bitemporal area.[1]

Patients with androgenetic alopecia see hair loss as a distressing process. The problems with androgenetic alopecia are primarily cosmetic with negative impact on quality of life, such as decreased self-confidence because of the cosmetic concerns, social taunting, worries about ageing and how others would perceive them, and feelings of lost beauty[2].

Male pattern hair loss affects 50% of men by the age of 50 and is most prevalent in Caucasian men. The rate of progression differs from individual to individual and clinical heterogeneity is also observed in affected family members. Racial variations with respect to prevalence and clinical presentations of AGA are recognized.

AGA is most likely a multifactorial disorder caused by complex interplay of androgens, genetics and age factors. The pathogenesis involves progressive and gradual miniaturization of hair follicles, which clinically translates into the transformation of terminal follicles into vellus-like hair. Follicle miniaturization is accompanied by progressive decrease in the duration of anagen with reduction of anagen to telogen ratio[3].

Numerous classification systems have been proposed by various researchers for grading of androgenetic alopecia. These systems vary from the simpler ones based on recession of the hairline to the more advanced multifactorial systems based on the morphological and dynamic parameters that affect the scalp and hair itself. Currently, the Hamilton-Norwood classification system for males and the Ludwig system for females are most commonly used to describe patterns of hair loss[4]. The Hamilton-Norwood classification categorises male androgenetic alopecia as grade I,II,III,III vertex, IV,V, VI,VII[5].

The diagnosis of androgenetic alopecia is clinical most of the times. But the investigations which can be used are hair pull test, trichoscopy and biopsy of scalp to differentiate from other causes of alopecia.

Numerous treatment options are available for androgenetic alopecia, but their efficacy is limited. Currently, only two medications have been approved by the US Food and Drug Administration for treatment of male AGA: finasteride, a 5α-reductase inhibitor, 1 mg daily and topical minoxidil (2% solution, 5% solution and 5% foam)[6]. Recently Low-level laser (light) therapy got FDA approval for the treatment of AGA. Other treatment modalities include intralesional PRP, hormonal therapy, prostaglandin analogues, herbal medications, microneedling, hair mesotherapy which are not yet FDA approved.

Minoxidil, an antihypertensive drug acts by its active metabolite i.e. minoxidil sulphate which is formed by an enzyme sulfotransferase. Inter-individual variation in sulfotransferase activity may be the cause of the discrepancy in minoxidil efficiency[7]. Minoxidil, has been tested for a wide range of concentrations (2% to 12.5%). In comparison to 2% or 10% minoxidil solutions, a standard 5% solution was found to be more effective. With a side effect profile essentially identical to that of 2% minoxidil, 5% minoxidil had a quicker response and better hair regrowth[8], moreover 10% minoxidil caused noticeable irritation[9]. Combined use of oral finasteride and topical minoxidil has also shown better therapeutic efficacy during 1-year follow-up, compared to the use of each single treatment[6].

Systemic FNS, a 5α-reductase inhibitor, 4-aza-3-oxosteroid compound, works by competitively inhibiting 5α-reductase type 2, resulting in the inhibition of the conversion of testosterone to DHT, markedly suppressing serum DHT levels[10]. Finasteride is available as topical formulations (0.10%, 0.25%, 0.5%) and oral tablet (1mg). A study comparing oral finasteride 1 mg per day with a topical finasteride solution of 0.25% showed similar inhibition of DHT production after a week of treatment (lower plasma levels) in both treatment arms[11]. Finasteride's effectiveness when applied topically is identical to that when taken orally, although it has significantly less systemic exposure and has less of an effect on serum DHT[12].

Biotin is an essential water soluble vitamin required as a cofactor for carboxylase enzymes. Biotin deficiency can be either congenital or acquired secondary to increased raw egg consumption, alcoholism, pregnancy, or other medications such as isotretinoin and valproic acid. Typical dermatological symptoms of biotin deficiency include alopecia, eczematous

skin rashes, and seborrheic dermatitis. Biotin's function in protein synthesis and keratin production, explains its contribution to healthy nail and hair growth[13].

Use of oral supplement for hair loss is prevalent, but the evidence supporting such practice is limited. Vitamins and minerals are among the most popular supplements taken by patients – despite most randomized clinical trials not demonstrating any clear benefits. Biotin is one of the most commonly prescribed nutritional support in patient with hair loss but the evidence supporting such practice is limited.

We intend to look at the efficacy of oral biotin supplement in combination with topical minoxidil on hair regrowth in male androgenetic alopecia.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

As early as can be traced, written documents testify endeavours shown by humanity to please by means of the hair. Hair care, colour, and style play an important role in people's physical appearance and self-perception.[1]

Hair is a unique characteristic found in all mammals but not in other animals. In humans, it is a special and cherished feature, but its main functions are to protect the skin from mechanical insults and to facilitate homeothermy. Eyebrows and eyelashes, for example, stop things from entering the eyes, while scalp hair prevents sunlight, cold, and physical damage to the head and neck. It also has a sensory function, increasing the perception of the skin surface for tactile stimuli, and serves important roles in sexual and social communication.[15]

Healthy scalp hair grows at a rate of 0.35 mm/day, which equates to about 0.5 inches/month or 6 inches/year, with vertex hair growing faster and scalp margin hair growing slower. Other parts of the body have rates ranging from 0.1 mm. on the trunk and limbs to 0.38 mm. on the beard, but there is significant individual variation. The average healthy man or woman has 80,000 to 120,000 terminal hairs on their scalp.

TYPES OF HAIRS

1. Lanugo hairs - These are fine, soft, unmedullated, and usually unpigmented hairs that form prenatal hair coat.
2. Vellus hairs- These are the fine hair of post-natal life; also, soft and unmedullated, but sometimes pigmented, and seldom exceeding 2 cm. in length.
3. Terminal hairs- Terminal hairs are longer, coarser, pigmented, and often medullated.

Intermediate hairs—Vellus and terminal hair cannot be sharply defined, for between them lie a series of hair types usually classified as intermediate.[16] Some follicles undergo a rapid transition from vellus to coarse terminal hair, such as the scalp before birth or the pubic region during puberty. In other follicles, a series of intermediate hairs, each slightly coarser than the one before it, is followed by terminal hair.[16]

ANATOMY OF HAIR FOLLICLE

The anatomic features of a hair will differ depending on whether it is in the anagen, catagen, or telogen phase. Follicle size varies as well, from minute vellus hair to long, thick terminal hair.[17]

Each follicle is divided into four distinct regions:

1. Bulb
2. Suprabulbar zone
3. Isthmus: It extends from below the area of sebaceous duct insertion to the bulge or arrector pili muscle attachment area
4. Infundibulum: it extends from the skin's surface to the junction of the sebaceous duct insertion and the isthmus.[17]

The lower segment of the hair follicle is where the majority of the hair follicle's activities take place. The three layers of the lower segment of the hair follicle, from innermost to outermost layers, are the hair shaft (HS), inner root sheath (IRS), and outer root sheath (ORS).[17]

The dermal papilla (DP) is an invagination of the hair bulb at its base in the inner part. The dermal papilla, along with its surrounding epithelial cells, forms the hair bulb matrix, also known as the germinative epithelium, which gives rise to the hair shaft (HS) and IRS. The size of the dermal papilla is dynamically controlled during the hair cycle. Dermal papilla cells emigrate during the catagen phase and then repopulate during the anagen phase. The hair follicle's proximal end has a condensation of mesenchymal cells that form the DP, which determines the size of the hair shaft and regulates matrix cell proliferation and differentiation. The dysfunctional dermal papilla cells cause hair follicle miniaturization in AGA.[17]

HAIR CYCLE

The hair growth cycle is divided into three distinct phases:

1. Anagen (growth phase):[14]

- The anagen phase is an active growth phase, during which the hair follicle enlarges.
- It can be divided into six stages (I–VI).
- During anagen I–V (proanagen), hair progenitor cells proliferate, envelope the growing dermal papilla, grow downwards into the skin, and begin to differentiate into the hair shaft and IRS; then, the newly formed hair shaft begins to develop, and the melanocytes located in the hair matrix show pigment-producing activity.
- Anagen VI (Metanagen) involves the complete restoration of the hair fibre-producing unit, which is characterised by the formation of the epithelial hair bulb surrounding the dermal papilla, located deep in the subcutaneous tissue, and the new hair shaft appears from the skin surface. This phase can last for several years in hair follicles.

2. Catagen (Transitional phase):[14]

- During catagen, differentiation and proliferation of hair matrix keratinocytes decrease significantly, melanocyte pigment-producing activity ceases, and hair shaft production is completed.
- The hair follicle undergoes apoptosis-driven regression resulting in a reduction of about one-sixth of the normal diameter.
- During catagen, a specialized structure, the club hair, is formed.
- The keratinized brush-like structure at the base of the club hair is surrounded by ORS epithelial cells and anchors the hair in the telogen follicle. The dermal papilla transforms into a cluster of quiescent cells close to the regressing hair follicle epithelium and travels from the subcutis to the dermis subcutis border to maintain contact with the distal portion of the hair follicle epithelium, including the secondary hair germ and the bulge. This phase lasts a few weeks.

3. Telogen (resting phase):[14]

- The telogen phase follows the catagen phase and can last anywhere from a few weeks (eyelashes) to 8 months (scalp hairs).
- During this stage, hair does not grow, and the dermal papilla remains dormant.
- Telogen follicles are distinguished by the absence of pigment-producing melanocytes and the IRS.
- At any given time, 10-15% of all hairs are in the telogen phase.
- Exogen phase begins at the end of telogen, during which hair shedding occurs.
- Hair follicles re-enter the growth phase after a few weeks by stimulating stem cells in the bulge area.

ALOPECIA

Hair loss disorders are a large, heterogeneous group of conditions that have various clinical features, pathologic findings, and aetiologies. Hair loss can be caused by hair cycle disorders, inflammatory diseases that damage the hair follicles, or inherited or acquired abnormalities of the hair shafts. Alopecia can be cicatricial or non-cicatricial.

1. **Cicatricial alopecia** - Primary cicatricial alopecias are inflammatory disease of the scalp that results in permanent hair loss. Loss of hair follicle stem cells in the bulge

region of the hair follicle contribute to the development of scarring alopecia. The type of inflammation detected on histologic examination distinguishes the primary scarring alopecias. The three main types are lymphocytic primary cicatricial alopecia, neutrophilic primary cicatricial alopecia, and mixed primary cicatricial alopecia.

2. **Non cicatricial alopecia** - In non-scarring alopecia, clinical signs of inflammation are usually mild or absent, and destruction of hair follicles does not occur. This category includes androgenetic alopecia and alopecia areata.

ANDROGENETIC ALOPECIA

The term "androgenetic alopecia (AGA)" was coined by Orentreich in 1960, but the same condition in men is also called male alopecia, common baldness, male pattern baldness, and male pattern hair loss (MPHL). Androgen dependence and hereditary factors are less obvious in affected females than in males; therefore, the preferred term for females is pattern hair loss, which is broader than AGA. AGA is the most common form of alopecia, occurring in both sexes after puberty.[15]

AGA is a nonscarring progressive miniaturization of the hair follicle with a usually characteristic pattern distribution in genetically predisposed men and women. AGA in men typically exhibits a pattern distribution, most commonly a male pattern, but a female pattern can be seen occasionally. AGA in women typically manifests as a diffuse loss of hair density over the frontal and central areas, but the parietal and occipital regions may also be involved. AGA can occur in women in a male pattern on rare occasions.[1]

The prevalence of androgenetic alopecia increases with age. Although it is common in the elderly, androgenetic alopecia frequently begins during puberty. The prevalence is highest in caucasians, reaching around 80% in men over the age of 70. Males over the age of 70 have a prevalence of 46.9%-60.0% in the asian population. Although the asian prevalence of AGA is lower than that of caucasians, AGA is a common disorder in asia. According to epidemiologic data from China, India, Korea, Taiwan, and Thailand, it is estimated that 41-73% of Asians will develop PHL at some point in their lives, with the incidence increasing with age.[15] In India, the prevalence rate in men aged 30-50 years is 58%. In all cases, the incidence increases with age.[16]

Cotton et al. first proposed AGA as a risk factor for coronary artery disease (CAD). Patients with AGA, who developed the disease before age 30, and patients with more severe grading

had a higher risk of developing coronary artery disease.[17] Patients with vertex baldness had a higher risk of myocardial infarction than patients with forehead alopecia.[18] Patients with AGA at a young age had a higher incidence of benign prostatic hyperplasia, and AGA at a young age could be an early indicator of developing benign prostatic hyperplasia later in life.[19] Several other studies have found a positive correlation between AGA and prostate cancer.[20] In an Indian study, patients with early onset AGA had a higher prevalence of obesity, hypertension, diabetes, and dyslipidaemia. Metabolic syndrome has been associated with early-onset androgenetic alopecia, which may contribute to the fact that patients with androgenetic alopecia are predisposed to cardiovascular disease.[21]

PATHOGENESIS

Hamilton first pointed out the interaction of androgens, genetic factors, and age in the development of AGA in 1951. Men castrated before puberty did not develop AGA, and AGA could be induced in castrated men by injection of testosterone. Hamilton accounted for the observations that androgens were necessary for the pathogenesis of male baldness. Osborn suggested in 1916 that the AGA pattern could be explained by an autosomal dominant trait. Later, Kuster and Happle studied the genetics of AGA and concluded that Osborn's hypothesis had not been thoroughly tested and therefore had questionable validity. They concluded that a polygenic mode of inheritance was more likely. Current scientific evidence supports the hypothesis of a polygenic trait at AGA, but the pathophysiology and genetics remain unknown.[3]

1. Role of genetics

The exact cause of androgenetic alopecia is unknown, but genetics plays an important role in its etiopathogenesis, which includes a complex interplay of various genes. Individual differences in gene expression explain why some people experience premature hair loss while others only show signs of AGA in their 60s. In genome wide association studies, strong associations have been found with the androgen receptor (AR) gene and the ectodysplasin A2 (EDA2R) gene on the X chromosome. The presence of AR on the X chromosome and the strong association signal of EDA2R highlight the importance of the maternal lineage in the inheritance of androgenetic alopecia. Regional differences in gene expression profiles of the AGA vertex and frontal scalp exist.[3]

2. Role of androgens

Androgenetic alopecia is thought to be caused by an abnormal sensitivity of scalp hair follicles to circulating androgens caused by an increase in the number of androgen receptors (AR). The enzyme 5 α Reductase (5R) converts testosterone into DHT, which has a tenfold higher affinity for AR than testosterone. Dihydrotestosterone (DHT) binds strongly to AR in the cytoplasm of target cells, and the AR-DHT complex is translocated to the nucleus after dimerization, where AR coactivators are recruited to bind to the androgen response element, a consensus sequence on DNA. Coactivators act as a link between AR and basal transcription factors such as RNA polymerase, resulting in target gene transcription and, eventually, translation into proteins with biological activity.[3]

3. 5 alpha (5 α) reductase isozymes role

There are two 5a-reductase isozymes in hair follicles, Type I and Type II, encoded by two different genes, SRD5A1 on chromosome 5 and SRD5A2 on chromosome 2, respectively which catalyse the conversion of testosterone to 5 alpha dihydrotestosterone. Type I 5 α -reductase is found in sebaceous glands, epidermis, eccrine sweat glands, apocrine sweat glands, and hair follicles (outer root sheath, dermal papilla, and matrix). The Type 2 enzyme has been found in the dermal papilla, the inner layer of the outer root sheath, the sebaceous ducts, and proximal inner root sheath of scalp hair follicles. Type II 5-alpha reductase accounts for about 80% of circulating DHT. This converted dihydrotestosterone binds the androgen receptor of the genetically marked hair follicles with five times the avidity of testosterone. 5α-reductase I activity is ubiquitously distributed in hair follicles, whereas 5α-reductase II is located at DPC from beard and AGA hair follicles, pointing out the dermal papilla as an androgenetic target. It is believed that both isoforms play a role in the metabolism and action of androgen, and their expression varies depending on the site of the body.[3]

4. Role of androgen receptors

Androgens, such as testosterone and DHT, affect the skin primarily through the androgen receptor (AR). The gene AR is located on the X chromosome. Steroid hormones, including androgens, act through intracellular nuclear receptors that function as hormone-induced transcription factors. AR is found in the dermal papilla of the hair follicle, but not in the outer root sheath (ORS) or hair bulb, suggesting that the dermal papilla is the primary target of the androgen in the hair follicle. Hair follicles in the frontal and parietal regions are androgen

sensitive due to increased expression of androgen receptors (ARs), whereas hair follicles in the occipital and temporal regions are androgen insensitive.[3]

5. Role of dermal papilla and signalling pathways

The hair follicle is a dynamic mini organ composed of different cell populations derived from the ectoderm, mesoderm, and neural crest. The epithelium gives rise to the outer root sheath, matrix, inner root sheath and hair shaft, while the mesenchyme gives rise to the dermal sheath and dermal papilla (DP). The hair cycle is based on cyclic activation and suppression of hair follicle stem cells that regulate proliferation, differentiation, and apoptosis of a variety of cells in the follicular niche. Many reciprocal signalling cascades between epithelial and mesenchymal cells in the hair follicle, such as Wnt/catenin, Sonic Hedgehog, Bone morphogenetic protein, Notch, Transforming growth factor 2 (TGF 2), NFB(Nuclear factor beta), and Fibroblast growth factors, are involved in hair follicle morphogenesis and regeneration during the hair cycle. In a co culture study of DPCs(Dermal papilla cells)with outer root sheath keratinocytes (KCs), the DHT treatment caused the DPCs to secrete Dikkopf-related protein 1 (DKK-1), which inhibits the growth of ORS cells. DKK-1 is a catagen inducer and regulates the transition from anagen to catagen in hair follicles. DKK-1 is one of the genes most upregulated by DHT in balding DPCs. It is evident that DHT induces hair follicles in the balding scalp to enter the catagen phase through the paracrine factor DKK-1. DHT also up-regulate interleukin-6 (IL-6) secretion in balding DPCs. IL -6 has also been shown to suppress proliferation of KC and inhibit hair shaft lengthening. Injection of IL -6 resulted in early onset of catagen in mice. Consequently, IL -6 is another paracrine factor secreted by balding DPCs stimulated by DHT and promotes the regression phase at hair follicle. Conclusively, in androgenetic alopecia, it is substantiated that aberrant regulation of Wnt signalling leads to hair loss. Wnt signalling is inhibited externally by the paracrine mediator DKK1 and within cells by CXXC5 and increased GSK3 activity. AR signalling is responsible for dephosphorylation of GSK3 and secretion of the paracrine factors DKK1, IL6, TGF1, and PGD2, which inhibit keratinocyte growth and promote hair follicle into the catagen phase. Androgen action inhibits Wnt/B-catenin, leading to miniaturization of follicles. The Notch signalling pathway is also involved in androgenetic alopecia. Both Notch and Wnt signalling pathways are directly affected by androgen expression in androgenetic alopecia.[3]

6. **Role of oxidative stress**

In biological systems, oxidative stress and inflammation are inextricably linked. Upton et al investigated the effects of physiologically relevant oxygen and oxidative stress on the growth and cellular signalling potential of glabrous dermal papilla cells (DPCs). Environmental oxygen significantly alters the morphology, migration, proliferation, senescence, and TGF signalling of DPC in vitro, which could inhibit hair follicle remodelling. Consequently, the role of oxidative stress in the dermal papilla of patients with androgenetic alopecia was confirmed. Prei et al detected decreased total antioxidant activity and increased malondialdehyde levels in plasma samples from patients with androgenetic alopecia, indicating the presence of oxidative stress. Significantly decreased activity of superoxide dismutase (SOD) in erythrocytes but no change in catalase, glutathione peroxidase, or total antioxidant activity indicate the presence of oxidative stress in these patients. Research also shows that oxidative stress plays a direct role in mediating DHT-stimulated TGF-B secretion by dermal papilla cells.[3]

7. Role of micro inflammation

Evidences suggest that AGA is associated with dysregulation of inflammatory cytokine expression, and chronic micro inflammation is thought to be an exacerbating factor. This inflammation is caused by microbial flora, oxidative stress, ageing, smoking, UV radiation, and pollutants. Nirmal et al found mild perifollicular inflammation in 76% of AGA patients and 30% of normal control subjects and more advanced inflammation with perifollicular fibrosis in AGA patients but not in normal control subjects. This inflammation and subsequent fibrosis around the follicles may eventually lead to irreversible follicular atrophy. High insulin levels in patients with metabolic syndrome cause vasoconstriction, which impairs hair follicle nutrition. In addition, insulin promotes the action of DHT on follicles, contributing to follicle miniaturisation. As a result, the subclinical micro inflammation around the hair follicles could be a local manifestation of the systemic inflammation, which explains why these patients are more likely to have AGA symptoms.[3]

8. Role of arrector pili:

Contact between the arrector pili muscle (APM) and the bulge may be required to reverse miniaturisation of hair follicles. In AGA, follicular miniaturisation is either irreversible or partially reversible, whereas alopecia areata is reversible. This is due to the fact that in AGA, the connection between the arrector pili muscle and the outer root sheath of the miniaturised

vellus hair follicles is usually lost. In vitro studies have shown that hair follicles can self-regulate their response to androgens by controlling the expression of 5-alpha-reductase and androgen receptors. This means that in people with AGA hair can grow back even without treatment. Detachment of the arrector pili muscle from a miniaturized follicle is considered a histopathological sign of AGA irreversibility for that follicle.[3]

9. Role of environment and lifestyle

Excessive endogenous free radicals, environmental factors (ultraviolet radiation, pollutants, chemical irritants and microbes) and lifestyle (e.g smoking) are all sources of oxidative stress that affect hair both during and after production. Su et al studied 26,226 women aged 30 years and older to determine risk factors for AGA. High body mass index, high fasting blood glucose, earlier puberty, three or fewer births, use of oral contraceptives for a year or more and UV exposure of more than 16 hours per week were all associated with increased risk for AGA. Epigenetic studies show that lifestyle can cause different responses in identical twins who develop different AGA stages despite having the same genetics, due to differences in their lifestyle. Gatherwright et al discovered frontal, temporal, and vertex hair loss in a distinct spatiotemporal pattern associated with smoking, excessive alcohol consumption and increased exercise duration in a study of 92 identical male twins. Smoking causes the production of free radicals. This allows DHT to enter the dermal papilla cells of the hair and cause an increase in sebaceous gland and 5 alpha reductase activity and the release of proinflammatory cytokines from follicular keratinocytes, which inhibits hair growth. Smoking also impairs blood flow and eventually causes local ischemia, which affects hair follicle nutrition. It also promotes androgen-dependent hair thinning by hydroxylating estradiol and inhibiting the aromatase enzyme.[3]

CLINICAL FEATURES

The primary clinical feature in both sex is patterned hair loss on vertex. The terminal hairs are gradually replaced by fine, short hairs. The replaced hairs are also lighter in colour. After puberty, the whole process can start at any age. Hamilton and Norwood observed and documented this progressive patterned hair loss. Hair density is reduced because the affected hairs are miniaturised. The terminal hairs on the affected areas of the scalp are replaced by vellus hairs, resulting in a decrease in hair density on the affected areas, which is a precursor

to complete baldness. Otherwise, the examination of the scalp is normal, except during periods of increased hair loss, when the hair pull test may be positive.

CLASSIFICATION OF ANDROGENETIC ALOPECIA

Several classification systems for grading purposes have been proposed by various researchers. These systems range from simple systems based on hairline recession to more advanced multifactorial systems based on morphological and dynamic parameters that affect the scalp and the hair itself. Currently, the Hamilton-Norwood classification system for males and the Ludwig system for females are the most commonly used to describe patterns of hair loss. Numerous researchers have proposed various classification systems for patterned hair loss in both males and females based on the evolutionary stage of hair loss, ranging from a simple two-stage classification proposed by Beek in 1950 to the more recent advanced basic and specific (BASP) classification.[4]

After studying patterned hair loss in 1,000 men, Dr. O'Tar Norwood, a dermatologist and hair transplant surgeon, revised Hamilton's classification in 1975. The Norwood classification is the most widely used classification for male pattern hair loss. Norwood observed that thinning begins at the temples as well as the crown area and gradually spreads throughout the scalp. Below are Norwood's descriptions of the different degrees of hair loss.

Type I: There is minimal or no recession of the hairline.
Type II: There are triangular, usually symmetrical, areas of recession at the frontotemporal hairline.
Type III: There is deep symmetrical recession at the temples that are bare or only sparsely covered by hair. In type III vertex, the hair loss is primarily from the vertex with limited recession of the frontotemporal hairline that does not exceed the degree of recession seen in type III.
Type IV: The frontotemporal recession is more severe than in type III and there is sparse hair or no hair on the vertex. The two areas of hair loss are separated by a band of moderately dense hair that extends across the top. This band connects with the fully haired fringe on the sides of the scalp.
Type V: The vertex hair loss region is still separated from the frontotemporal region but it is less distinct. The band of hair across the crown is narrower and sparser and the vertex and frontotemporal regions of hair loss are bigger.

Type VI: The bridge of hair that crosses the crown is gone with only sparse hair remaining. The frontotemporal and vertex regions are joined together and the extent of hair loss is greater.

Type VII: The most severe form of hair loss and only a narrow band of hair in a horseshoe shape remains on the sides and back of the scalp.

Norwood also defined a Type A variant from his standard classification system, which is distinguished by two major features and two minor features.

The major features are:

1) The anterior border of the hairline progresses to the rear without leaving an island of hair in the mid-frontal region.

2) There is no simultaneous development of a bald area on the vertex. Instead, the frontal hairline recession keeps progressing to the rear of the scalp.

The minor features are:

1) There is a persistent sparse hair scattering in the area of hair loss and

2) The horseshoe-shaped fringe areas of hair that remain on the side and back of the scalp tend to be wider and reach higher on the head compared to Norwood's standard.

The various Type A variants described by Norwood are as follows:

Type IIA: The hairline is anterior to the coronal plane, 2 cm anterior to the external auditory meatus.

Type IIIA: The hairline has receded back to a point between the limit of type IIA and the level of the external auditory meatus.

Type IVA: The hairline has receded beyond the external auditory meatus but has not reached the vertex.

Type VA: The area of denudation includes the vertex. Hair loss more severe than Type VA cannot be distinguished from Types VI or VII.

The Norwood classification is one of the most detailed classification systems for male patterned hair loss and is the most widely used classification in the world, but it is too detailed, divided, and complicated to be used for various surgical procedures. Furthermore, it excludes some unusual types of baldness (a balding patch on the vertex or occiput with a preserved anterior hairline) and is ineffective in determining the surgical method.[5] Hamilton-Norwood grade along with type A variant and Ludwig scale is shown in figures 1, 2 and 3 respectively.

FIGURE 1: **Hamilton Norwood's classification of pattern hair loss in males**.

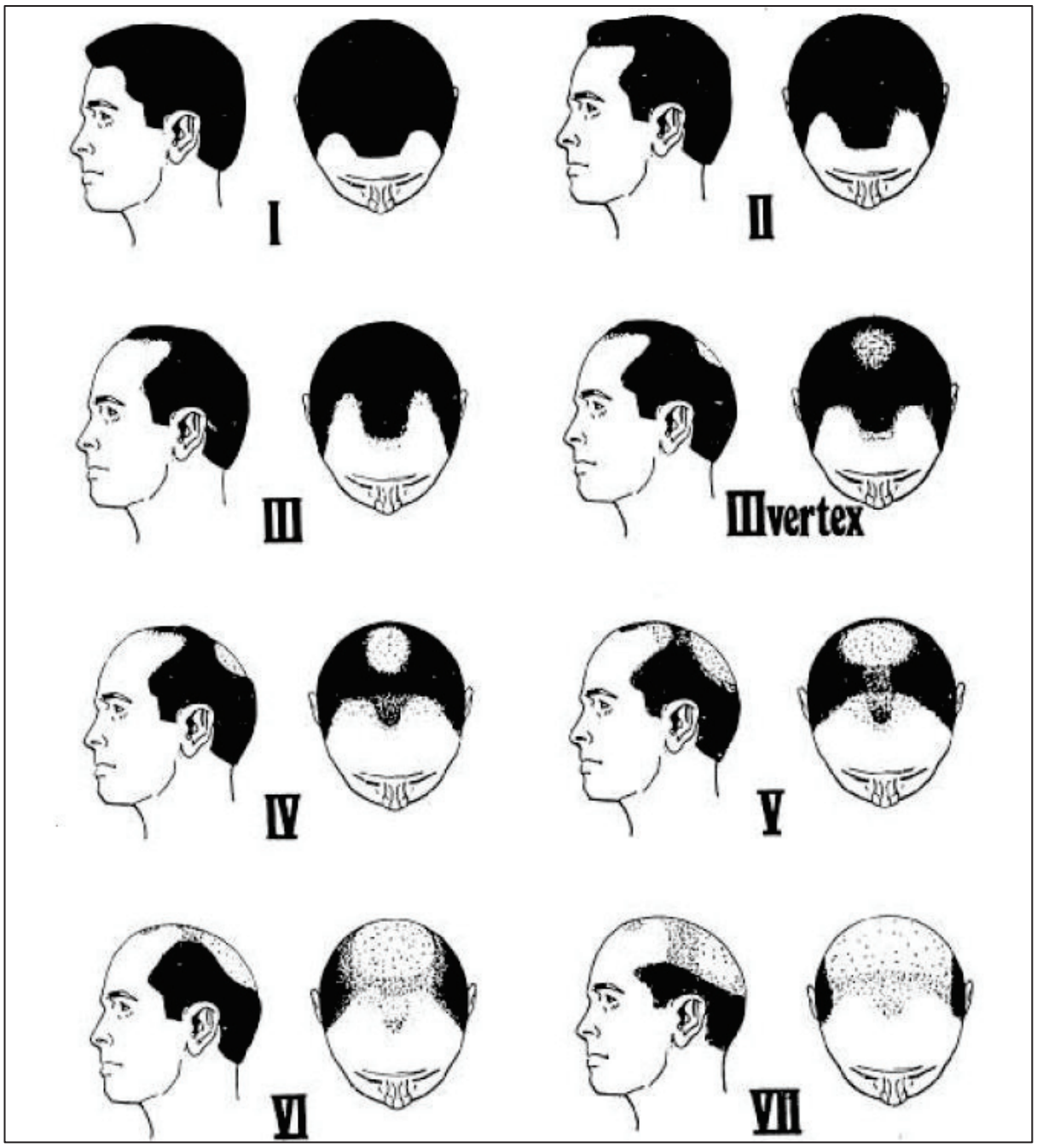


FIGURE 2: Type A variants described by Norwood.

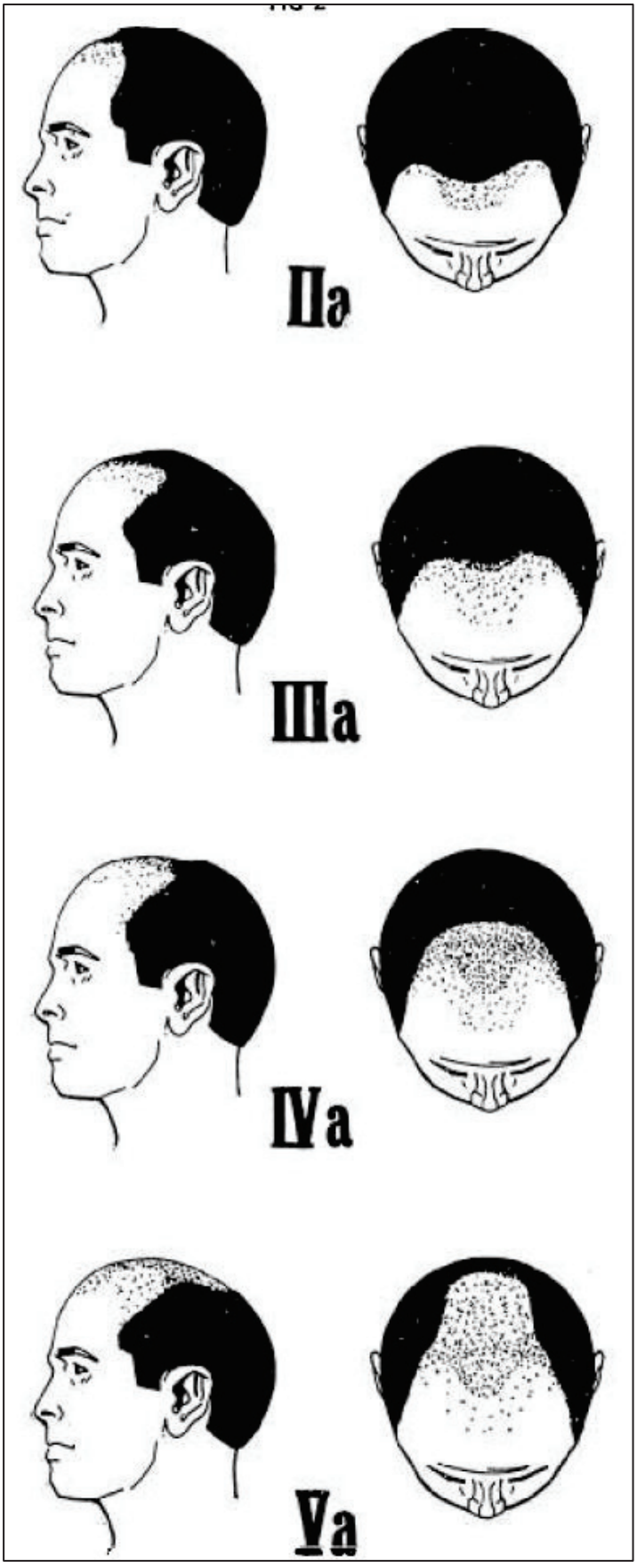
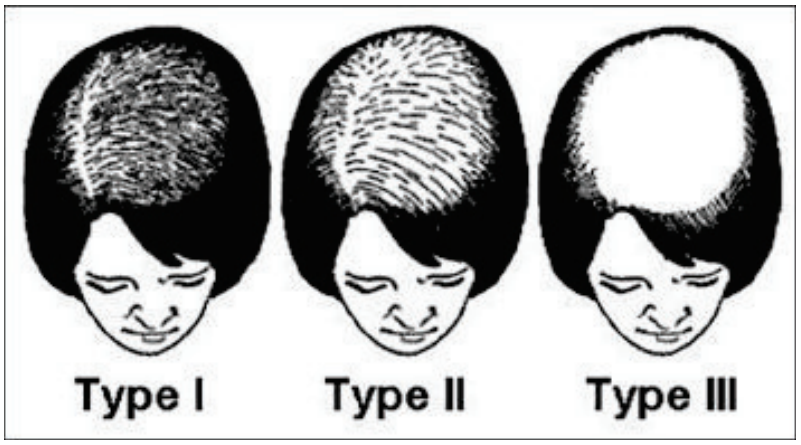


FIGURE 3: Ludwig's classification of hair loss among females.



INVESTIGATIONS

There are numerous tools available to evaluate patients presenting with hair loss. Non-invasive examinations (e.g., hair counting, microscopic evaluation, trichoscopy), semi-invasive (e.g., trichograms) and invasive are available (e.g., scalp biopsies).[22]

1. **Trichoscopy**: Trichoscopy is a relatively new technique used to aid in the diagnosis of hair and scalp disorders. Trichoscopy findings in AGA included hair shaft thickness heterogeneity (HSTH), brown peripilar sign (BPPS), white peripilar sign (WPPS), yellow dots, pinpoint white dots, focal atrichia, scalp honeycomb pigmentation, epidermal scaling, and arborizing red lines (vertex, frontal, and temporal hair lines in MAGA patients and crown area in FAGA patients). The most common trichoscopic features in MAGA were HSTH of more than 20%, BPPS, WPPS, yellow dots, focal atrichia, and scalp honeycomb pigmentation, with pinpoint white dots being the least common.[23]

(A) **Hair shaft thickness heterogeneity (HSTH):** Hair diameter diversity, also known as "anisotrichosis," is seen in the affected scalp region of all AGA patients and is caused by progressive and unsynchronized miniaturisation of hair follicles in genetically predisposed scalp regions. Thus, terminal hairs are replaced by vellus hairs that are less than 30 micrometres thick and 2-3 mm long, while single-haired pilosebaceous units increases. HSTH of more than 20%, which means that vellus hairs account for more than 20% of all the hairs in the same view, is regarded as a hallmark of AGA.[23]

(B) **Brown peripilar sign (BPPS):** A brown halo around the exiting hair shaft about 1 mm in diameter identifies the brown peripilar sign. This symptom is associated with

superficial perifollicular infiltrates composed mainly of lymphocytes. It occurred most frequently in the early AGA.[23]

(C) **White peripilar sign (WPPS)**: White peripilar sign is more common in advanced AGA patients and is associated with perifollicular fibrosis in the late stage of AGA. WPPS is a poor predictor of outcome.[23]

(D) **Pinpoint white dots**: Histopathology revealed that punctate white dots 0.2-0.3 mm in diameter, evenly distributed between the follicular ostia, corresponded to the empty hair follicular ostia or the epidermal part of the eccrine sweat ducts and were positively related to the severity of the disease.[23]

(E) **Yellow dots**: In AGA, it corresponds to sebum.[23]

(F) **Scalp honeycomb pigmentation**: SHCP is caused by sun exposure and is characterised by hypomelanotic areas bordered by hyperchromic lines, which is typically seen in thinning or completely balding areas. Patients with severe AGA are more likely to develop this condition.[23]

(G) **Epidermal scaling and arborizing red lines:** These are classified as non-specific findings because they are also present in the normal scalp.[23]

(H) **Focal atrichia:** Atrophic follicles can cause focal atrichia. Focal atrichia is associated with an advanced stage of AGA.[23]

2. **Scalp biopsy**: Biopsies are usually useful when the pattern of AGA is unusual or to rule out telogen effluvium or early scarring alopecia. In the early stages of the disease, 4 mm punch biopsies of affected and unaffected areas are ideal to compare follicle size and number of hairs[24]

(A**) Early disease**: Two 4mm punch biopsies (lesional - frontal or vertex scalp and uninvolved - occiput scalp) processed horizontally to allow for hair counts.[24]

(B) **Late disease**: One 4mm punch biopsy from the involved scalp is processed horizontally to allow for hair counts.[24]

AGA is characterised by a near-normal total number of hairs, and a decrease in terminal hairs and an increase in vellus hairs, resulting in a terminal: vellus ratio of approximately 2:1. Another sign of AGA is an increase in telogen hairs.[25]

TREATMENT

There are a variety of options in a practitioner's armamentarium for treating AGA which include oral and topical medications, hormonal therapies, nutraceuticals, platelet rich plasma (PRP), exosomes, microneedling, and more invasive techniques such as hair transplantation.

AGA treatment can be particularly difficult because patients do not respond uniformly to conventional therapies and the exact pathogenesis of the disease itself is also poorly understood. Patients must adhere to lifelong therapy because AGA progresses if treatment is interrupted. The only FDA-approved treatments for this disease are oral finasteride (1mg), topical minoxidil (2% and 5%), and LLLT (2007 in male and 2011 in female), all of which may be effective in treating certain AGA patients. However, the patient's age and aesthetic concerns, lifestyle and preferences, access to treatment, compliance, extent of hair loss, and financial budget should be considered when selecting an appropriate therapy for a patient.[26] Various treatment modalities for AGA are shown in table 1.

Table 1: Treatment modalities available for AGA are:[26]

Topical therapies	1. Topical minoxidil (2%, 5%, 10%) 2. Topical finasteride (0.10%, 0.25%)
Oral therapies	1. Oral finasteride (1 mg) 2. Oral dutasteride (0.02, 0.1, 0.5, 2.5 mg) 3. Oral minoxidil (2.5-5mg)
Hormonal therapies	1. Spironolactone (100-200mg) 2. Flutamide and bicalutamide 3. Cyproterone acetate
Light therapies	1. Low-level laser therapy (LLLT) 2. Light-emitting diode (LED) devices
Injectable	1. Platelet rich plasma (PRP) 2. Mesenchymal stem cell-derived exosomes (MSC-Exosomes)
Adjuvant therapy	1. Microneedling
Supplements and over the counter preparations	1. Oral nutraceutical supplement containing Synergen Complex 2. Marine complex supplement 3. Serenoa repens 4. Plant-based oils (rosemary oil, tea tree oil, pumpkin seed oil, coconut oil, castor oil, amla oil) 5. Ketoconazole

Combination therapy	1. Topical minoxidil + topical finasteride 2. Topical minoxidil + oral finasteride 3. Topical retinoid+ topical minoxidil + oral finasteride 4. Topical minoxidil + spironolactone gel 5. Low-dose oral minoxidil + oral spironolactone 6. LLLT + medical therapy 7. Hair transplantation + PRP + medical therapy
Hair transplantation	1. Follicular unit extraction (FUE) 2. Follicular unit transplantation (FUT)
Newer modalities	1. Topical Clascoterone 7.5% (topical antiandrogen) 2. Oral JAK inhibitors 3. Topical Prostaglandin analogue (latanoprost 0.1%.)

1. <u>Minoxidil:</u>

Topical minoxidil is one of only three FDA approved treatments for male and female pattern hair loss. It was approved specifically for AGA in 1988 as a first line treatment for men with mild to moderate AGA. In the 1960s, the oral formulation was used as a vasodilator to treat hypertension. Hypertrichosis was discovered as a side effect of chronic oral minoxidil use, prompting the development of a topical formulation for hair growth stimulation. Minoxidil is widely available in 2% and 5% foam and liquid solutions.[26]

Topical minoxidil stimulates hair growth. Its mechanism of action is not well understood. Minoxidil sulphate, the active form of the molecule, is converted by sulfotransferase in the scalp. Individual differences in sulfotransferase activity may explain the difference in minoxidil efficacy. Minoxidil acts by shortening the telogen phase so that dormant hair follicles enter the anagen phase prematurely. After starting minoxidil therapy, the telogen phase may be shortened, resulting in telogen effluvium. In addition, minoxidil prolongs the anagen phase. Finally, the clinical effects of minoxidil include increased hair length and diameter. Minoxidil appears to act on the potassium channels of vascular smooth muscles and hair follicles, which may induce the following effects:

- Minoxidil stimulates microcirculation near hair follicles by inducing arteriolar vasodilation, which may result in hair growth.

- It induces the expression of vascular endothelial growth factor (VEGF), which increases vascularization around hair follicles and thus promotes hair growth.
- Minoxidil also stimulates hair growth by activating the prostaglandin-endoperoxide synthase.
- Minoxidil reduces hair fall by inhibiting androgen effects on androgen-sensitive hair follicles.
- Minoxidil may act on matrix cells as an "epidermal growth factor," delaying ageing and thus extending the duration of the anagen phase via the beta-catenin pathway.
- Minoxidil has been shown to have anti-fibrotic properties as a result of its effect on collagen synthesis.

The effects of minoxidil begins after about eight weeks of treatment and peaks after four months. Approximately 1.4% of topical minoxidil is absorbed through a normal scalp, with increased absorption associated with drug concentration, frequency of drug application, and stratum corneum barrier function damage. Minoxidil has no affinity for plasma proteins and does not cross the blood-brain barrier. Within four days, the kidney excretes approximately 95% of the systemically absorbed drug and its metabolites.[27]

Olsen et al conducted a randomised controlled trial comparing minoxidil 5% versus minoxidil 2% for male patients with patterned hair loss. At the end of 48 weeks, 5% minoxidil outperformed 2% minoxidil and placebo in terms of increasing hair regrowth in men with AGA. Men who used 5% minoxidil had an earlier response to treatment and better psychosocial perceptions than men who used 2% minoxidil. The patients in this trial tolerated topical minoxidil (5% and 2%) well, with no evidence of systemic effects.[8]

Ghonemy et al demonstrated that topical 5% minoxidil was moderately superior to 10% minoxidil and placebo in increasing hair regrowth, whereas 10% minoxidil caused significant irritation.[9]

2. <u>Topical finasteride</u>:

Systemic finasteride (FNS), a 5-reductase inhibitor, 4-aza-3-oxosteroid compound, has been extensively studied and is currently used in the treatment of benign prostatic hyperplasia and AGA. Finasteride works by competitively inhibiting 5-reductase type II, thereby inhibiting the conversion of testosterone to DHT and significantly suppressing serum DHT levels. The average terminal half-life of finasteride is five to six hours in men aged 18 to 60, and eight

hours in men aged 70 and older. DHT levels return to normal within 14 days of stopping treatment. It is expected that hair regrowth will be reversed within 12 months of discontinuing systemic finasteride treatment for AGA.

Mazarella et al conducted a single-blind, placebo-controlled study on 28 male and 24 female AGA patients in 1997. Subjects were randomly assigned to either 1.0 mL of topical FNS 0.005% solution or a placebo twice daily to the affected scalp for 16 months. The pharmacodynamics data revealed no significant difference in total testosterone, free testosterone, or DHT plasma levels between the groups. Researchers observed a significant decrease in the rate of hair loss in the topical finasteride group compared to the placebo group at six months. Patient's perceptions of treatment effectiveness were generally positive in the finasteride group, with 73% of treated patients reporting "high effectiveness" and 60% of placebo patients reporting "no effect".[28]

A subsequent RCT comparing the efficacy of 0.1% finasteride solution to a placebo in 20 males with moderate-to-severe AGA by Charuwichitratana et al discovered that the finasteride group had a greater mean hair count increment from baseline, despite no significant difference in response rates. Clinical improvements in the topical finasteride group were significantly greater than in the placebo group, according to patients and investigators.[29]

Another placebo-controlled study of 50 AGA males done by Sittichareoenchai et al confirmed the efficacy of topical finasteride. As early as 3 months into treatment, patients who received 0.5% topical solution twice daily had significantly higher hair counts and mean terminal hair changes from baseline than those who received placebo. At the end of the study, global photography revealed that 53.8% of finasteride-treated patients had sustained their hair loss and 46% had improved clinically, while 83.3% of placebo-treated patients had no change in their hair loss and 16.7% reported a worsening condition.[29]

To compare the clinical outcomes of topical and oral formulations, Hajheydari et al conducted a double-blind, placebo controlled trial in 45 males with AGA and randomly assigned them to two groups: 1% finasteride gel twice daily with placebo tablets daily and vehicle gel twice daily plus 1 mg oral finasteride tablet daily for 6 months. After 6 months of treatment, both groups showed comparable results in terms of changes in hair counts from baseline and an increase in terminal hair counts. Although patients taking oral tablets improved faster, there was no statistically significant difference in therapeutic response between the two groups.[30]

3. Topical minoxidil with finasteride:

Because topical minoxidil is currently the first-line treatment for AGA, a novel combination therapy of finasteride and minoxidil has been investigated to improve therapeutic efficacy.

In 2012, a pilot RCT compared the efficacy and safety of 3% minoxidil lotion and a combination of 3% minoxidil and 0.1% finasteride lotion in 40 men with AGA. After 24 weeks of application, the increase in hair counts from baseline was significantly different only in patients treated with the combined treatment (58.09 ± 13.39 vs 62.91 ± 13.34, p = 0.044). Additionally, the global assessment scores graded by three blinded physicians were significantly greater in the combination group (1.84 ± 0.79 vs 1.02 ± 0.69, p = 0.003).[31]

Most recently, two RCTs were performed by Suchonwanit et al, who further examined the synergistic effect of topical finasteride admixed with minoxidil solution in both male and female patients. Both studies determined the clinical efficacy of a topical formulation of 0.25% finasteride in combination with 3% minoxidil (FMX) twice daily compared to 3% minoxidil solution twice daily for 24 weeks. The study in 40 men with AGA exhibited significant superiority of combination over 3% minoxidil in increasing hair density after 16 and 24 weeks of treatment and in increasing the hair diameter after 24 weeks of treatment. The mean change from baseline in total hair density (61.84 ± 15.65 hairs/cm^2 vs 34.88 ± 10.24 hairs/cm^2, p = 0.003) and in hair diameter (17 ± 5.24 μm vs 13 ± 4.15 μm, p = 0.034) at week 24 was significantly greater in FMX group than the minoxidil group. Global photographic assessment graded by both patients and physicians displayed a significantly higher improvement in hair growth for the FMX group at 24 weeks.[6]

4. Oral biotin:

Biotin is a necessary cofactor for carboxylase enzymes, which are activated after being linked together by holocarboxylase synthase. These enzyme complexes are involved in a variety of metabolic processes, such as gluconeogenesis, fatty acid synthesis, and amino acid catabolism. Biotin's role in protein synthesis, specifically keratin production, explains how it contributes to healthy nail and hair growth. Biotin can be found in a variety of foods and is also produced by normal gut flora. Nuts, legumes, whole grains, unpolished rice, and egg yolk have been found to be high in biotin. Biotin recommended daily allowances range between 35 and 70 ug/day.

A biotin deficiency can be acquired or inherited. Although acquired biotin deficiency is possible, it is uncommon. Increased raw egg consumption is a commonly documented

secondary cause of acquired biotin deficiency. Cooking can denature the protein avidin, which is found in raw egg whites, but when uncooked, this protein tightly binds to biotin, preventing it from being used as an essential cofactor. Patients taking anticonvulsant medications, such as valproic acid, can also become deficient and are therefore given biotin prophylactically. Other factors that contribute to acquired biotin deficiency include alcoholism, pregnancy, other medications such as isotretinoin, impaired intestinal absorption, or long-term antibiotic use that disrupts normal gut flora.[32]

Congenital Biotin deficiency is caused by an autosomal recessive trait that results in a lack of either holocarboxylase synthase or biotinidase. This deficiency is known as the neonatal type when it occurs within the first 6 weeks of life. The enzyme holocarboxylase synthetase is absent in this type of biotin deficiency, and patients typically have severe, life-threatening conditions. Beyond the age of three months, the infantile form predominates and is defined by a biotinidase deficiency, which is involved in the absorption of free biotin after carboxylase degradation.

Alopecia, eczematous skin rashes, seborrheic dermatitis, conjunctivitis, and multiple neurological symptoms, such as depression, lethargy, hypotonia, and seizures, are common symptoms of biotin deficiency, whether acquired or congenital. While neurological symptoms appear at higher levels of biotin deficiency, dermatological manifestations frequently appear first and are thus an important indicator. Biotin plasma concentrations typically range from 400 to 1,200 pg/ml. A level of less than 200 pg/ml is technically considered deficient. However, because plasma biotin levels fluctuate on a daily basis, it is not considered a sensitive marker. An increased urinary excretion of the metabolite 3-hydroxyisovaleric acid (normal level: 195 mol/24 h) is a more validated measure of biotin deficiency.[32]

Supplementation improved hair and nail growth in patients with established biotin deficiency, according to the literature. Biotin supplementation at higher doses (10,000 to 30,000 ug/day) is recommended for patients with inherited enzyme deficiencies. Brittle nail syndrome and other underlying hair pathologies, such as uncombable hair syndrome, necessitate much lower biotin supplementation doses ranging from 300 to 3,000 ug/day.[32]

Patel et al examined 18 case reports of biotin and hair and nail growth and discovered clinical improvement in all cases after receiving biotin, and three reported cases of uncombable hair syndrome showed improvement in hair quality after a few months of biotin treatment.[32]

In a study conducted by El Esawy et al, serum biotin levels were found to be suboptimal in AGA patients when compared to controls, with non-significant correlations with patients'

age, BMI, disease duration, and severity.[13] Studies on topical and oral medications as shown in table 2 and 3.

Table 2: Clinical studies using topical therapies in AGA:

Author/ Year	Study design/ sample size	Arms (groups)/ Treatment duration	Population	Efficacy parameter
Mozzarella et al,1997[28]	Retrospective n=52	Topical Finasteride 0.005% 16 Months	18-38 years; men & pre-menopausal women, Hamilton-Norwood scale I-III in men, Ludwig scores I-II in women	Clinically improved hair density & increased hair regrowth in finasteride group.
Charuwichi tratana et al, 2003[29]	RCT n=12	0.1% finasteride solution vs placebo twice daily. 12 months	Male with Hamilton Norwood scale III-VI	-Higher proportions of improved patients in finasteride group (100%) than placebo group (40%). -28.6% of finasteride treated patients & 20% placebo-treated patients had increase in hair counts.
Sitticharoenchai et al, 2006[29]	RCT n=50	0.5% finasteride vs placebo twice daily. 6 months	18-60 years; men; Hamilton Norwood scale III-V	-Increased terminal hair counts of 34.2 & 80.9 in finasteride group at 3 months & 6 months respectively. - 46.2% of patients in finasteride group showed improved GPA % & 53.8% remained unchanged - 83.3% of patients in placebo group remained unchanged & 16.7% had decreased GPA.
Hajheydari et al, 2009[30]	RCT n=45	1% finasteride gel twice daily vs 1 mg oral finasteride daily. 6 months	22.8 ± 3.3 years; men	Both groups had significantly increased hair counts and the number of terminal hairs from baseline.
Tanglertsampan et al, 2012[31]	RCT n=40	3% MNX with 0.1% finasteride vs 3% MNX	men; Hamilton Norwood scale III-V	-Increased in hair count from baseline of 4.8 ± 9.1 in finasteride + MNX group & 2.9 ± 6.9 in MNX group at 6 months. -GPA showed scores of 1.8 ± 1 in

		lotion twice daily 6 months		finasteride + MNX group & 1 ± 1 MNX group.
Chandrashe kar et al, 2015[33]	Retrospective n=50	5% MNX fortified with 0.1% finasteride 12 months	20-40 years; men; previously treated with MNX & oral finasteride	-84.4% maintained a good hair density - 4 of 5 patients who discontinued oral finasteride within 1 year responded to topical MNX+ finasteride combination.
Sheikh et al, 2015[34]	RCT n=104	5% MNX with 0.1% finasteride vs 5% MNX solution 6 months	18-45 years; men; Hamilton Norwood II-V	-The investigator scores of patients in the finasteride + MNX group were significantly higher (64.7% vs 25.5%). -A significantly greater number of patients with finasteride + MNX (88.9%) than with MNX (60%) improved in GPA. -Patients with finasteride + MNX had greater satisfaction related to hair growth, slowing down hair loss & hairline at the front.
Rai et al, 2018[35]	RCT n=50	5% MNX with 1 mg oral finasteride (group A) vs 5% MNX with 0.1% topical finasteride (group B) 12 months	Hamilton Norwood grade III-IV	-Mean quality of life was significantly higher in patients of group A (46.3) than patients in group B (40.5) at 12 months ($p < 0.05$). -65% of patients in group A & 83% of patients in group B had clinical improvement.
Narasimhalu et al, 2018[29]	RCT n=300	0.1% finasteride with 5% MNX vs 5% MNX solution. 6 months	18-45 years; men; maximum diameter of bald area < 10 cm	Comparable response in extent of bald area, hair count & number of terminal hairs.
Suchonwanit et al, 2018[6]	RCT n=40	0.25% topical finasteride with 3% MNX vs 3% MNX solution twice	Hamilton Norwood scale III-V	- Mean increased hair density of 61.8 ± 15.6 hairs/cm2 in finasteride + MNX group & 34.9 ± 10.2 hairs/cm2 in MNX group at 6 months. Mean increased hair diameter of 17 ± 5.2 µm in finasteride + MNX group & 13 ± 4.2 µm in MNX group at 6 months.

		daily. 6 months		-63.1% & 56.2% of patients in finasteride + MNX group & 22.2% 16.7% of patients in MNX group had marked improvement in GPA by investigators & patients, respectively.
Panchaprateep et al, 2015[36]	RCT n=30	Single-arm Oral minoxidil 5 mg 6 months	24-59 years; men Hamilton-Norwood scale III vertex to V	-Compared to baseline, the Target Area Hair Count (Total and Non-Vellus Hair Counts) was significantly higher at 12 weeks ($P = 0.023$) and 24 weeks ($P = 0.003$) of treatment. -Hair diameter significantly increased from baseline by 10.6% at 12 weeks ($P = 0.002$) and by 15.21% at 24 weeks ($P < 0.001$).

Table 3: Clinical studies using oral therapies in AGA:

Author/ Year	Study design/ sample size	Arms (groups)/ Treatment duration	Population	Efficacy parameter
Trüeb Et al, 2016[37]	Retrospective cohort n=541	Single cohort	Female complaining of hair loss of age 9 years to 92 years	Biotin deficiency was found in 38% of women complaining of hair loss.
Patel et al, 2018[32]	Review article n=18	18 case reports		Clinical improvement in all 8 cases after receiving biotin treatment, and three reported cases of uncombable hair syndrome showed improvement in hair quality after a few months of biotin treatment.
El Esawy et al, 2019[13]	Cohort study n=120	2 arms: 60 AGA patients, 60 control	Male AGA patients and healthy control	Zinc (g/dl) levels were significantly lower in patients compared to controls (P = 0.01), and biotin (ng/L) levels were suboptimal in patients but within normal ranges in controls (P = 0.01). Serum zinc and biotin levels had no significant relationship with age or disease duration. MAGA grades were found to have a non-significant relationship with zinc (P = 0.485) and biotin levels (P = 0.367).
Danane et al, 2021[38]	Longitudinal follow up study n=50	Single arm	Male with premature AGA	Significant correlation was fount between vitamin D deficiency and severity of AGA.
Gupta et al, 2022[39]	RCT n=40	Two arms: Intradermal PRP every 15 days with oral biotin (group A) versus oral biotin (group B)	21-40 years male with Hamilton Norwood grade III to VI	At six months there was a statistically significant increase in hair growth ($p<0.05$) in individuals in group A as compared to group B; this change was progressive at both nine and 12 months using 7-point global photographic assessment. ($p<0.001$)

AIM & OBJECTIVES

AIM AND OBJECTIVES

AIM OF THE STUDY

To study and to compare the efficacy and safety of minoxidil 5% solution plus oral biotin versus minoxidil 5 % solution plus oral placebo versus minoxidil 5 % solution with finasteride 0.10% plus oral placebo in hair growth in male with androgenetic alopecia.

OBJECTIVES

Primary objective:

1. To compare the efficacy of hair regrowth in the 3 groups using global photography and dermoscopy.

Secondary objectives:

1. To compare the efficacy of hair regrowth in the 3 groups using the subjective VAS scale.
2. To compare the side effects experienced in each group

MATERIALS & METHODS

MATERIALS AND METHODS

1. STUDY SETTING:

This study was conducted on consenting male patients with androgenetic alopecia, who attended Dermatology, Venereology and Leprology OPD at AIIMS JODHPUR.

2. STUDY DESIGN:

An open labelled, three arm randomised controlled trial

3. STUDY PARTICIPANTS: Written informed consent was taken from all the patients.

Inclusion criteria

- Age more than or equal to 18 years and less than or equal to 50 years
- Clinical diagnosis of androgenetic alopecia
- Grade I, II, III, III vertex and IV of Hamilton Norwood grading.

Exclusion criteria

Patient having any of the following criteria were excluded from the study.

- Known sensitivity to either topical minoxidil or topical finasteride or oral biotin.
- Known hypotensive
- Absent oral intake
- Uncontrolled gastrointestinal infection/malabsorption syndrome
- Had used topical minoxidil/topical finasteride/oral supplement/oral herbal extract/any OTC medication for hair growth within the past 1 month
- Patients with diffuse hair loss (telogen effluvium)
- Patient with scarring alopecias
- Patient with severe/uncontrolled seborrheic dermatitis.

Primary efficacy parameters:

- A global photographic assessment was performed at baseline as well as at the end of weeks 4, 8, 12, 16, 20, and 24.
- Trichoscopic findings for target area hair count (TAHC) and target area hair width (TAHW) were recorded at 50X-70X at baseline and at the end of weeks 4, 8, 12, 16, 20, and 24.

Secondary efficacy parameters:

- Subjective VAS scoring was performed at baseline, as well as at the end of weeks 12 and 24.
- Adverse drug reaction reports were completed at each visit.

4. SAMPLING:

The sample size was calculated using G power software v3.0™. Based on literature search sample size was calculated[6]. We estimate a sample size of 44 per group based on alpha error of 0.05, power of 80%, 95% confidence interval, 10% contingency, and the number of groups being three.

Formula used:

$$N_1 = \left\{ z_{1-\alpha/2} * \sqrt{\bar{p} * \bar{q} * (1 + \frac{1}{k})} + z_{1-\beta} * \sqrt{p_1 * q_1 + (\frac{p_2 * q_2}{k})} \right\}^2 / \Delta^2$$

$$q_1 = 1 - p_1$$

$$q_2 = 1 - p_2$$

$$\bar{p} = \frac{p_1 + kp_2}{1 + K}$$

$$\bar{q} = 1 - \bar{p}$$

$$N_1 = \left\{ 1.96 * \sqrt{0.4265 * 0.5735 * (1 + \frac{1}{1})} + 0.84 * \sqrt{0.631 * 0.369 + (\frac{0.222 * 0.778}{1})} \right\}^2 / 0.409^2$$

$$N_1 = 22$$

$$N_2 = K * N_1 = 22$$

p_1, p_2 = proportion (incidence) of groups #1 and #2
$\Delta = |p_2 - p_1|$ = absolute difference between two proportions
n_1 = sample size for group #1
n_2 = sample size for group #2
α = probability of type I error (usually 0.05)
β = probability of type II error (usually 0.2)
z = critical Z value for a given α or β
K = ratio of sample size for group #2 to group #1

5. STUDY DURATION:

Ethical clearance to the protocol was provided by the institutional ethics committee of AIIMS Jodhpur with letter no AIIMS/IEC/2021/3291 and recruitment was started from 01/05/2021 and followed up till 31 December 2022.

6 month long randomized controlled trial.

6. STUDY PROCEDURE:

Evaluation of patients

Full information regarding the investigation objectives and methods were explained to the patients, and consent was taken from the participants. At first all patients underwent clinical examination by a dermatologist in the OPD to confirm the diagnosis of androgenetic alopecia. After selecting the patients who satisfied the inclusion criteria, then a structured and approved case sheet /proforma was filled up which included points like demographic details (name, age, address, education, marital status, age of onset of disease, addiction history, family history) and other relevant details like history of medications along with their contact number. A complete examination was performed and the following were recorded at baseline: Hamilton Norwood grade, clinical photographs, target area hair diameter and target area hair density over frontal and vertex scalp, subjective visual analogue scale, baseline serum biotin levels. Baseline dermoscopy and clinical photographs were taken from fixed anatomical landmark. Dermoscopy was done in the midline after parting the subject's hair in the midline. Three sites on the scalp were then measured at 12, 24 and 30 cm from glabella, similar to in Birnbaum et al[40] study, which were identified as the frontal, vertex and occipital area. The hair density and diameter were evaluated at 50 -70 fold magnification.

After that patients were allocated randomly to 3 groups using research randomisation software (Microsoft excel version 16).

- Group A received topical 5% minoxidil solution 1ml twice a day plus placebo tablet once a day.
- Group B received topical combination of 5 % minoxidil and 0.10% finasteride solution twice a day plus placebo tablet once a day.

- Group C received topical 5% minoxidil solution 1ml twice a day plus 5 mg biotin tablet once a day.

Placebo- Elemental calcium (500 mg) with vitamin D_3 (250 IU).

Minoxidil preparation used in this study was alcohol based.

Dermoscopic features of androgenetic alopecia noted at each visit were:

- Hair shaft thickness heterogeneity (HSTH): >20% in Male AGA (MAGA) and >10% in Female AGA (FAGA) which corresponds to vellus hair transformation is the feature of AGA
- Brown peripilar sign (BPPS): is brown halo around the emergent hair shaft
- White peripilar sign (WPPS): larger in size as white halo at the follicular ostium
- Yellow dots are round or polycyclic best seen under polarized light. Reflect empty hair follicle
- Focal atrichia: They are areas of total hair loss on scalp, usually in a size of a pencil eraser
- Scalp honeycomb pigmentation (SHCP**)**.

Follow up

Patients were followed up every 4 weekly or as per emergent need of the patient, if any, preferably in physical OPD and appropriate clinical examination was done according to standard guidelines.

Follow up visits- Clinical photographs of the patients were taken on each visit with due consent and kept for record purpose. Target area hair density and target area hair diameter were calculated at the end of 12 weeks and 24 weeks over both frontal and vertex scalp over same predefined area. Subjective improvement assessed by visual analogue score done at the end of 12 weeks and 24 weeks. Treatment was continued for 6 months. The baseline parameters were compared with the parameters after 6 months. Clinical improvement was assessed using these serial photographs and efficacy parameter at every visit by a blinded assessor.

After 6 months, patient continued to get appropriate treatment for maintenance of remission

Treatment failure- In any group absence of hair regrowth on global photographic assessment, dermoscopy findings and subjective VAS in the predefined anatomical areas at the end of 3 months. These patients were started on oral finasteride treatment as per standard protocol.

Global photographic assessment

Paired (baseline and week 24) global photographs were evaluated by a blinded dermatologist, based on 7-point rating scale (-3 = marked deterioration, -2 =moderate deterioration, -1 = mild deterioration, 0 = no change, 1 = mild improvement, 2 =moderate improvement, 3 = marked improvement).[36]

Investigator global assessment:

Percentage improvement was calculated at the end of 24 weeks by two blinded dermatologists. No change and worsening were recorded as 0-point while improvement was scored between 1-100%. Average of Mean scores obtained from both the dermatologists was calculated.

Calculation of Target area hair density

Dermoscopy was done in the midline using Dinolite AM7515MZT (rare metabolic life science private limited, India) after parting the subject's hair in the midline. Three sites on the scalp were then measured at 12, 24 and 30 cm from glabella which were identified as the frontal, vertex and occipital area. The hair density was evaluated at 50 -70-fold magnification. Manually number of hairs in each field (0.36 cm square) were counted by the same assessor over the above points at baseline, end of 12 weeks and end of 24 weeks and then extrapolated to an area of 1 cm square.

Calculation of Target area hair diameter

Manually hair diameter was calculated by the same assessor over the above points (12cm, 24cm and 30cm from glabella) at baseline, end of 12 weeks and end of 24 weeks using following steps:

STEP-1: With Dinolite AM7515 MZT at 50-70 X, Scale provided with Dermoscope was focussed to calculate area under the field which was 0.36cm square (7*5.2).

STEP-2: Scale ruler was used and overlapped with the image captured by Dermoscope the length of which was 7 mm and value of 1 millimetre of scale ruler was calculated. 1 mm of ruler scale corresponded to 0.05 mm of Dermoscope scale.

STEP-3: The image captured by the Dermoscope was divided into four quadrants and hair diameters of the thickest hair in each quadrant was measured at the scalp-hair interface, using a scale ruler and the diameters measured.

STEP-4: The average of the hair diameters was then multiplied by the multiplication factor of 0.05 mm and the readings were taken in micrometres.

Visual analogue scale

subjective improvement was assessed using a visual analogue scale, and scoring was performed as follows: based on a 100-mm analogue scale in which a value of 0 = negative, 50 = neutral, and 100 = positive.[8]

Sample collection and processing:

Five ml of whole blood sample was collected in plain vacutainers or gel separators from the recruited subjects. The sample was allowed to clot for one hour. Serum was separated by centrifugation at 3000 rpm for 10 minutes at room temperature. After blood collection, samples stored at -80^0 C deep freezer in the Central Biochemistry Laboratory. All serum samples were processed for estimation of biotin at one time using ELISA kit.

Quantification of serum Biotin: Biotin was measured using commercially available Human Biotin ELISA kit purchased from ImmunoTag (Geno Technology Inc., USA). Properly thawed sera samples were analysed on an ELISA platform based on Competitive inhibition enzyme immunoassay technique.

Test Principle - This assay employs the competitive inhibition enzyme immunoassay technique. The wells were pre-coated with Biotin protein to which samples and standards were added with a biotin-conjugated antibody specific to Biotin. Following incubation, HRP streptavidin was added to wells, followed by addition of TMB substrate. The final addition of stop solution changed the colour of the solution from blue to yellow. The absorbance was measured using the Eon Microplate spectrophotometer (BioTek, USA). A wavelength of 450 nm was used for the measurements. The concentration of Biotin in the samples was then determined by comparing the OD of the samples to the standard curve. The analytical range of the kit ranges form 3.13 – 200 pg/mL, with a sensitivity of 4.7 pg/mL. The Intra-Assay and Inter-Assay CVs were < 8% and < 10% respectively.

Reagent preparation

1. Wash buffer was diluted into 1x working concentration with DDW from 25x

2. Biotinylated-Conjugate (1x) - Biotinylated-Conjugate requires a 100-fold dilution. And for that 10 µL of Biotinylated-Conjugate was mixed with 990 µL of Biotinylated-Conjugate Diluent.

3. Standard -Standard was reconstituted with 1.0 mL of Standard Diluent, kept for 10 minutes at room temperature, shaked gently (not to foam) which gave the concentration of standard in the stock solution to be 200 pg/mL. 7 tubes containing 0.5mL Standard Diluent was further diluted to produce a double dilution series to achieve the concentration of 200 pg/mL, 100 pg/mL, 50 pg/mL, 25 pg/mL, 12.5 pg/mL, 6.25 pg/mL, 3.13 pg/mL, and the last EP tubes with Standard Diluent was blank as 0 pg/mL.

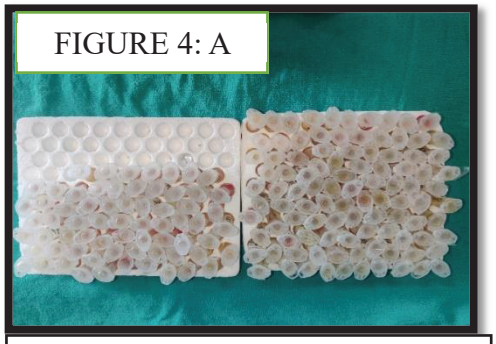
4. Streptavidin-HRP (1x) -Streptavidin-HRP requires a 100-fold dilution.10 µL of Streptavidin-HRP with 990 µL of HRP Diluent was used to obtain 1x.

Assay Procedure

Serial dilutions of the standard were added in the assay plate of concentration ranging from 200pg/ml to 0 pg/ml. 50 µL of sample was added to each well immediately after that 50 µL of Biotinylated -Conjugate(1x) was added, mixed well, covered with adhesive film provided and incubated for 1 hour at 37°C. After that, the liquid was aspirated from each well and washed three times using Wash Buffer (200 µL). After the last wash, excess wash buffer was removed. 100 µL of Streptavidin-HRP (1x) was then added to each well. The plate was covered with the adhesive films provided and again Incubated at 37°C for 1 Hour. The wash protocol was repeated again. 90 µL of Substrate Solution was added in all the well. The plate was covered with the adhesive films provided and again Incubated at 37°C for 20 min. The wash protocol was repeated once more. 50 µL of Stop Solution was added to each well to terminate the enzyme substrate reaction. The first four wells containing the highest concentration of standards developed blue colour. Colour change was measured spectrophotometrically at a wavelength of 450 nm using a microplate reader. The concentration of Biotin in samples was calculated by comparing the OD of the samples with the standard curve.

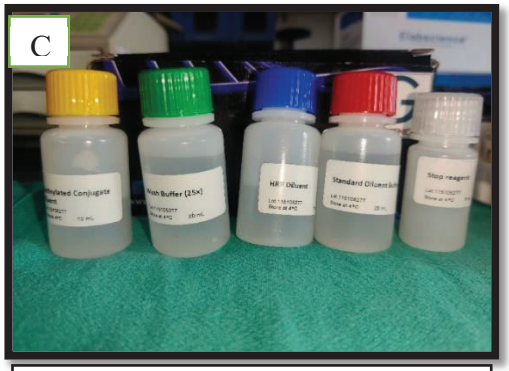
ESTIMATION OF SERUM BIOTIN:

Figure 4:



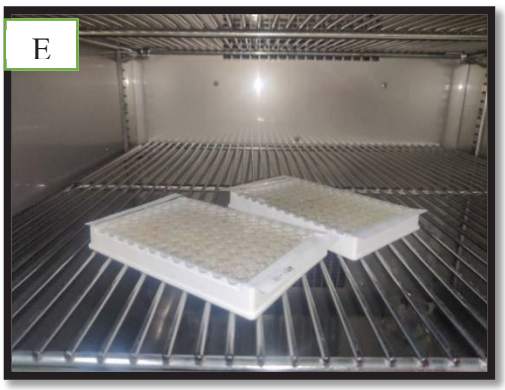
Samples
(stored at -80 degree centigrade)

Biotin ELISA kit

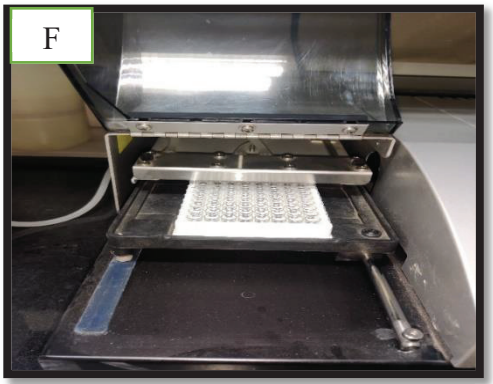


Reagents

ELISA plate



Elisa plate on incubator



Automated washer

STATISTICAL ANALYSIS

Data was entered in Microsoft excel, and all the entries were checked for errors. The data was analysed using Microsoft Excel ver. 2016 and Statistical Package for Social Sciences ver. 26.0 (IBM SPSS, Inc., Chicago, IL).

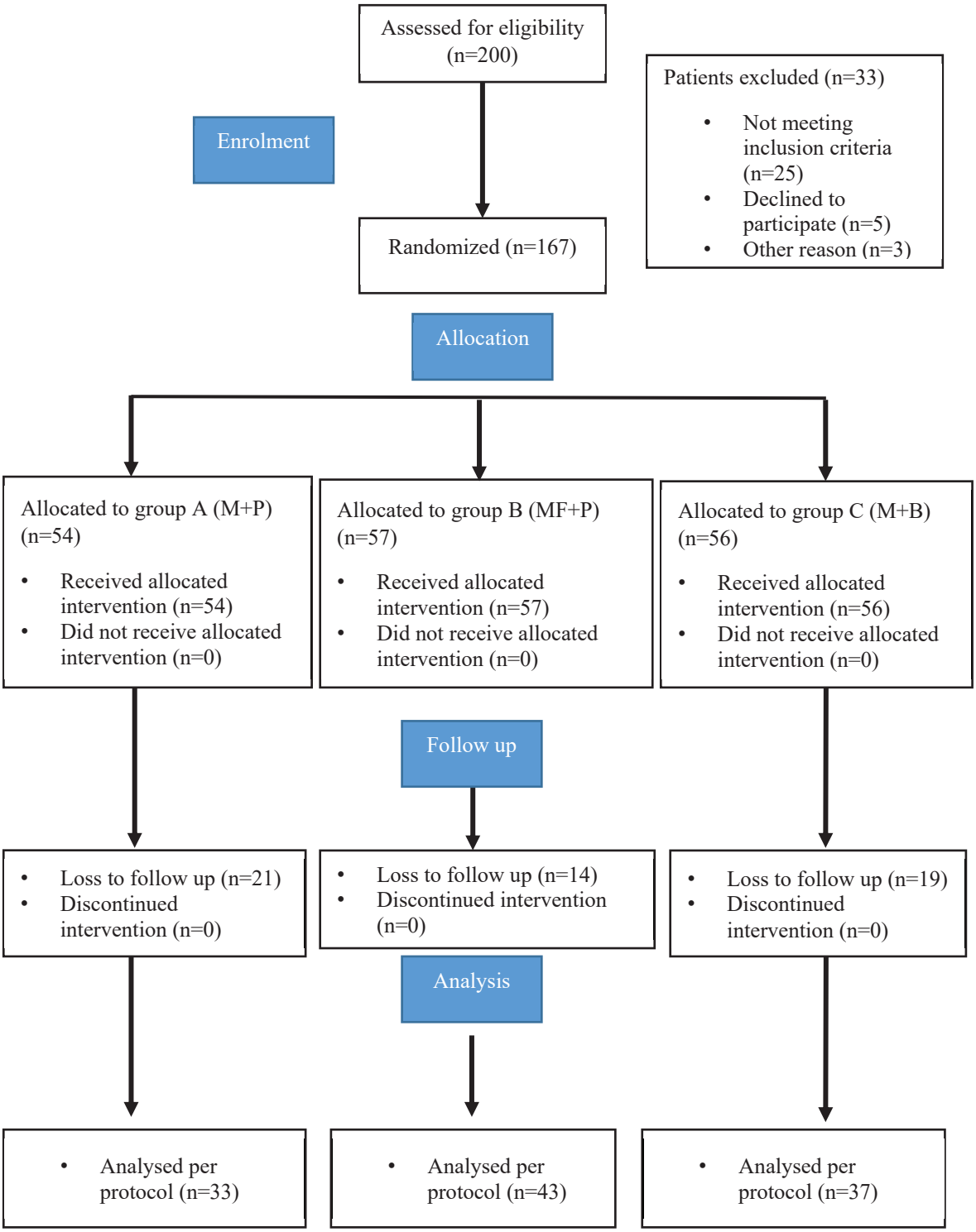
Descriptive analysis, including frequency distribution, proportion, and mean +/- standard deviation (SD) was performed to summarize the baseline qualitative and quantitative characteristics of the three treatment groups.

The following statistical tests were applied for the results:

1. The qualitative variables for the three groups were compared using Chi-Square test.
2. The percentage improvement in investigator global assessment from baseline was calculated using Excel and One-way ANOVA was applied to look for any statistically significant inter-group difference in the means.
3. For continuous variables i.e. target area hair density, target area hair diameter and visual analogue scale, repeat Measure ANOVA was applied to look for any intra-group significance in variation among the means, and One-way ANOVA was employed for similar inter-group significance.

For statistical significance, p value of less than 0.05 was considered significant.

Figure 5: Consort Diagram



RESULTS

RESULTS

Baseline characteristics are presented as intension to treat analysis (ITT), and efficacy parameters as per protocol analysis (PP) and the same has been specified further.

(1) BASELINE CHARACTERISTICS-

1. DEMOGRAPHIC DATA AND BASIC CLINICAL DETAILS:

Total number of patients in the study were 167 out of which 54 patients received treatment as per Group A protocol (Topical minoxidil 5%, 1 ml local application twice a day with oral placebo i.e. calcium 500 mg plus vitamin D 250 IU) i.e. M+P, 57 patients received treatment as per Group B protocol (topical minoxidil 5% plus finasteride 0.10% , 1 ml local application twice a day with oral placebo) i.e. MF+P and 56 received treatment as per group C protocol (Topical minoxidil 5% , 1 ml local application twice a day with oral biotin 5 mg) i.e. M+B. Overall the mean age of patients in our study was 24.55 ± 4.32 years (18-46 years) and the mean duration of illness was 2.26 ± 2.27 years (1 month -20 years). Total 129/167 (77.24%) patients were unmarried. Past history of treatment for androgenetic alopecia was present in 31/167 (19%) patients and positive family history for androgenetic alopecia was found in 103/167 (62%) patients. All groups were comparable (p>0.05) in terms of demography and basic clinical data. This is shown in tables 4,5 and figures 6,7.

TABLE 4: Baseline demographic details of study participants (as per ITT analysis):

S.NO	PARAMETER	GROUP A (N=54)	GROUP B (N=57)	GROUP C (N=56)	TOTAL (N=167)	p* VALUE
1.	Age (years) Mean ± SD	24.62±4.8	24.50±4.31	24.53±3.94	24.55 ± 4.32	0.988
2.	Total duration of illness (years) Mean ± SD	2.24±2.70	2.3±2.2	2.3±2	2.26 ± 2.27	0.995
3.	Past treatment for AGA n (%)	10(19%)	10(18%)	11(20%)	31(19%)	0.960
4.	Family history n (%)	36(67%)	33 (58%)	34(61%)	103(62%)	0.626
p*values calculated using One Way ANOVA						

TABLE 5: Age distribution of study participants (as per ITT analysis):

S.NO	AGE GROUP	NUMBER OF PATIENTS N (%)
1.	10-20 years	25/167 (15%)
2.	21-30 years	128/167 (77%)
3.	31-40 years	13/167 (8%)
4.	41-50 years	1/167 (0.5%)

FIGURE 6:

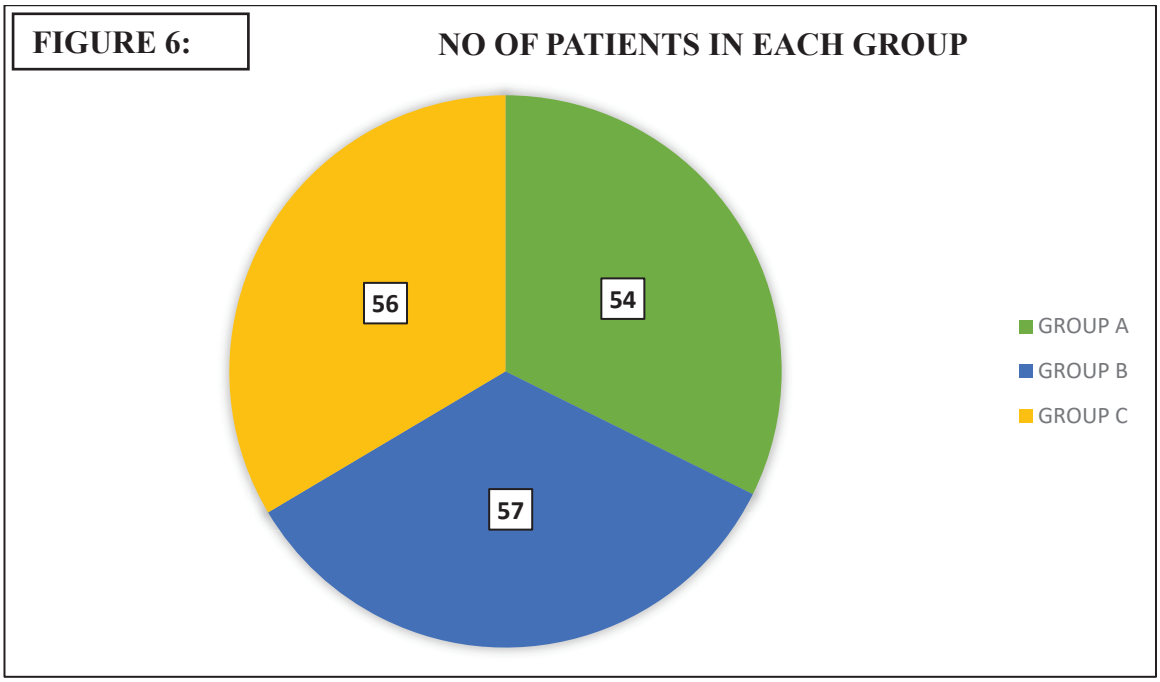
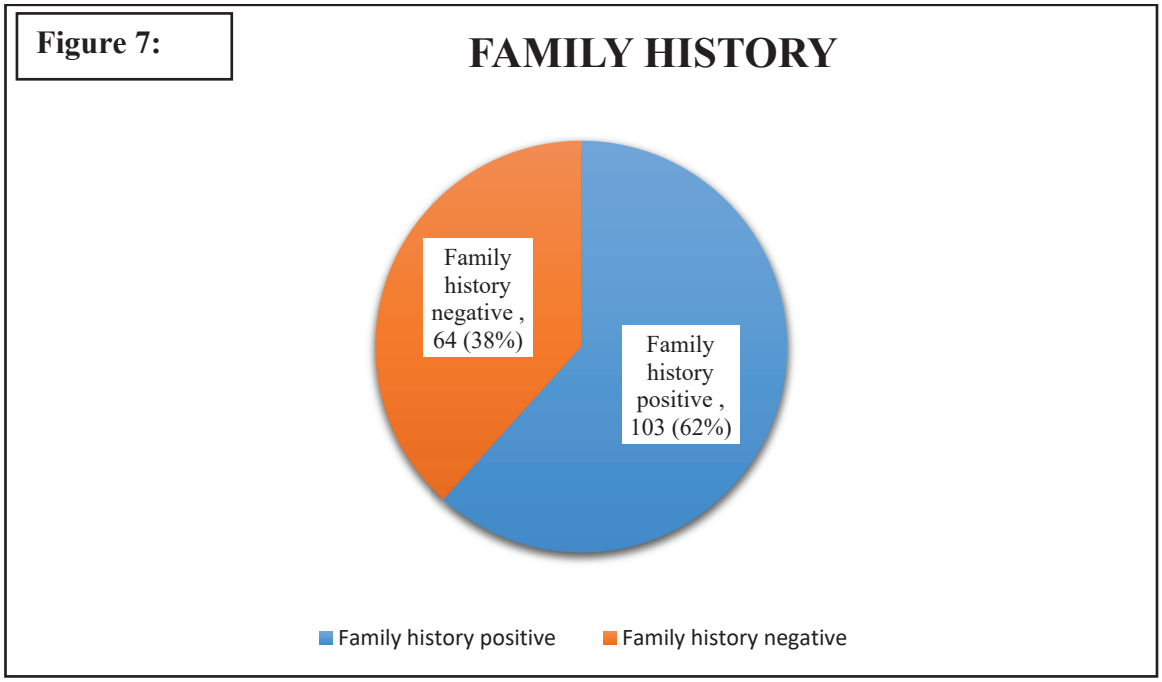


Figure 7:



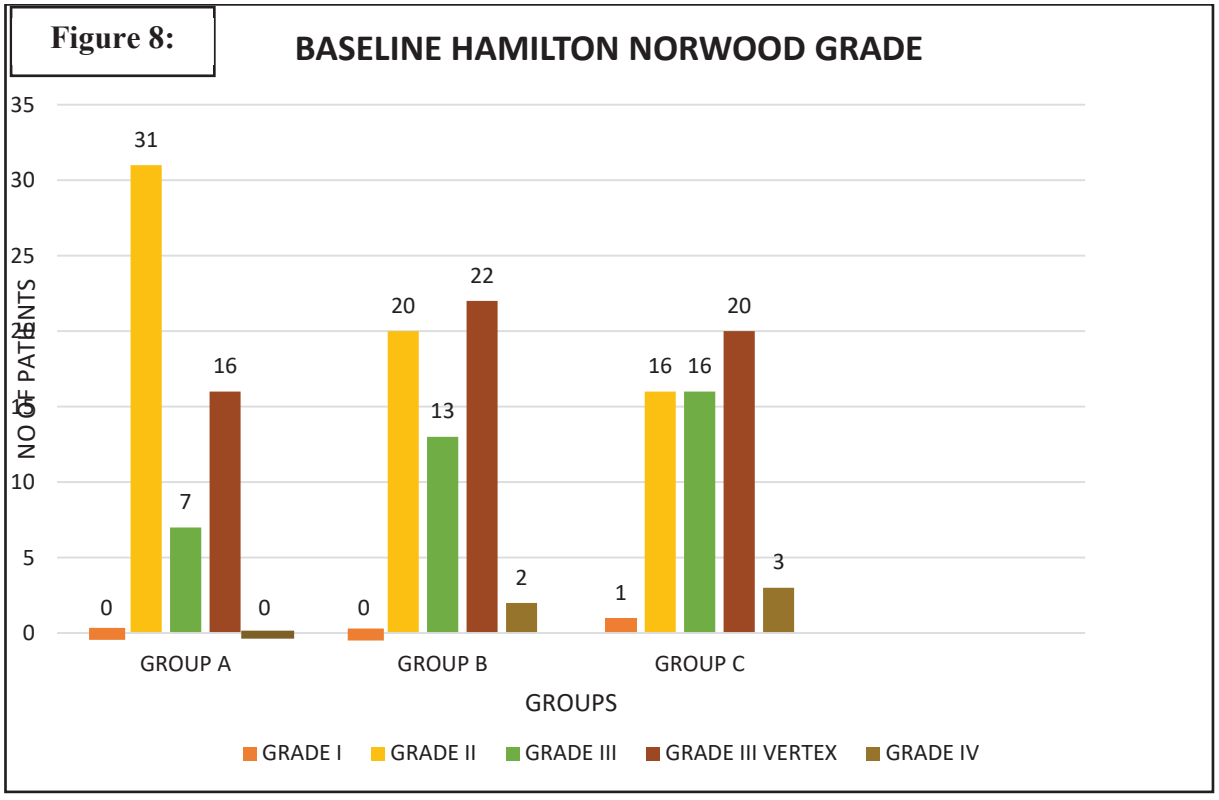
2. BASELINE HAMILTON NORWOOD GRADE:

All recruited 167 patients complained of hair loss in a patterned distribution, of which Hamilton Norwood grade I was found in 1/167 (0.5%) patients, grade II in 67/167 (40%) patients, grade III in 36/167 (22%) patients, grade III vertex in 58/167 (35%) patients, and grade IV in 5/167 (3%) patients. Overall, grade II, followed by grade III vertex, was the most common pattern detected. Comparing among different groups grade II was the most common pattern in group A, and grade III vertex was the most common in groups B and C. The overall findings are mentioned in table 6 and figure 8.

TABLE 6: Baseline Hamilton Norwood grade of study participants (as per ITT analysis):

S.NO	BASELINE GRADE	GROUP A (N=54)	GROUP B (N=57)	GROUP C (N=56)	TOTAL (N=167)
1.	Grade I N (%)	0	0	1(2%)	1 (0.5%)
2.	Grade II N (%)	31(57%)	20(35%)	16(29%)	67 (40%)
3.	Grade III N (%)	7(13%)	13(23%)	16(29%)	36 (22%)
4.	Grade III vertex N (%)	16(30%)	22(38%)	20(36%)	58 (35%)
5.	Grade IV N (%)	0	2(4%)	3(5%)	5 (3%)

Figure 8:



3. BASELINE SERUM BIOTIN (PICOGRAMS/MILILITER, pg/ml):

Biotin plasma concentrations typically range from 400 to 1,200 pg/ml and level less than 200 pg/ml is considered deficient.[32] In our study the mean baseline total serum biotin value was 17.43±33.02 pg/ml, while the mean values in groups A, B, and C were 13.9, 28.1, and 20.3, respectively, with a non-significant p value of 0.201. This is shown in table 7.

TABLE 7: Baseline serum biotin levels (pg/ml) of study participants (as per ITT):

GROUP	BASELINE SERUM BIOTIN (pg/ml) Mean±SD	p* value
Group A	13.9±25.3	0.201
Group B	28.1±48.8	
Group C	20.3±45.7	
p* values calculated using One way ANOVA		

(II) EFFICACY PARAMETERS:

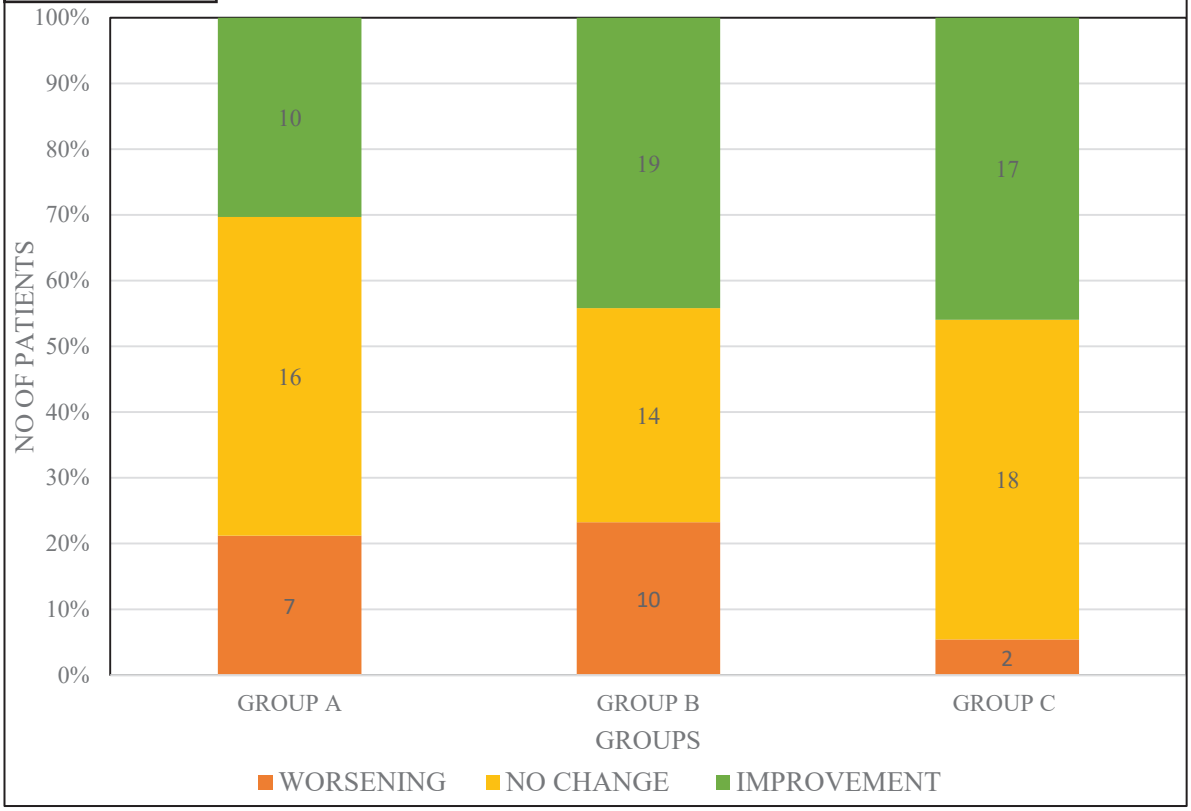
1. **GLOBAL PHTOGRAPHIC ASSESSMENT**:

Global photographic assessment was stratified into 3 groups, i.e. worsening (-3 to -1), no change (0), and improvement (+1 to +3). A blinded dermatologist rated 17/37 (45.94%) of Group C, 19/43 (44.1%) of Group B, and 10/33 (30.3%) of Group A treated patients as improved after 24 weeks of treatment (score +1, +2, +3). Chi-square test was applied for the comparison of Global photographic assessment across the 3 groups. p value came out to be 0.127 which is not significant. This is shown in table 8 and figure 9.

TABLE 8: Global Photographic Assessment (according to PP):

GLOBAL PHOTOGRAPHIC ASSESSMENT GROUP CROSSTABULATION							
			Group			**Total N=113**	**p* value**
			A N=33	**B N=43**	**C N=37**		
Global photographic Assessment	Worsening	Count	7	10	2	19	0.127
		% within group	21.2%	23.2%	5.4%	16.8%	
	No change	Count	16	14	18	48	
		% within group	48.4%	32.5%	48.6%	42.4%	
	Improvement	Count	10	19	17	46	
		% within group	30.3%	44.1%	45.9%	40.7%	
p* values calculated using Chi-Square test.							

Figure 9: **GLOBAL PHOTOGRAPHIC ASSESSMENT**



2. INVESTIGATOR GLOBAL ASSESSMENT

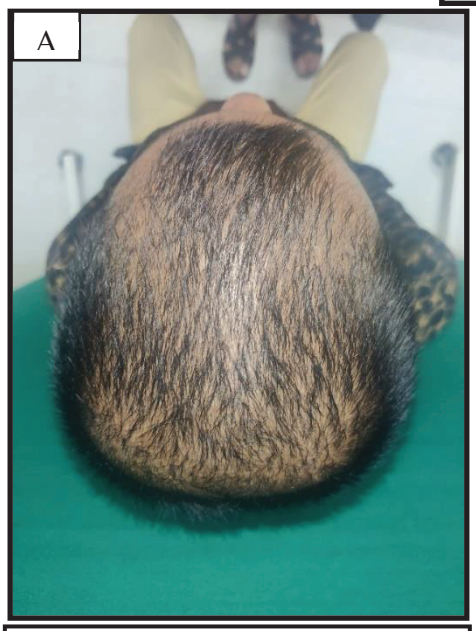
Based on the clinical photographs, patients were rated as worsening and no change with 0, while improvement was rated between 1-100% by 2 blinded dermatologists. The average of mean percent improvement from baseline calculated for all three groups was 6.9% in group B, followed by 6.7% in groups A and C. The percentage improvement was not statistically significant in all three groups. This is shown in table 9.

TABLE 9: Percentage improvement in global photographic assessment from baseline (according to PP):

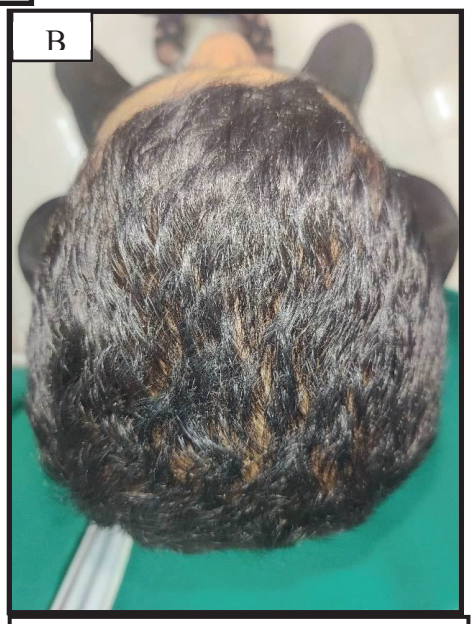
GROUP	**INVESTIGATOR GLOBAL ASSESSMENT**	**p* value**
Group A	6.7±2.3	0.263
Group B	6.9±2.7	
Group C	6.7±2.3	
p* values calculated using one way ANOVA		

GROUP A

Figure 10

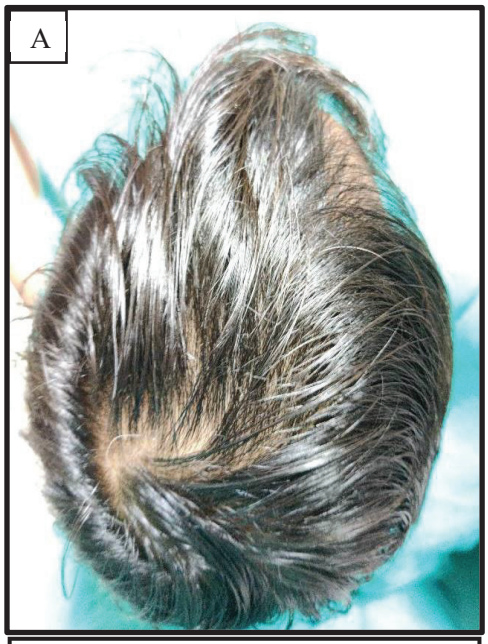


Baseline:
Patient number: 72
Hamilton Norwood: Grade III vertex

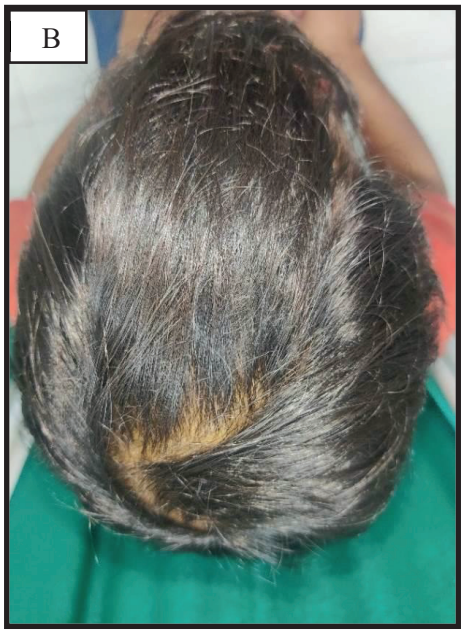


At 24 weeks:
GPA: +3 (Excellent improvement)
VAS (V24-V0): 20

Figure 11



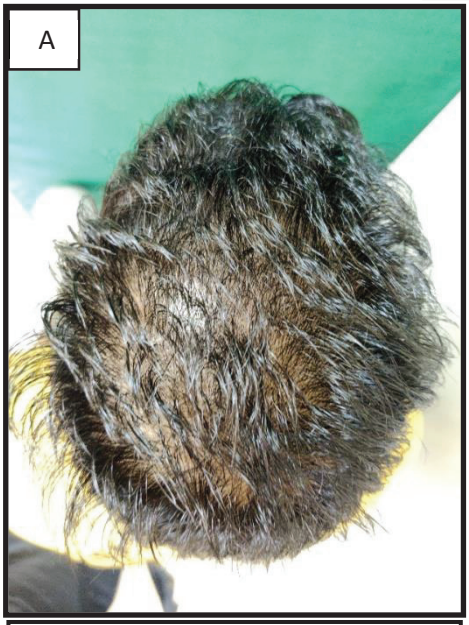
Baseline:
Patient number: 84
Hamilton Norwood: Grade III vertex



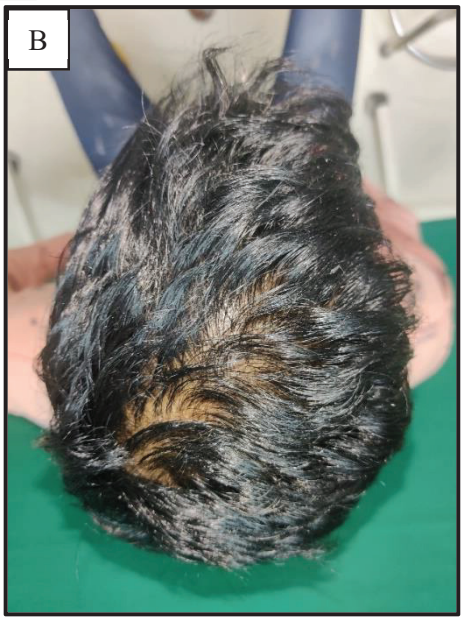
At 24 weeks:
GPA: +1 (Mild improvement)
VAS (V24-V0): 10

GROUP A

Figure 12

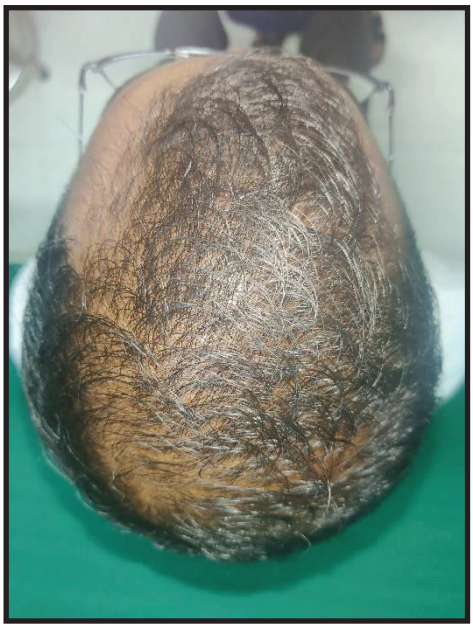


Baseline:
Patient number: 15
Hamilton Norwood: Grade III vertex

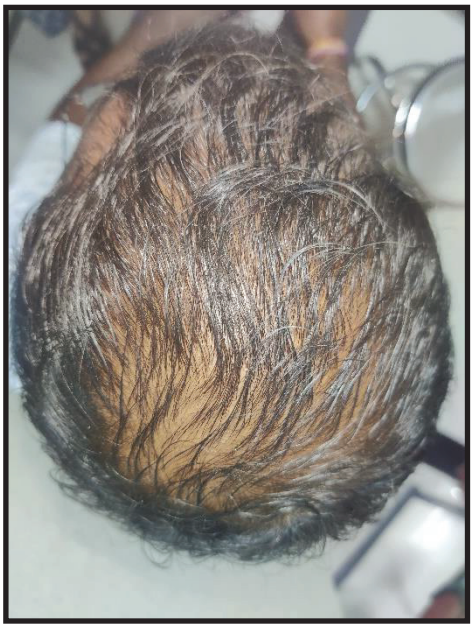


At 24 weeks:
GPA: +2 (Moderate improvement)
VAS (V24-V0): 0

Figure 13



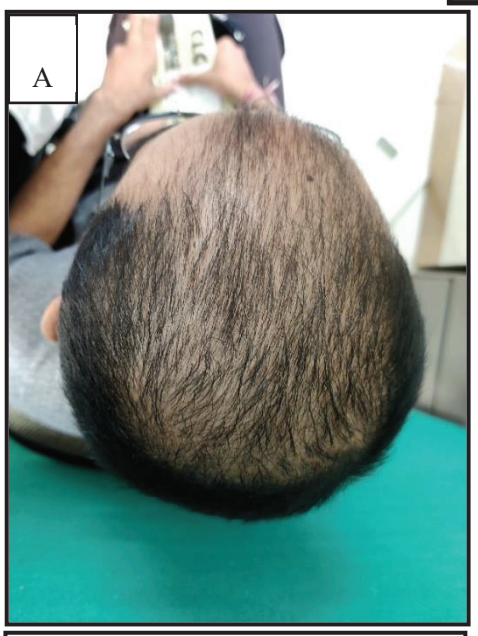
Baseline:
Patient number: 71
Hamilton Norwood: Grade III vertex



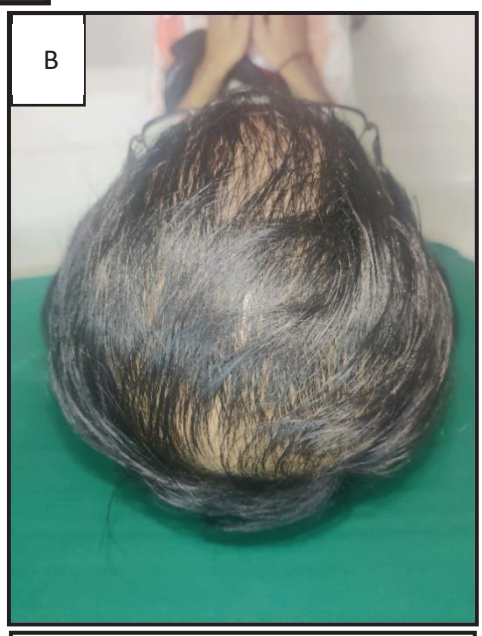
At 24 weeks:
GPA: -1 (Mild worsening)
VAS (V24-V0): 0

GRPOUP B

Figure 14

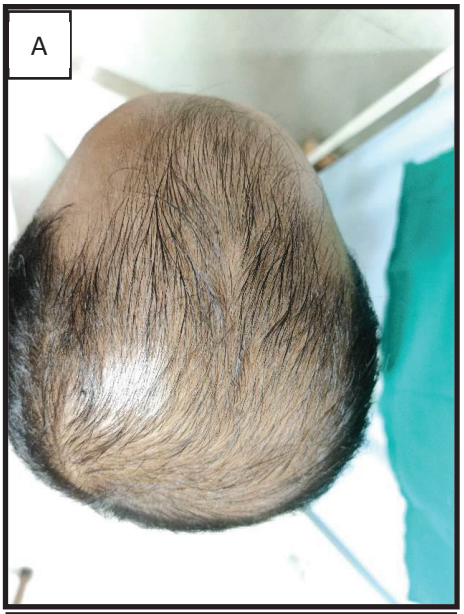


Baseline:
Patient number: 50
Hamilton Norwood: Grade III
Vertex

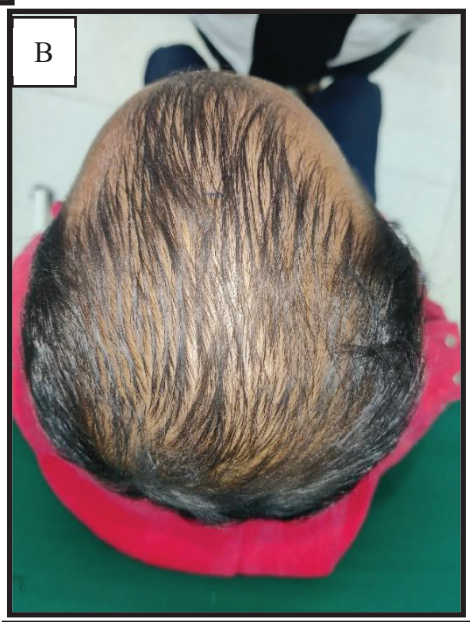


24 weeks:
GPA: +3 (Excellent improvement)
VAS (V24-V0): 7

Figure 15



Baseline:
Patient number: 11
Hamilton Norwood: Grade III
vertex

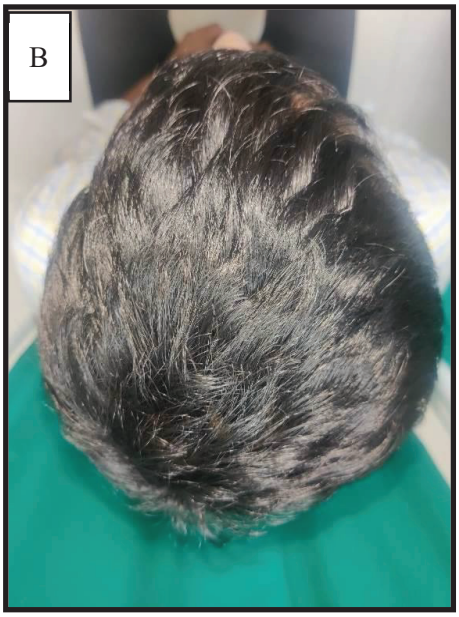


At 24 weeks:
GPA: +2 (Moderate improvement)
VAS (V24-V0): 25

GRPOUP B

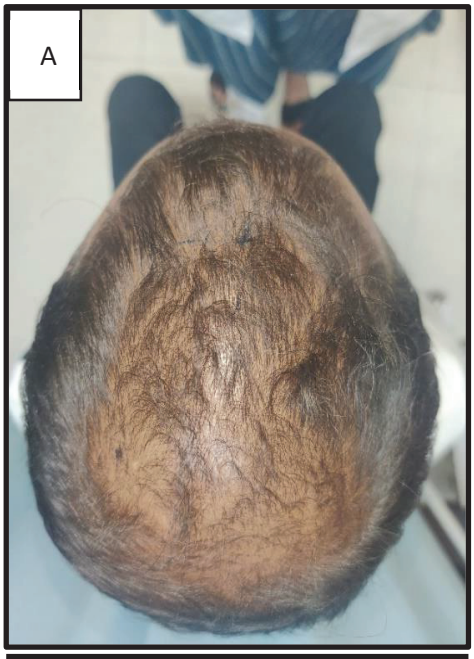
Figure 16

Baseline:
Patient number: 79
Hamilton Norwood: Grade III vertex

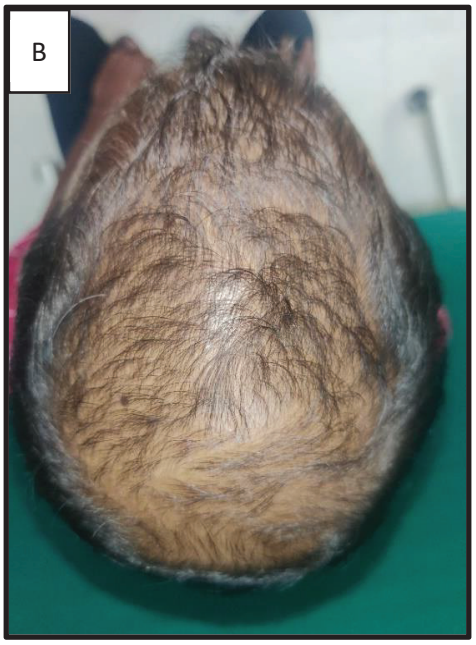


At 24 weeks:
GPA: +2 (Moderate improvement)
VAS (V24-V0): 5

Figure 17



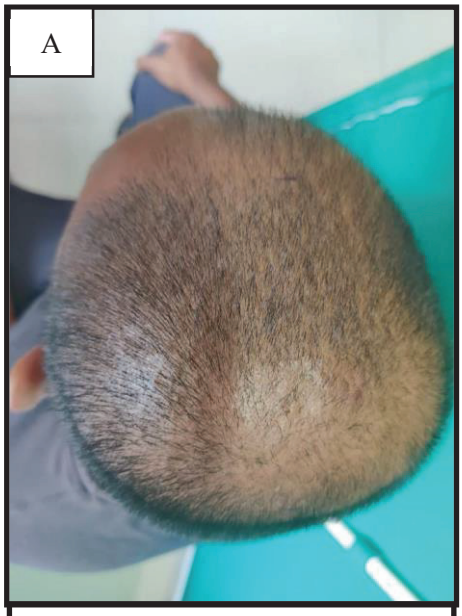
At baseline:
Patient number: 58
Hamilton Norwood: Grade III vertex



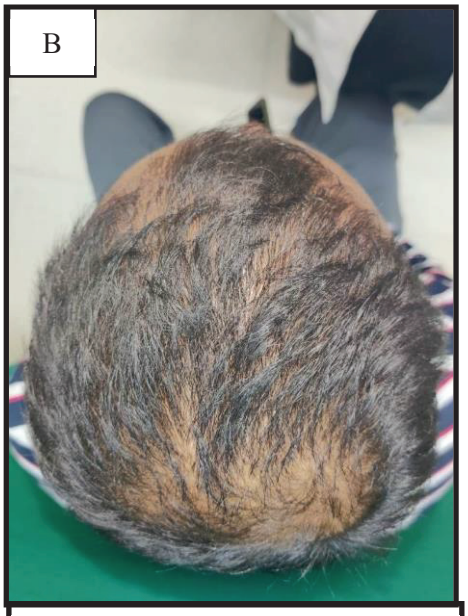
At 24 weeks:
GPA: -1 (Mild worsening)
VAS (V24-V0): 10

GRPOUP C

Figure 18

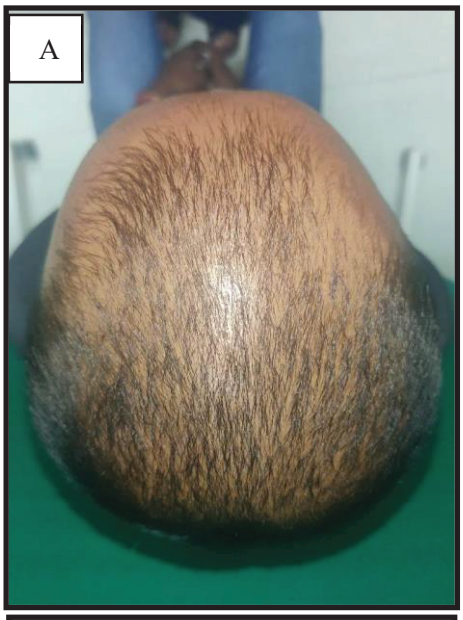


Baseline:
Patient number: 6
Hamilton Norwood: Grade III vertex

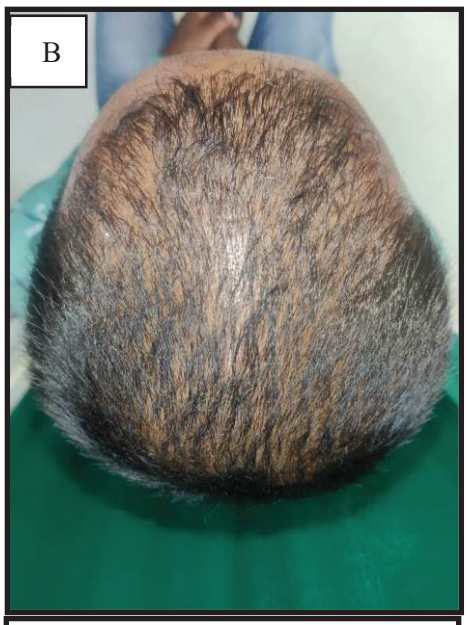


24 weeks:
GPA: +2 (Moderate improvement)
VAS (V24-V0): 35

Figure 19



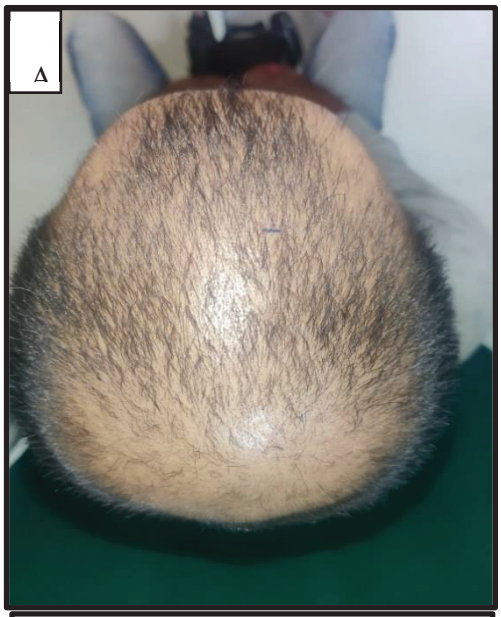
Baseline:
Patient number: 66
Hamilton Norwood: Grade III vertex



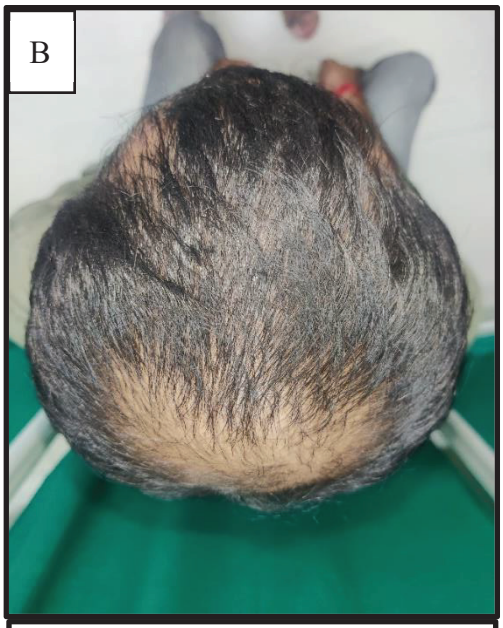
24 weeks:
GPA: +2 (Moderate improvement)
VAS (V24-V0): 10

GROUP C

Figure 20

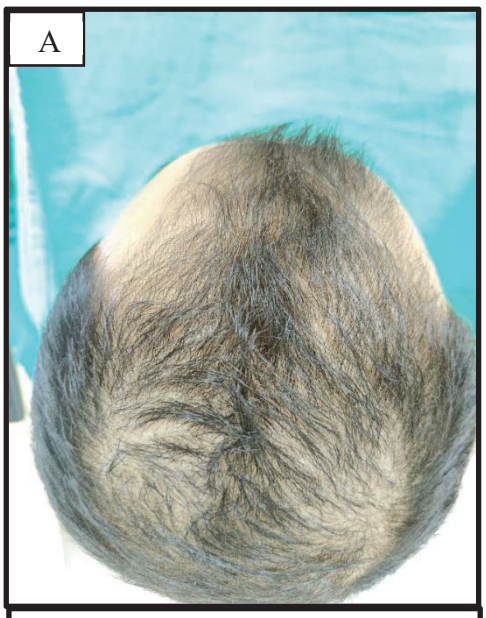


Baseline:
Patient number: 93
Hamilton Norwood: Grade III vertex

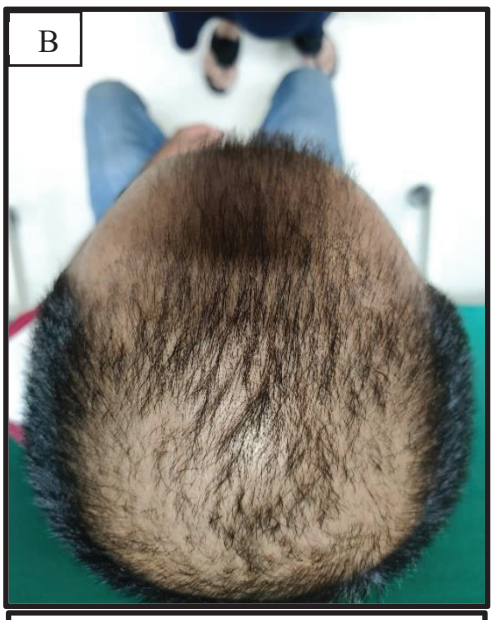


24 weeks:
GPA: +3 (Excellent improvement)
VAS (V24-V0): 10

Figure 21



Baseline:
Patient number: 2
Hamilton Norwood: Grade II vertex



At 24 weeks:
GPA: -1 (Mild worsening)
VAS (V24-V0): 0

1. DERMOSCOPY PARAMETERS:

Hair shaft thickness heterogeneity was the most common dermoscopic parameter present in all 3 groups over both frontal and vertex scalp followed by brown peripilar sign. More than 20% hair diameter diversity, corresponding to hair follicle miniaturisation, was observed in 87.6% (99/113) and 67.2% (76/113) of patients, respectively, over the frontal and vertex scalps. Brown Peripilar sign, corresponding to perifollicular inflammation, was observed in 33.6% (38/113) of patients over the frontal scalp and 21.2% (24/113) of patients over the vertex scalp. White peripilar sign was observed in 15% (17/113) of patients over the frontal scalp and 10.6% (12/113) of patients over the vertex scalp. Scalp honey comb pigmentation was observed in 7% (8/113) of patients over the frontal and vertex scalp each. This is shown in tables 10 and 11.

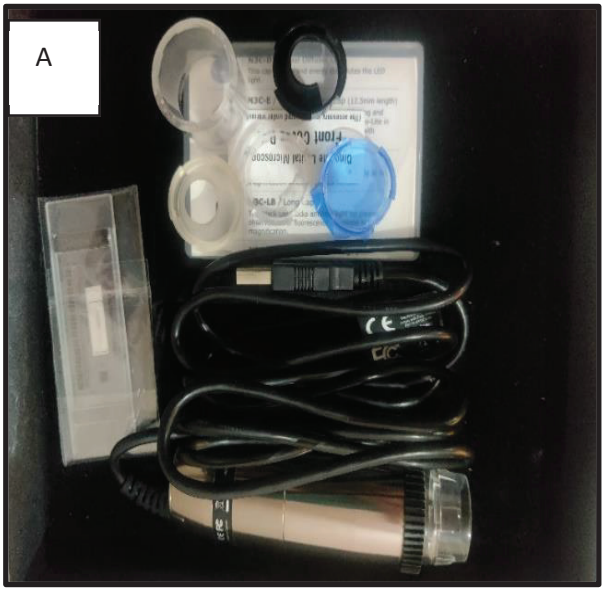
TABLE -10: Dermoscopic parameters over frontal scalp (according to PP):

S.NO	PARAMETER	Group A N=33	Group B N=43	Group C N=37	Total N=113	p* value
1.	Hair shaft thickness heterogeneity	29 (87.8%)	38 (88.37)	32 (86.4%)	99 (87.6%)	0.966
2.	Brown peripilar sign	12 (36.3%)	17 (39.53)	9 (24.3%)	38 (33.6%)	0.330
3.	White peripilar sign	5 (15.1%)	6 (13.9%)	6 (16.2%)	17 (15.0%)	0.961
4.	Yellow dots	1 (3.0%)	4 (9.3%)	1 (2.7%)	6 (5.3%)	0.332
5.	Focal atrichia	0 (0.0%)	1 (2.3%)	0 (0.0%)	1 (0.8%)	0.440
6.	Scalp honey comb pigmentation	2 (6.0%)	4 (9.3%)	2 (5.4%)	8 (7.0%)	0.766
p* values calculated using Chi-Square test.						

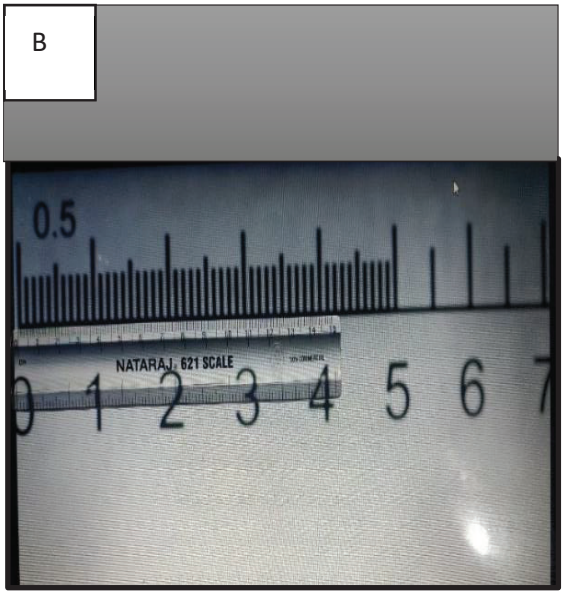
TABLE-11: Dermoscopic parameters over vertex scalp (according to PP):

S.NO	PARAMETER	Group A N=33	Group B N=43	Group C N=37	Total N=113	p* value
1.	Hair shaft thickness heterogeneity	20 (60.6%)	32 (74.4%)	24 (64.8%)	76 (67.2%)	0.415
2.	Brown peripilar sign	9 (27.2%)	12 (27.9%)	3 (8.1%)	24 (21.2%)	0.059
3.	White peripilar sign	2 (6.0%)	4 (9.3%)	6 (16.2%)	12 (10.6%)	0.364
4.	Yellow dots	1 (3.0%)	6 (13.9%)	0 (0.0%)	7 (6.1%)	**0.024**
5.	Focal atrichia	0 (0.0%)	1 (2.3%)	0 (0.0%)	1 (0.8%)	0.440
6.	Scalp honey comb pigmentation	2 (6.0%)	3 (6.9%)	3 (8.1%)	8 (7.0%)	0.945
p* values calculated using Chi-Square test.						

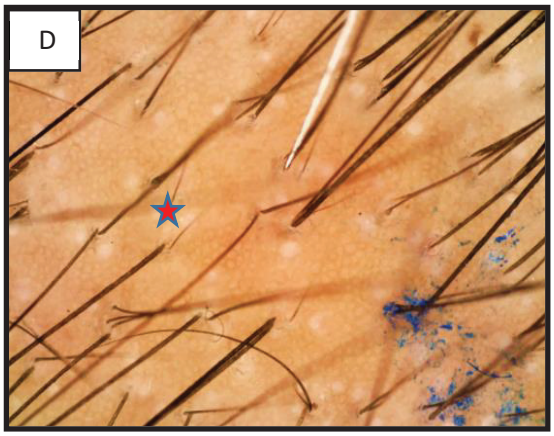
Figure 22

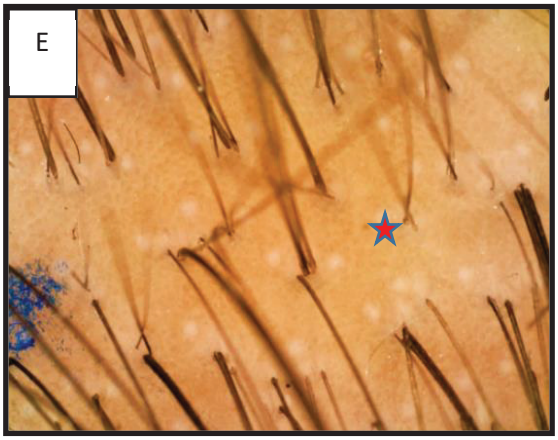


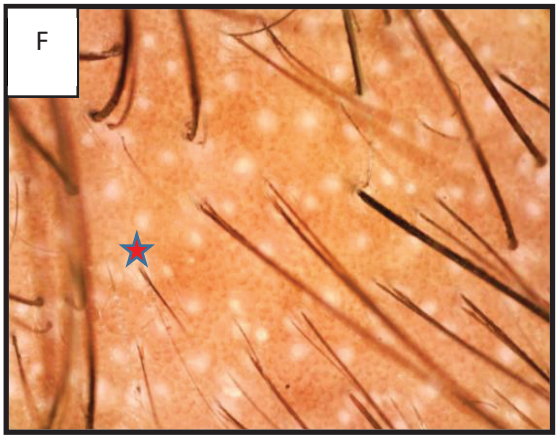
Dinolite AM 7515 MZT with accessories

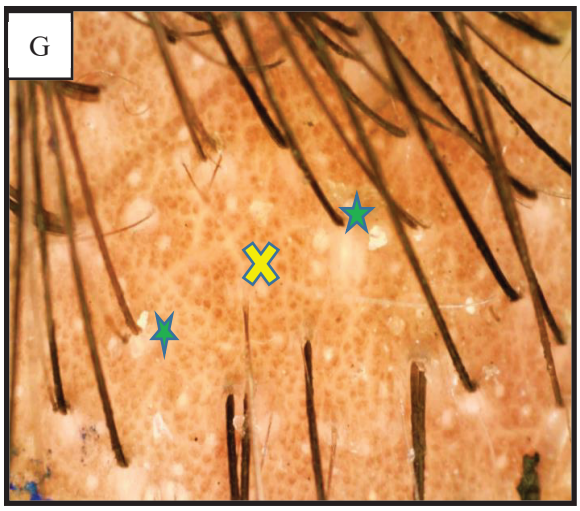


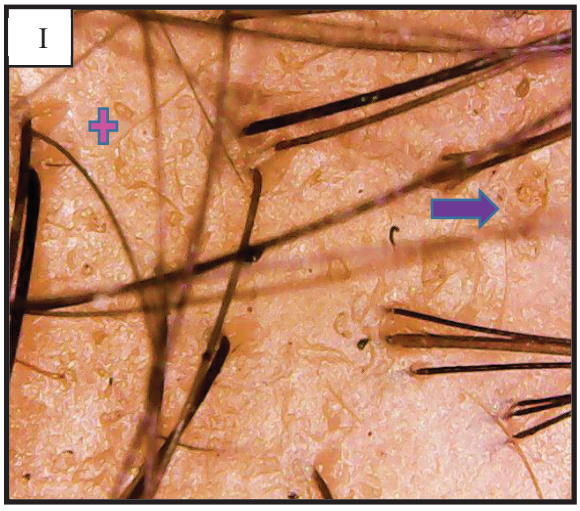
Ruler scale used to measure TAHW (1 mm of ruler scale=0.05 mm of dermoscopic scale)

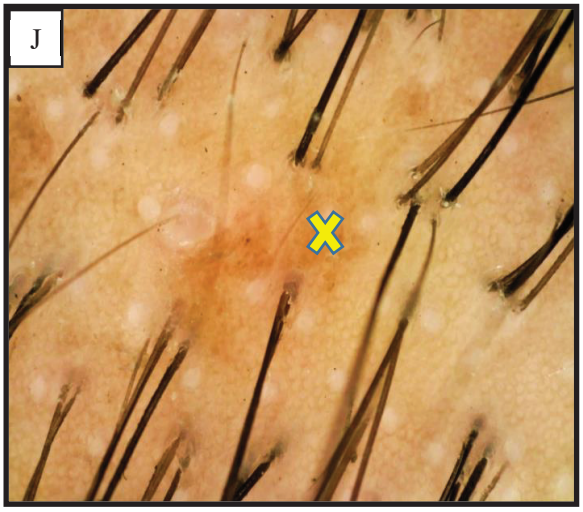












HAIR SHAFT THICKNESS HETEROGENEITY

BROWN PERIPILAR SIGN

WHITE PERIPILAR SIGN

SCALP HONEY COMB PIGMENTATION

YELLOW DOT

FOCAL ATRICHIA

A: NORMAL SCALP DERMOSCOPY

1. TARGET AREA HAIR COUNT (NUMBER OF HAIRS PER SQUARE CENTIMETER) OVER FRONTAL SCALP:

Mean TAHC at baseline was similar among all treatment groups, which is as follows: 156±37.7 hairs for Group A, 146.7±34.9 hairs for Group B and 158.2±28.5 hairs for Group C. All groups were comparable (p>0.05, one way ANOVA) in terms of TAHC at baseline.
In frontal area changes in mean number of total hair count was 18.1, 16.3, 14.3, for group C, B, A respectively. On repeated measures ANOVA, there was a significant improvement in TAHC over frontal area at 6 months as compared to baseline in all 3 groups [F (1,14770) = 45.836, **p<0.001**] but neither of the groups had a significantly better outcome as compared to others [F (2,129.891) =0.202, p=0.818]. This is shown in table 12 and figures 23, 24.

TABLE 12: **Target area hair density (number of hairs per square centimetre) over frontal scalp (according to PP):**

	GROUP A N=33		**GROUP B N=43**		**GROUP C N=37**			
PARAMETER	**MEAN**	**SD**	**MEAN**	**SD**	**MEAN**	**SD**	**p* value**	**p# value**
TAHC FRONTAL BASELINE	156.0	37.7	146.7	34.9	158.2	28.5	0.272	0.818
TAHC FRONTAL VISIT6	170.3	28.9	163.0	33.8	176.3	30.2	0.166	
MEAN CHANGE IN HAIR COUNT (HC 24-HC0)	14.3	26.1	16.3	25.3	18.1	24.6		
p value by repeated measures ANOVA showed [F (1,14770) = 45.836, **p<0.001]** in each of the 3 groups (intragroup).								
p*: One-way ANOVA. p#: Repeat measure ANOVA								

Figure 23:

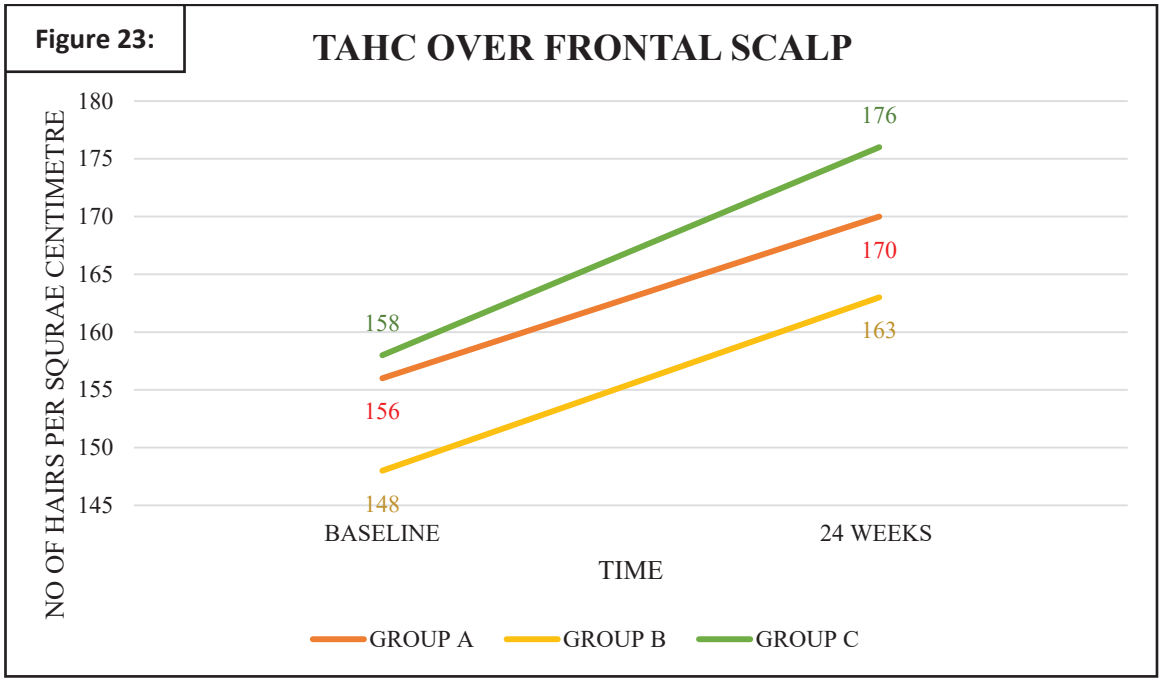
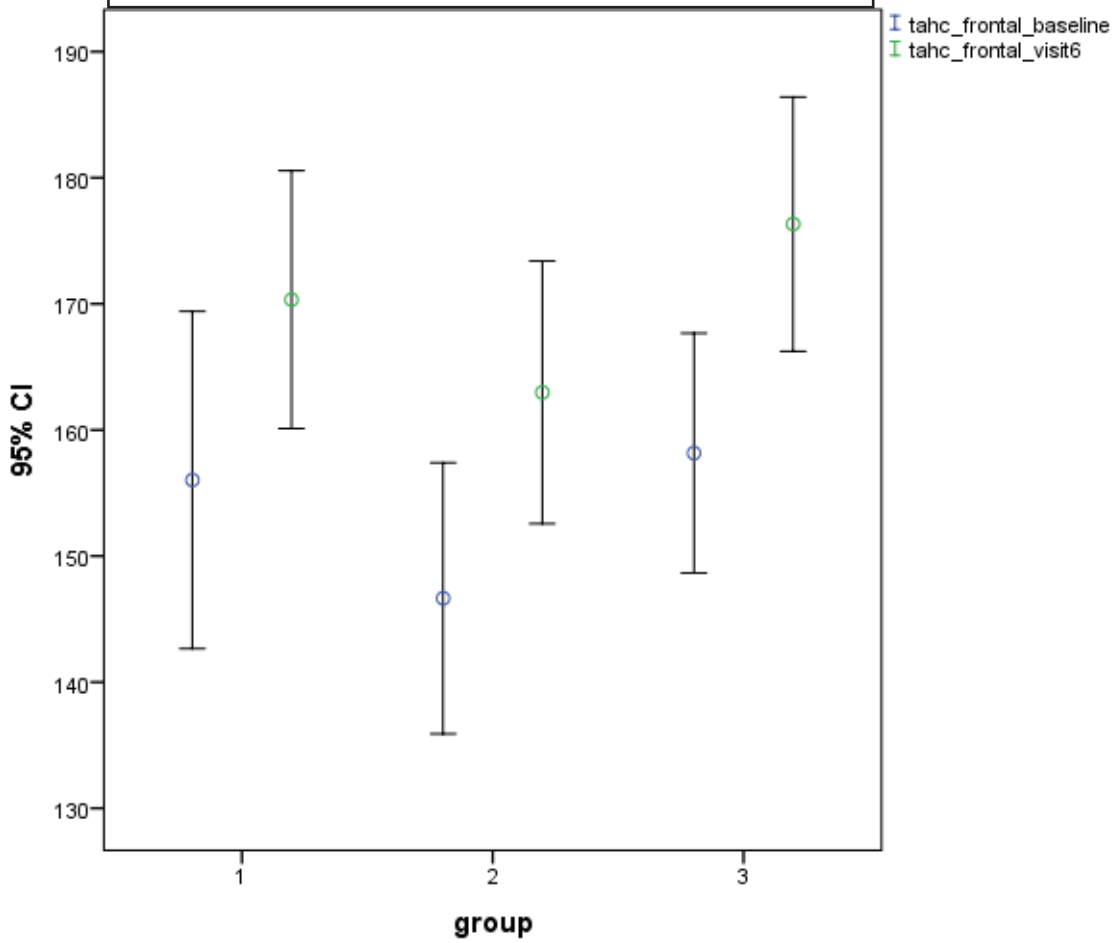


Figure 24: Error Plot showing TAHC over frontal scalp across 3 groups.



2. TARGET AREA HAIR COUNT (NUMBER OF HAIRS PER SQUARE CENTIMETER) OVER VERTEX SCALP:

Mean TAHC over vertex at baseline was similar among all treatment groups, which is as follows: 146.6±29.8 hairs for Group A, 145.8±32 hairs for Group B and 145.6±34.3hairs for Group C. All groups were comparable (p>0.05, one way ANOVA) in terms of TAHC at baseline. Over vertex area changes in mean number of total hair count were 18.9, 17.5, 14 for group C, A, B respectively. On repeated measures ANOVA, there was a significant improvement in TAHC vertex at 6 months as compared to baseline in all 3 groups [F (1,15760.267) = 31.593, **p<0.001**] but neither of the groups had a significantly better outcome as compared to others [F (2,246.606) =0.247, p=0.781]. This is shown in table 13 and figures 25, 26.

TABLE 13: Target area hair density (number of hairs per square centimetre) over vertex scalp (according to PP) :

	GROUP A N=33		**GROUP B N=43**		**GROUP C N=37**			
PARAMETER	**MEAN**	**SD**	**MEAN**	**SD**	**MEAN**	**SD**	**p* value**	**p# value**
TAHC VERTEX BASELINE	146.6	29.8	145.8	32.0	145.6	34.3	0.991	0.781
TAHC VERTEX VISIT6	164.1	35.5	159.8	32.7	164.5	35.3	0.797	
MEAN CHANGE IN HAIR COUNT (HC 24-HC0)	17.5	26.7	14.0	30.1	18.9	36.8		
p value by repeated measures ANOVA showed [F (1,15760.267) = 31.593, **p<0.001**] in each of the 3 groups (intragroup).								
p*: One-way ANOVA. p#: Repeat measure ANOVA								

Figure 25:

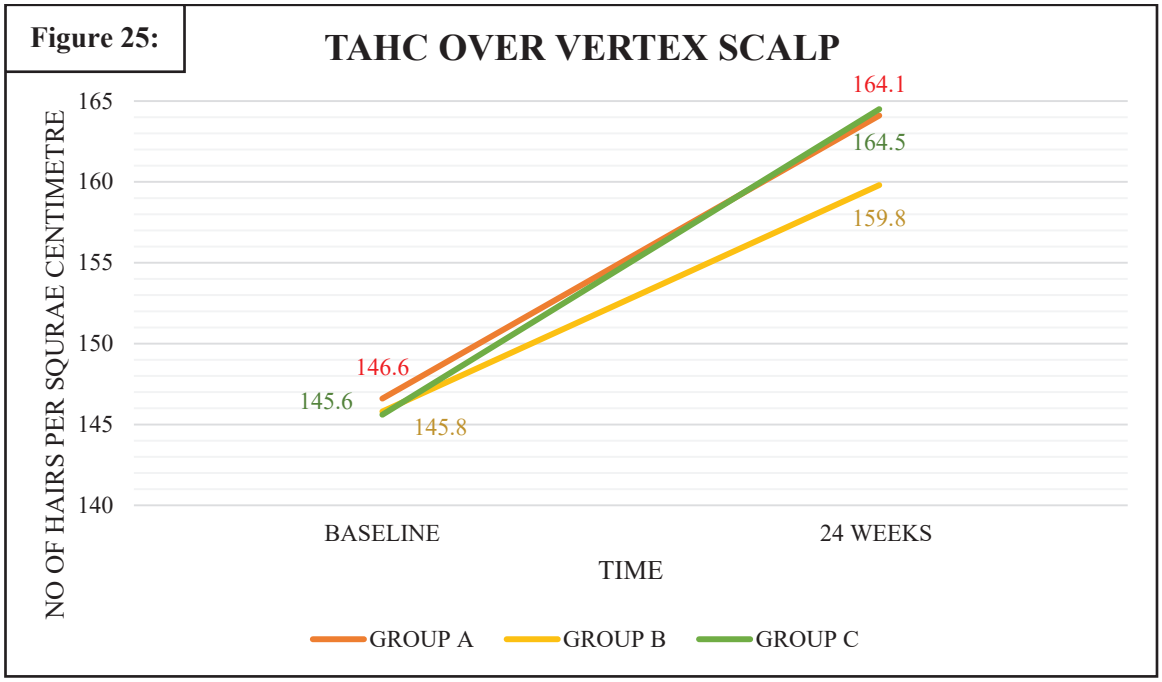
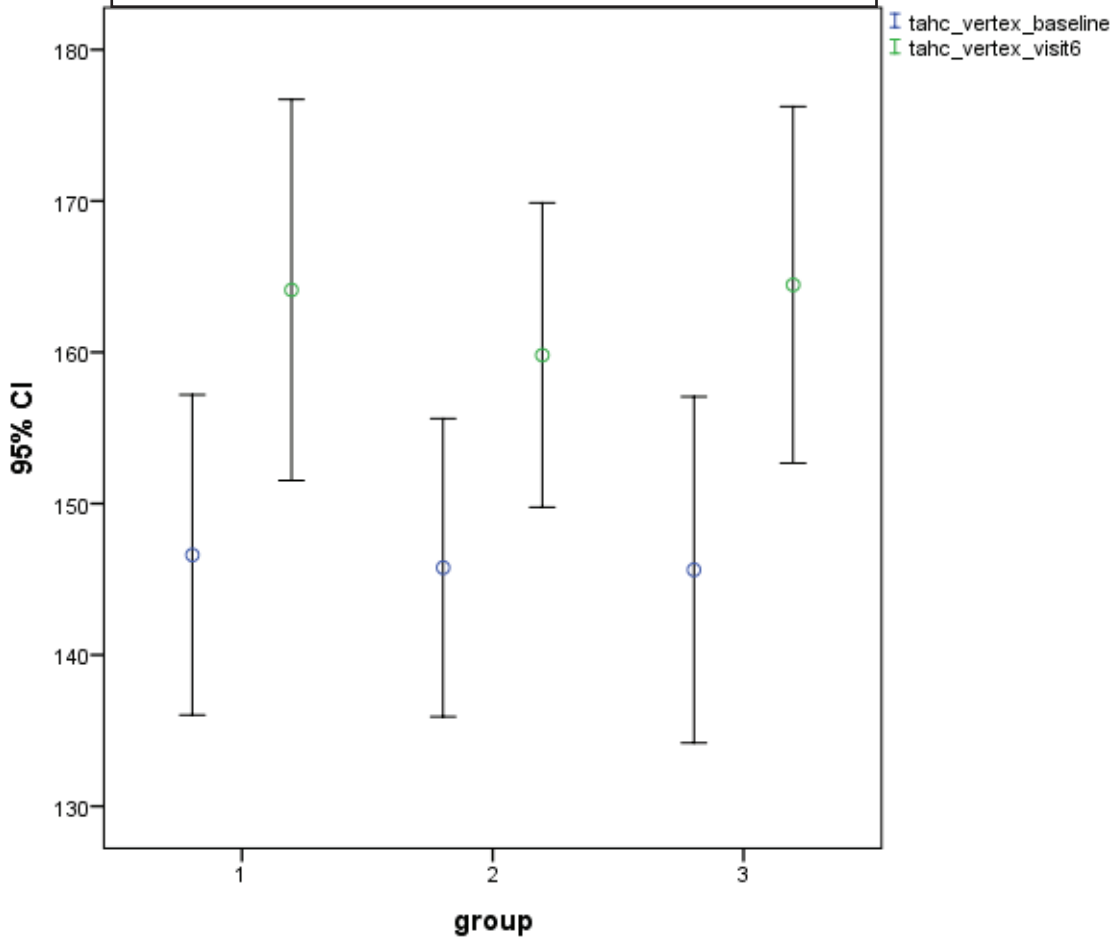
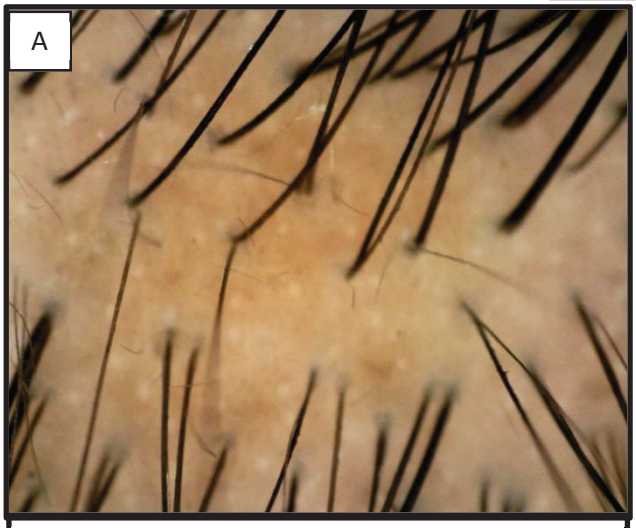


FIGURE 26: Error Plot showing TAHC over vertex scalp among 3 groups.

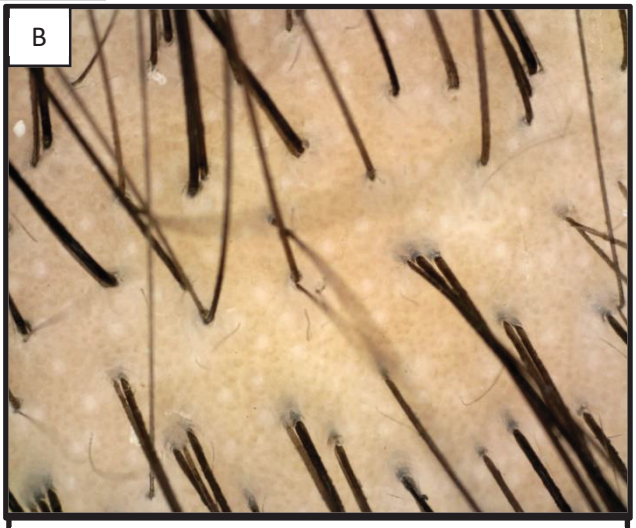


GROUP A

Figure 27



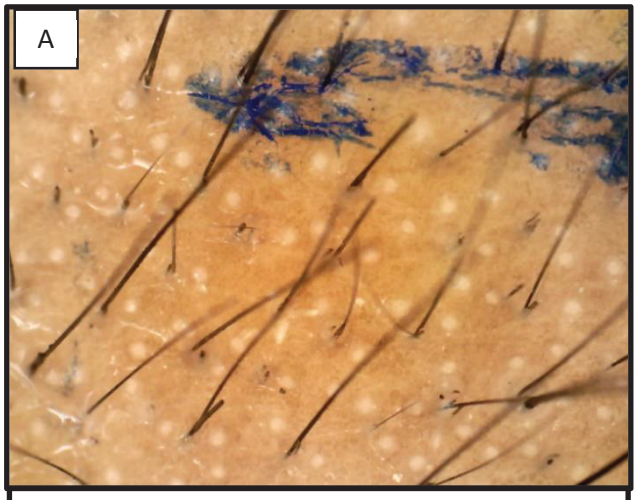
Baseline:
Patient number: 70
TAHC: 156 hairs per cm^2



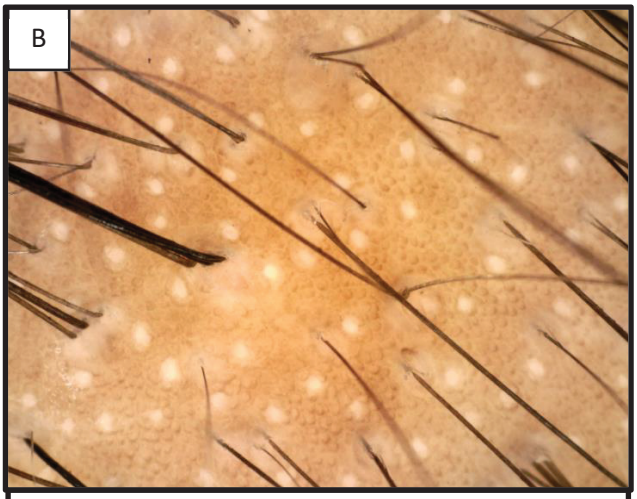
At 24 weeks:
TAHC: 203 hairs per cm^2

GROUP B

Figure 28



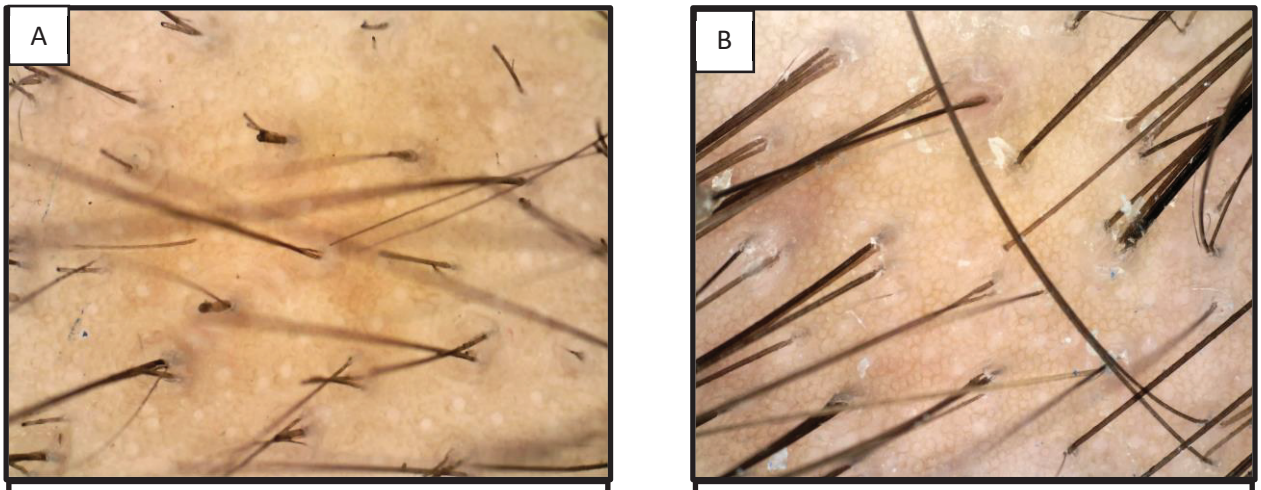
Baseline:
Patient number: 61
TAHC: 134 hairs per cm^2



At 24 weeks:
TAHC: 142 hairs per cm^2

GROUP C

Figure 29

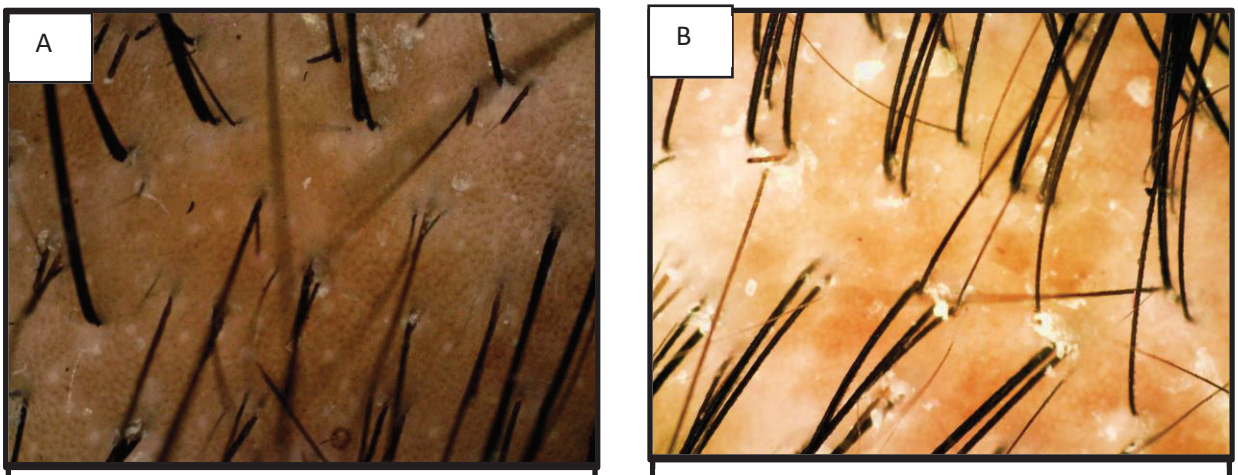


Baseline:
Patient number: 93
TAHC: 151 hairs per cm^2

At 24 weeks:
TAHC: 164 hairs per cm^2

GROUP A

Figure 30



Baseline:
Patient number: 93
TAHC: 137 hairs per cm^2

At 24 weeks:
TAHC: 159 hairs per cm^2

3. TARGET AREA HAIR WIDTH OVER FRONTAL SCALP

At baseline, the mean TAHW for all treatment groups was 88.5±16.5 micrometre for group A, 84.1±22.6 micrometre for group B, and 91.1±15.6 micrometre for group C. In terms of TAHW at baseline, all groups were comparable ($p > 0.05$, one way ANOVA). The changes in mean hair thickness over frontal scalp was maximum for group B (9.7) followed by group A (5.9) and group C (4.7). On repeated measures ANOVA, there was a significant improvement in mean hair thickness over frontal scalp at 6 months as compared to baseline in all 3 groups [$F(1,25590.56) = 18.841$, **$p<0.001$**] but neither of the groups had a significantly better outcome as compared to others [$F(2,274.086)=1.009$, $p=0.368$]. This is shown in table 14 and figures 31, 32.

TABLE 14: Target area hair diameter over frontal scalp (according to PP):

	GROUP A N=33		**GROUP B N=43**		**GROUP C N=37**			
PARAMETER	**MEAN**	**SD**	**MEAN**	**SD**	**MEAN**	**SD**	**p* value**	**$p^{\#}$ value**
TAHW FRONTAL BASELINE	88.5	16.5	84.1	22.6	91.1	15.6	0.244	0.368
TAHW FRONTAL VISIT6	94.5	22.0	93.8	22.1	95.8	19.0	0.915	
MEAN CHANGE IN HAIR WIDTH (HW 24-HC0)	5.9	23.2	9.7	12.4	4.7	13.0		
p value by repeated measures ANOVA showed [$F(1,25590.56) = 18.841$, **$p<0.001$**] in each of the 3 groups (intragroup).								
p*: One-way ANOVA. $p^{\#}$: Repeat measure ANOVA								

Figure 31:

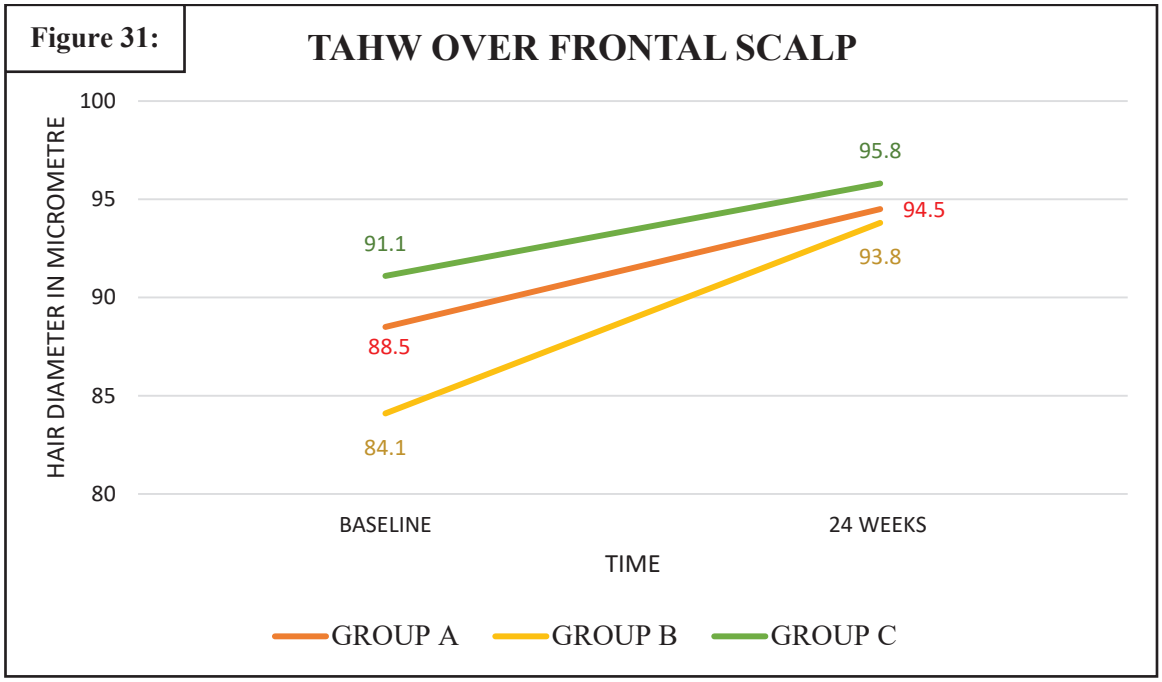
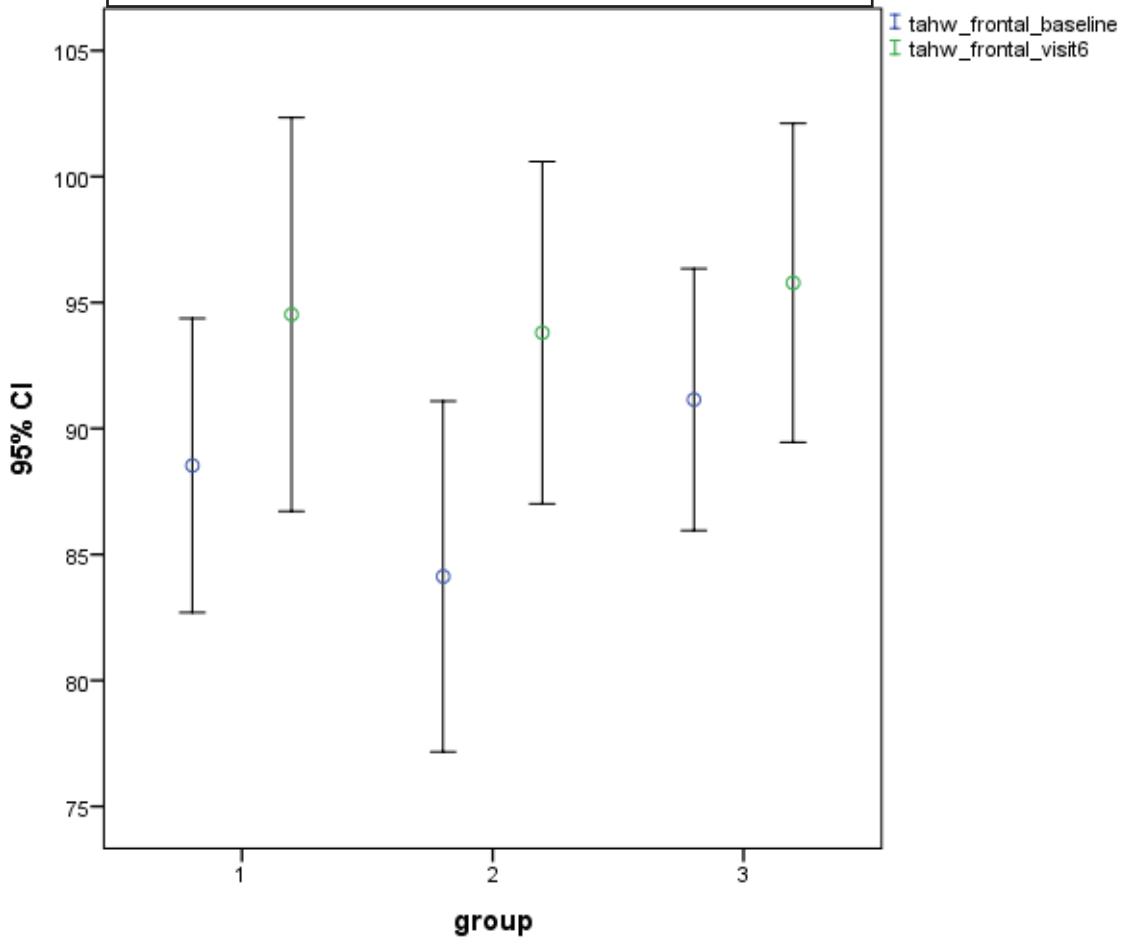


Figure 32: Error Plot showing TAHW over frontal scalp among 3 groups.



4. TARGET AREA HAIR WIDTH OVER VERTEX SCALP:

The mean TAHW for all treatment groups at baseline was 95.6±17.5 micrometres for group A, 90.2±25.2 micrometres for group B, and 95.4±16.6 micrometres for group C. All groups were comparable in terms of TAHW at baseline ($p > 0.05$, one way ANOVA). Group A had the greatest change in mean hair thickness over vertex scalp (7.9), followed by Group B (4.3) and Group C (4.2). On repeated measures ANOVA, there was a significant improvement in TAHW vertex at 6 months as compared to baseline in all 3 groups [$F(1,1699.564) = 21.814$, **$p<0.001$**] but neither of the groups had a significantly better outcome as compared to others [$F(2,158.382)=1.016$, $p=0.365$]. This is shown in table 15 and figures 33, 34.

TABLE 15: Target area hair diameter over vertex scalp (according to PP):

	GROUP A N=33		**GROUP B N=43**		**GROUP C N=37**			
PARAMETER	**MEAN**	**SD**	**MEAN**	**SD**	**MEAN**	**SD**	**p* value**	**p# value**
TAHW VERTEX BASELINE	95.6	17.5	90.2	25.2	95.4	16.6	0.409	0.365
TAHW VERTEX VISIT6	103.5	17.9	94.5	27.2	99.6	20.0	0.219	
MEAN CHANGE IN HAIR WIDTH (HW 24-HC0)	7.9	12.3	4.3	13.7	4.2	11.0		
p value by repeated measures ANOVA showed [$F(1,1699.564) = 21.814$, **$p<0.001$**] in each of the 3 groups (intragroup).								
p*: One-way ANOVA. p#: Repeat measure ANOVA								

FIGURE 33:

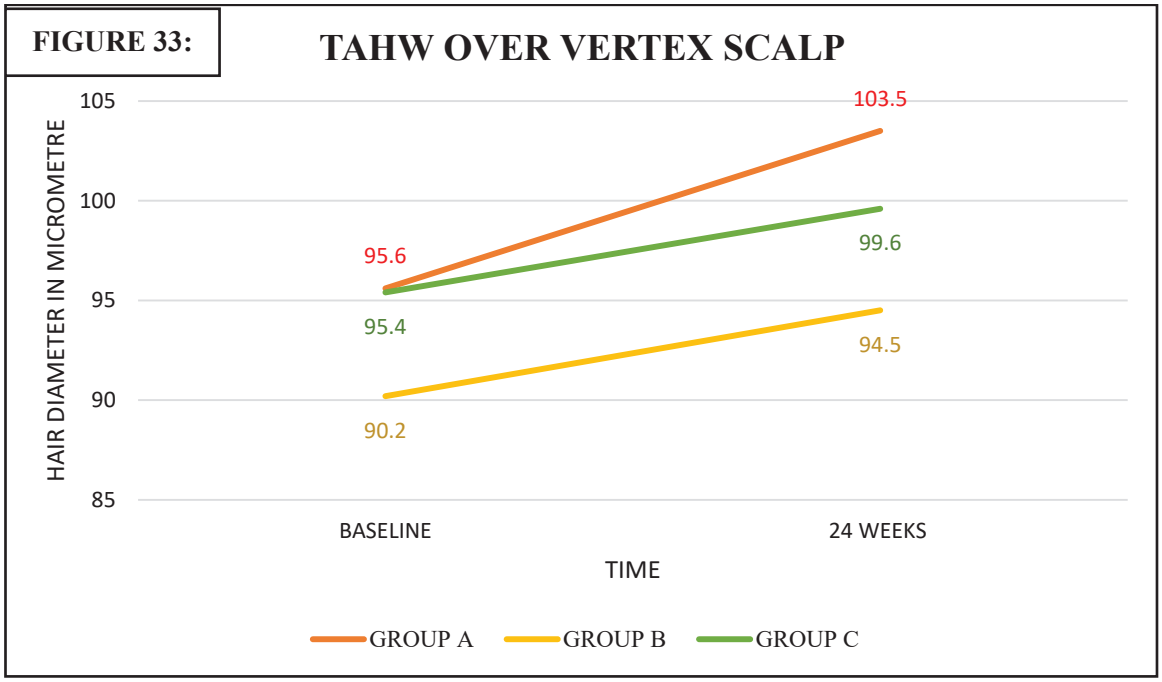
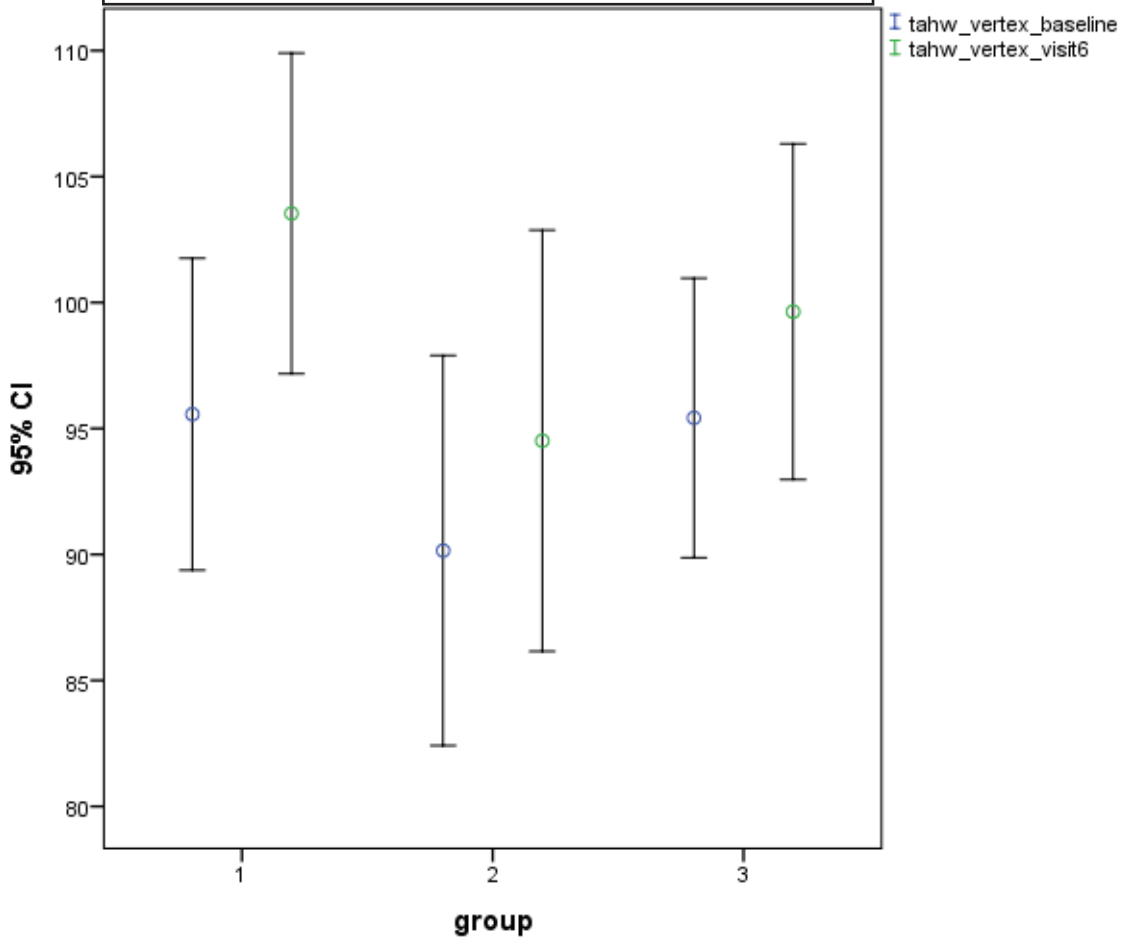


FIGURE 34: Error Plot showing TAHW over Vertex scalp among 3 groups.



5. SUBJECTIVE VISUAL ANALOGUE SCALE:

Mean subjective visual analogue scale at baseline was similar among all treatment groups, which is as follows: 28.1±13.9 for Group A, 25.7±10.2 for Group B and 27.9±12.1 for Group C. All groups were comparable (p>0.05, one way ANOVA) in terms of subjective VAS at baseline. On repeated measures ANOVA, there was a significant improvement in VAS at 6 months as compared to baseline in all 3 groups [F (1,2560.295) = 97.295, p<0.001] but neither of the groups had a significantly better outcome as compared to others [F(2,52.849)=1.004, p=0.370]. This is shown in table 16 and figures 35, 36.

TABLE 16: Subjective visual analogue scale (according to PP):

	GROUP A N=33		**GROUP B N=43**		**GROUP C N=37**			
PARAMETER	**MEAN**	**SD**	**MEAN**	**SD**	**MEAN**	**SD**	**p* value**	**p# value**
VAS BASELINE	28.1	13.9	25.7	10.2	27.9	12.1	0.600	0.370
VAS VISIT6	33.5	12.0	33.3	8.6	35.2	11.0	0.677	
Mean improvement (24 weeks- 0 weeks)	5.4	6.7	7.6	7.4	7.3	7.4		
p value by repeated measures ANOVA showed [F(1,2560.295) = 97.295, **p<0.001**] in each of the 3 groups (intragroup).								
p*: One-way ANOVA. p#**:** Repeat measure ANOVA								

Figure 35:

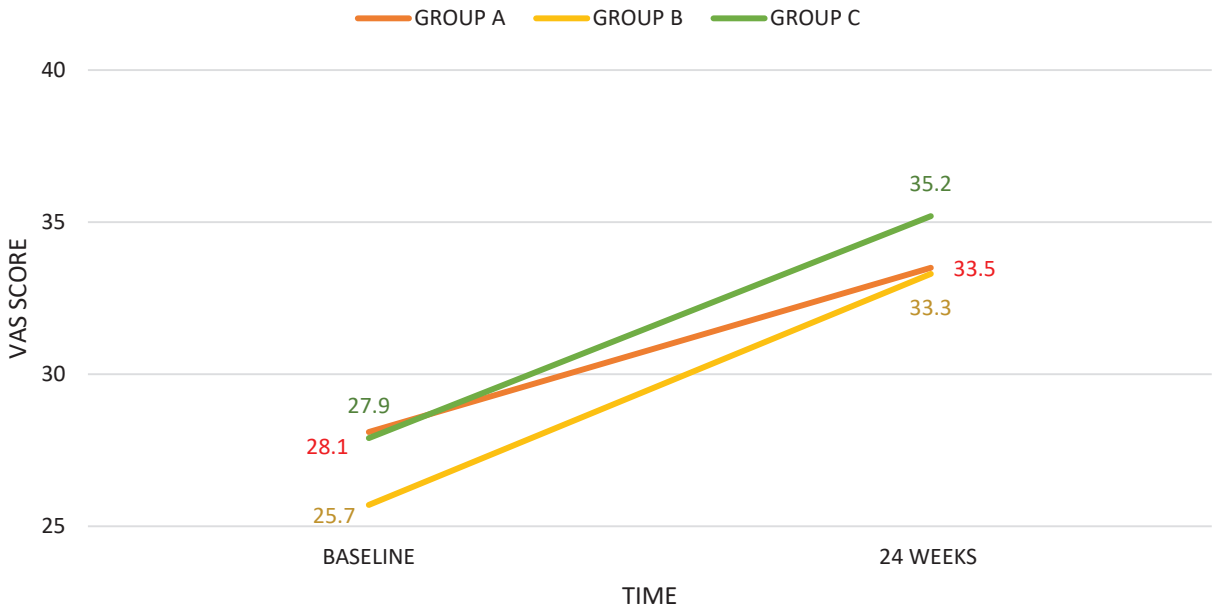
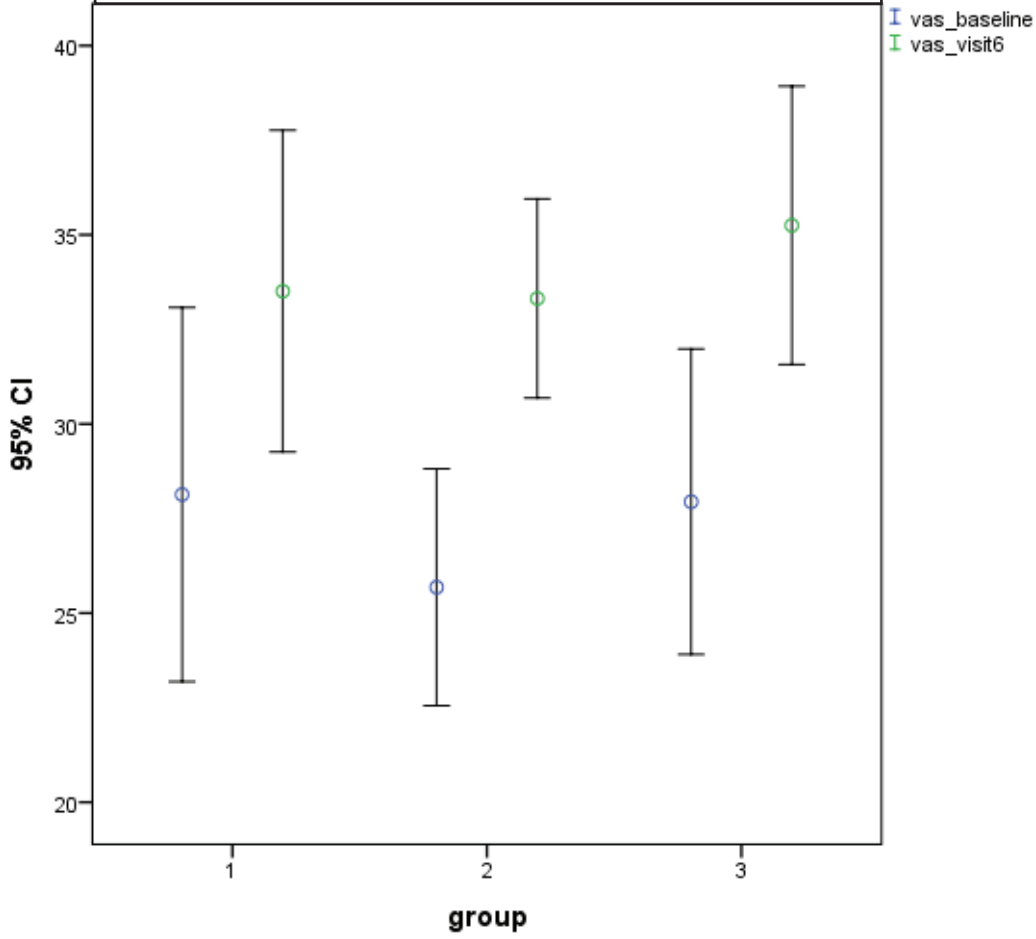


FIGURE 36: Error Plot showing Visual analogue scale among 3 groups.



6. ADVERSE DRUG REACTIONS:

Overall, 62/113 (54.8%) patients had intervention related side effects. The most common adverse reaction observed in all three groups was dryness and scaling, which occurred in 8 out of 33 (24.2%) patients in group A, 19 out of 43 (44.2%) patients in group B, and 17 out of 37 (44.9%) patients in group C. Chi-square was used for intragroup group analysis, and all three groups were comparable with a p value of >0.05.

Itching was reported as the second most common adverse event in 7/33 (21.2%), 14/43 (32.6%), and 16/37 (43.2%) patients in groups A, B, and C, respectively.

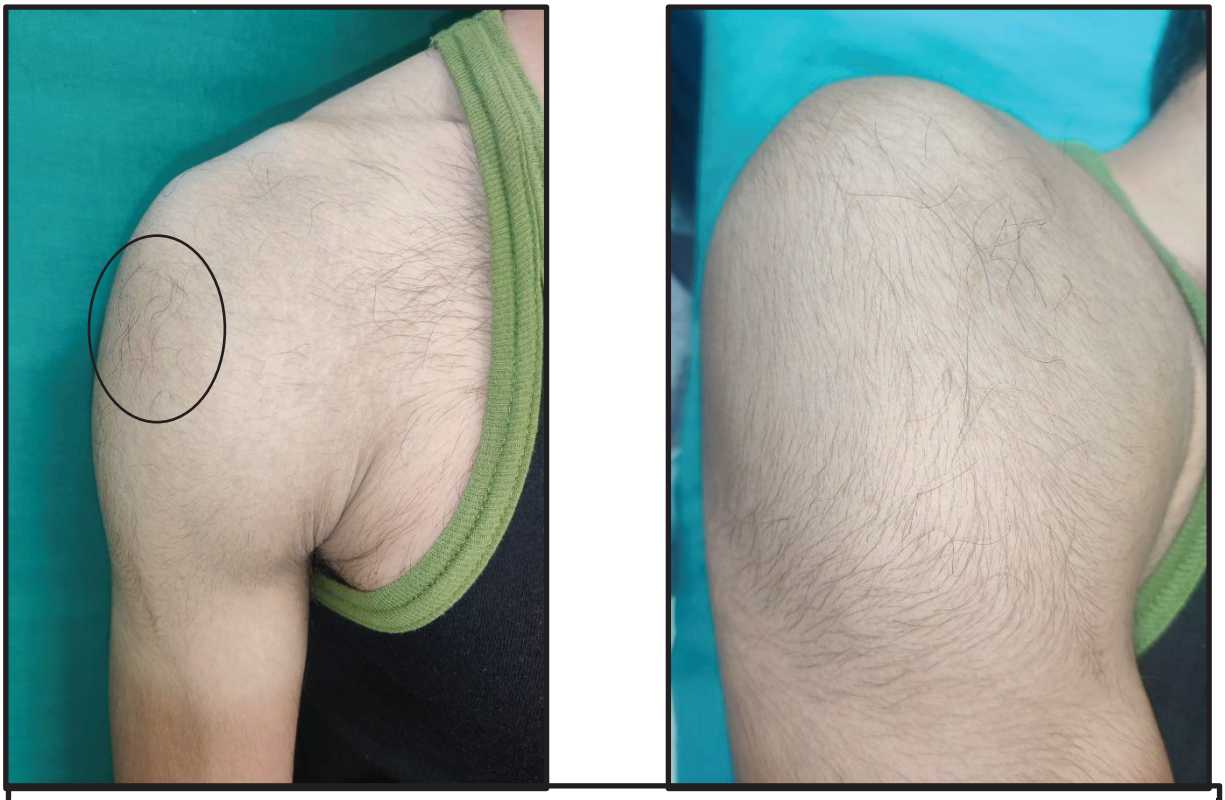
Other common side effects included headache followed by burning sensation on the scalp.

5 of 113 people reported papules and pustules on their scalp and forehead, with 3 in group B and 2 in group C. Out of 113 patients, 1 had hypertrichosis (Figure 36), 1 had periorbital itching and edema. All adverse effects were rated mild in severity, and there were no differences between treatment groups. There were neither serious adverse events nor sexual problems reported in both groups. This is shown in table 17.

TABLE 17: Adverse effects (according to PP):

	GROUP A N=33	GROUP B N=43	GROUP C N=37	TOTAL N=113	p* value
Dryness Or Scaling	8 (24.2%)	19 (44.2%)	17 (45.9%)	44 (38.9%)	0.119 [b]
Itching	7 (21.2%)	14 (32.6%)	16 (43.2%)	37 (32.7%)	0.146 [b]
Headache	1 (3.0%)	6 (14%)	4 (10.8%)	11 (9.7%)	0.271[a]
Stinging Or burning	1 (3.0%)	1 (2.3%)	2 (5.4%)	4 (3.5%)	0.829 [a]
Papules And Pustules Over Scalp and Forehead	0	3 (7%)	2 (5.4%)	5 (4.4%)	-
Hypertrichosis	0	1 (2.3%)	0	1 (0.8%)	-
Periorbital Itching and Edema	1 (3.0%)	0	0	1(0.8%)	-
Palpitations/Chest Pain/Tachycardia	0	0	0	0	-
	p*: a: Fisher's Exact test Freeman Halton extension; b: Chi Square test				

Figure 37



Hypertrichosis over the shoulder noted in patient number 1.

DISCUSSION

DISCUSSION

Based on our selection criteria 167 consenting and consecutive males with androgenetic alopecia were recruited from Dermatology, Venereology and Leprology OPD at AIIMS, Jodhpur after ethical clearance from May 2021 to April 2022. The patients were randomly distributed into three groups namely Group A, group B and Group C. For 6 months, 54 patients were given topical minoxidil 5% (1 ml local application twice a day) with oral placebo (group A), 57 patients were given topical minoxidil 5% plus finasteride 0.10% (1 ml local application twice a day) with oral placebo (group B), and 56 patients were given topical minoxidil 5% (1 ml local application twice a day) with oral biotin 5 mg (group C). Six months of follow-up was completed by 33 patients in Group A, 43 patients in Group B, and 37 patients in Group C.

The patients in this study were most commonly in the age group of 21- 30 years which includes 128/167 (77%) patients. The second most common age group was 18-20 years with 25/167(15%) patients. The mean age in this study was 24.55 years which was in agreement with Khandpur et al[41] (24.86 years), Hajheydari et al[30] (22.80 years). Androgenetic alopecia is most common after puberty, and the majority of patients who presented for treatment were students and young people who are extremely sensitive to cosmetic disfigurement. Total duration of illness in our study ranged from 1 month to 20 years with a mean of 2.26 years (27 months) which was in agreement with Hajheydari et al[30] with mean hair loss duration of 23.10 months.

Family history of AGA was present in 62% of recruited patients, which agrees with Khandpur et al[41] (68%), Shah et al[42] (54%) and Paik et al[43] (48.5%) and is much lower than the 81.60% positivity in a study done by Hajheydari et al[30] A positive family history predisposes to the early onset and rapid progression of male-pattern baldness. More patients with a positive family history suggest a role for genetics in the pathogenesis of AGA. AGA was previously thought to be an autosomal dominant condition, but the presence of many second-degree relatives with AGA suggests a complex polygenic inheritance.

Past history of treatment for androgenetic alopecia was found in 19% of our patients, indicating that majority of our patients were treatment naïve. Patients with Hamilton Norwood grades I through IV was included in this study. In this study, the most common grade of AGA at presentation was grade II, which was found in 67/167 (40%) patients, followed by grade III vertex, which was found in 58/167 (35%). This was in line with the findings of Krupashankar et al[44], who found that grade 2 was the most common grade in

27.27% of patients. This was in contrast to Shah et al[42], who found that grade III was more common in 50% of patients. Based on this data, it can be assumed that the majority of patients recruited for this study are young, presenting early in their disease course, as hair loss has a significant impact on the self-esteem of young patients. p value was calculated using one-way ANOVA to compare three groups, and all three groups were compared in terms of baseline parameters with a non-significant p value (>0.05).

The use of oral supplements for hair loss is common, but the evidence to support this practise is limited. Despite the fact that most randomised clinical trials show no clear benefits, vitamins and minerals are among the most popular supplements taken by patients. Biotin is one of the most commonly prescribed nutritional supplements in patients suffering from hair loss, but evidence to support this practise is limited. This study was conducted to compare the efficacy of oral biotin supplementation in male patients with androgenetic alopecia, so as to fill the lack of knowledge about the same. Literature is available on many multivitamin combination products or herbal preparations showing improvement in male and female AGA, however there is insufficient data to support the use of specific micronutrient supplements or oral biotin supplements.

Post intervention clinical assessment of the patients showed that over the course of 6 months, there was improvement across all 3 groups. Global photographic assessment using a 7-point scale showed that 45.94% (17/37) of patients in group C, i:e, topical minoxidil 5% (1 ml LA BD) plus oral biotin 5 mg, improved, compared with 30.3% and 44.1% of patients in groups A and B, respectively. Topical minoxidil plus finasteride and topical minoxidil with oral biotin improved more than topical minoxidil alone (30.3% versus 45.9%), but the difference was not statistically significant. Chi-square test was used to compare global photographic assessment in the three groups. The p-value was 0.13, which is insignificant. This is consistent with the results of Suchonwanit et al[6], who found that topical minoxidil (3%) combined with finasteride (0.25%) produced 93.3% improvement compared with 85.7% with topical minoxidil (3%) alone, although the difference was not statistically significant. Tanglertsampan et al[31] found that minoxidil (3%) with finasteride (0.1%) had significantly higher efficacy than minoxidil (3%) by global photographic assessment (p = 0.003), and Sheikh et al[34] found that minoxidil (5%) with finasteride (0.10%) group had better improvement than minoxidil (5%) group (89% vs. 60%) with a significant p value (0.05).

In our study, improvement from baseline was noted for all three groups as per investigator global assessment, which was 6.9% in group B, followed by 6.7% in each of groups A and C.

In our study, we calculated target area hair density (TAHC), or the number of hairs per square centimetre, over the frontal and vertex scalps at baseline and subsequent visits, and we compared the baseline parameter with the parameter after 6 months. Intragroup analysis revealed a significant improvement in TAHC across both the frontal and vertex scalps at 6 months compared to baseline in all three groups ($p < 0.05$). Over frontal scalp mean change in TAHC from baseline was maximum for group C i.e. topical minoxidil (5%) with oral biotin followed by group B and group A (18.1 Versus 16.3 and 14.3). Similarly, over vertex maximum increase in TAHC was observed for group C followed by group A and B (18.9 versus 17.5 and 14). In our study mean change in hair count per square centimetre of scalp was maximum for group Cover both frontal and vertex scalp. Intergroup analysis showed no statistically significant difference between 3 groups in terms of increase in TAHC ($p>0.05$). This finding was in agreement with Suchonwanit et al[6] found a similar increase in TAHC after 6 months of treatment, more in the topical minoxidil with finasteride group than in the minoxidil alone group, but the difference was not statistically significant. Tanglertsampan et al[31] found that at week 24, hair counts increased from baseline in both the topical minoxidil plus finasteride and the minoxidil alone groups, but the difference was statistically significant only in the combination group ($p = 0.044$), with no statistical difference between the two groups in terms of change in hair counts at 24 weeks from baseline ($p = 0.503$).

In our study, we calculated target area hair width (TAHW) over the frontal and vertex scalps at baseline and subsequent visits, and we compared the baseline parameter to the parameter after 6 months. In all three groups, intragroup analysis revealed a significant improvement in TAHW across both the frontal and vertex scalps at 6 months compared to baseline ($p < 0.05$). Group B had the greatest change in mean hair thickness over the frontal scalp (9.7 micrometre), followed by Group A (5.9 micrometre), and Group C (4.7 micrometre). Group A, however, had the greatest change in mean hair thickness over the vertex scalp (7.9 micrometre), followed by Group B (4.3 micrometre), and Group C (4.2 micrometre). Group C showed minimum increment in TAHW over both frontal and vertex scalp when compared with group A and B. Intergroup analysis showed no statistically significant difference between 3 groups in terms of increase in TAHW ($p>0.05$). Our study evaluated both the frontal and vertex scalps, which was not the case in previous AGA studies, which only examined the vertex scalp. This finding was in disagreement with Suchonwanit et al[6] where increased hair diameter was observed in both topical minoxidil plus finasteride and topical minoxidil alone group but the difference was significantly in favour of combination group after 24 weeks of treatment ($p=0.02$).

Subjective improvement as assessed by the visual analogue scale (VAS) showed improvement in all three groups, with Group B (topical minoxidil with finasteride plus oral placebo) showing the greatest increase in score (7.6), followed by Group C (7.3) and Group A (5.4), but no significant difference between the three groups was found in the intergroup analysis (p=0.37). In our study the increase in score was maximum for group B but it was comparable with group C. This is consistent with the results of Suchonwanit et al[6] where 93.3% of participants in the FMX group and 92.9% of patients in the MX group reported improvement in their condition via self-evaluation on a seven-point rating scale. Nevertheless, there was no statistical significance between the two treatment groups.

In our study, the most common adverse drug reaction was dryness and scaling, which was observed in 44/113 patients, followed by pruritus, which was observed in 37/114 patients. Other common side effects included headache followed by burning sensation on the scalp.

5 of 113 people reported papules and pustules on their scalp and forehead. Out of 113 patients, 1 had hypertrichosis, 1 had periorbital itching and edema. No serious adverse reactions or sexual problems were reported. No of adverse reaction were not significantly different between groups. According to Suchonwanit et al,[6] the most common adverse effects were itching, followed by irritation. Local irritation in the form of itching, pruritus, and scaling of the scalp were the most common adverse effects observed in a study by Olsen et al.[8] Ghonemy et al[9] found that all cases of minoxidil 10% had irritation, while 22% of patients on minoxidil 5% had irritation. Minoxidil 10% caused more irritation than the other groups. This is confirmed by Olsen et al[8] when the investigators found symptoms and signs of contact dermatitis and severe scalp symptoms were more common with 5% topical minoxidil than with 2% topical minoxidil, suggesting that this was dose-dependent. They suggested that this was a function of the vehicle, increasing the percentage of propylene glycol in the formulation rather than the concentration of minoxidil in the formulation.

Biotin deficiency is uncommon because intestinal bacteria typically produce adequate levels of biotin.[13] In the absence of a deficiency, no clinical trials have demonstrated efficacy in treating hair loss with biotin supplementation. Despite this, biotin can be found in a variety of hair loss supplements marketed to consumers. This marketing strategy could have been chosen because biotin has been shown to be effective in the treatment of brittle fingernails and onychoschizia.[45] Recommended daily allowance of biotin for adults is 30 microgram per day but majority of preparations proposed for hair loss has 5 or 10 mg. Biotin plasma concentrations typically range from 400 to 1200 picogram per millilitre, with less than 200 picogram per millilitre considered deficient. In our study, the mean baseline total serum

biotin value was 17.43±33.02 picogram per millilitre which was deficient. This was in agreement with a study done by El-Esawy et al[13] where it was found that serum biotin was at the suboptimal level compared to controls (Mean ± SD = 339.4 ± 12.125, 532.82 ± 35.224, respectively; p = 0.01) and showed non-significant correlations with patients' age, BMI, disease duration, and severity. Oral biotin supplementation had no effect on increasing hair density or diameter in our study participants. This finding was in disagreement with Patel et al[32] where 18 case reports of biotin relation with hair and nail growth were analysed and it was found that patients improved clinically after receiving biotin in all cases, and three reported cases of uncombable hair syndrome showed improvement in hair quality after a few months of biotin treatment. Prager et al[46] reported 60% improvement in male androgenetic patients after treatment with an oral combination containing not only biotin but also niacin, ß-sitosterol and saw palmetto extract.

So, to conclude, the intra-group analysis at the end of 6 months showed a significant increase ($p<0.05$) in TAHC, TAHW and VAS score in all 3 groups. This corroborates the clinical experience that the 6-month application of topical minoxidil is an adequate period to achieve significant changes. However, all three groups were comparable in terms of change from baseline in Global photographic assessment (using 7-point scale and percentage improvement), TAHC, TAHW, VAS score at the end of 6 month with a non-significant p value ($p>0.05$). Hair shaft thickness heterogeneity followed by brown peripilar sign were the most common dermoscopic parameter present in recruited patients. An increased occurrence of Dryness and Scaling was noted in all three groups.

Of the 167 patients recruited for the current study, 54 subjects were lost to follow-up. The cost of treatment, the need for lifelong therapy, slow response to treatment, and unrealistic expectations were the reasons for study discontinuation. The topical preparation used in this study was alcohol-based because it was readily available and cheaper, which possibly may have led to adverse effects and eventual discontinuation of follow-up. This study was initiated during the COVID 19 pandemic, which unfortunately resulted in numerous losses to follow-up.

Limitations of the study included the small sample size and the fact that financial constraints made it impossible to recruit controls for serum biotin calculation. Dermoscopic parameters such as TAHC and TAHW were calculated manually in absence of a readily available photographic image analysis software, and this could have resulted in less accurate readings.

CONCLUSION

CONCLUSION

This study was an interventional, prospective, three arm randomized controlled trial, recruited patients from the Department of Dermatology, Venereology and Leprology OPD, AIIMS Jodhpur. The study included 167 male patients with a clinical diagnosis of androgenetic alopecia of age ranging from 18-50 years with Hamilton Norwood grade I to IV. They were randomly divided into three groups using research randomization software as follows: Group A (topical minoxidil 5% 1 ml twice a day with oral placebo i;e. vitamin D 250 IU plus calcium 500 mg), group B (topical minoxidil 5%+ finasteride 0.10% 1 ml twice a day with oral placebo), group C (topical minoxidil 5% 1 ml twice a day with oral Biotin 5 mg) with 54, 57 and 56 patients in groups respectively. All patients were followed monthly for a total of 6 months.

Overall, the mean age of patients in our study was 24.55 ± 4.32 years (18-46 years) and the mean duration of illness was 2.26 ± 2.27 years (1 month -20 years). Past history of treatment for androgenetic alopecia was present in 31/167 (19%) patients and positive family history for androgenetic alopecia was found in 103/167 (62%) patients. All groups were comparable ($p>0.05$) in terms of demography and basic clinical data.

Overall, grade II 67/167 (40%), followed by grade III vertex 58/167 (35%), was the most common pattern of AGA detected. Separately, grade II was the most common pattern in group A (M+P), and grade III vertex was the most common pattern in groups B (MF+P) and C (M+B).

In our study, serum biotin was calculated for all recruited patients using a biotin ELISA kit. The mean baseline total serum biotin value was 17.43±33.02 pg/ml, while the mean values in groups A, B, and C were 13.9, 28.1, and 20.3, respectively, with a non-significant p value of 0.201.

Out of 167 recruited patients, 113 completed 6 months of follow-up, with 33 in group A, 43 in group B, and 37 in group C completing follow-up. In total, 54 patients dropped out of follow up for various reasons.

The Global photographic assessment was stratified into 3 groups, i.e. worsening (-3 to -1), no change (0), and improvement (+1 to +3). A blinded dermatologist rated 17/37 (45.94%) of Group C, 19/43 (44.1%) of Group B, and 10/33 (30.3%) of Group A treated patients as improved after 24 weeks of treatment (score +1, +2, +3). In our study, improvement from

baseline was noted for all three groups, which was 6.9% in group B, followed by 6.7% in each of groups A and C.

In our study dermoscopy was done over both frontal and vertex scalp (12 and 24 centimetres from glabella) to look for Target area hair count (TAHC) i.e. number of hairs per square centimetre, Target area hair diameter (TAHW) and baseline parameters were compared with the parameters after 6 months of treatment.

Hair shaft thickness heterogeneity was the most common dermoscopic parameter present in all 3 groups over both frontal and vertex scalp followed by brown peripilar sign. HSTH was observed in 87.6% and 67.2% of patients, respectively, over the frontal and vertex scalps. Brown Peripilar sign was observed in 33.6% of patients over the frontal scalp and 21.2% of patients over the vertex scalp. White peripilar sign was observed in 15% of patients over the frontal scalp and 10.6% of patients over the vertex scalp. Scalp honey coomb pigmentation was observed in 7% of patients over the frontal and vertex scalp each.

In our study, TAHC rises from baseline in all three groups, as shown below: over frontal scalp changes in mean TAHC from baseline were 18.1, 16.3, and 14.3, for groups C, B, and A, respectively. Similarly, over vertex mean TAHC increments were 18.9, 17.5, and 14 for groups C, A, and B respectively.

When TAHW was examined over the frontal and vertex scalps, it showed an increase in all three groups when compared to the baseline. The changes in mean hair thickness over the frontal scalp were maximum for group B (9.7) followed by group A (5.9) and group C (4.7). Over the vertex area, group A had the greatest change in mean hair thickness (7.9), followed by Group B (4.3) and Group C (4.2).

Subjective improvement was assessed using a visual analogue scale in our study, and it showed improvement across all three groups. Group B had the greatest mean change in VAS from baseline, followed by groups C and A, with differences of 7.6, 7.3, and 5.4 points, respectively.

Overall, 54.8% patients had intervention related side effects. The most common adverse reaction observed in all three groups was dryness and scaling, which occurred in 24.2% patients in group A, 44.2% patients in group B, and 44.9% patients in group C. Itching was reported as the second most common adverse event in 21.2%, 32.6%, and 43.2% patients in groups A, B, and C, respectively. Other common side effects included headache followed by burning sensation on the scalp. 5 of 113 people reported papules and pustules on their scalp

and forehead, with 3 in group B and 2 in group C. Out of 113 patients, 1 had hypertrichosis, 1 had periorbital and edema. All adverse effects were rated mild in severity, and there were no differences between treatment groups. There were neither serious adverse events nor sexual problems reported in both groups.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Blume-Peytavi U, Blumeyer A, Tosti A, Finner A, Marmol V, Trakatelli M et al. S1 guideline for diagnostic evaluation in androgenetic alopecia in men, women and adolescents. *Br J Dermatol*. 2011;164(1):5-15.
2. Cash TF. The psychological effects of androgenetic alopecia in men. *J Am Acad Dermatol*. 1992;26(6):926-31.
3. Dhurat RS, Daruwalla SB. Androgenetic alopecia: Update on etiology. *Dermatological Reviews* 2021 Jun;2(3):115-21.
4. Gupta M, Mysore V. Classifications of Patterned Hair Loss: A Review. *J Cutan Aesthet Surg*. 2016;9(1):3-12.
5. Norwood OT. Male pattern baldness: classification and incidence. *South Med J*. 1975;68(11):1359-65.
6. Suchonwanit P, Srisuwanwattana P, Chalermroj N, Khunkhet S. A randomized, double-blind controlled study of the efficacy and safety of topical solution of 0.25% finasteride admixed with 3% minoxidil vs. 3% minoxidil solution in the treatment of male androgenetic alopecia. *J Eur Acad Dermatol Venereol*. 2018;32(12):2257-63.
7. Rossi A, Cantisani C, Melis L, Iorio A, Scali E, Calvieri S. Minoxidil use in dermatology, side effects and recent patents. *Recent Pat Inflamm Allergy Drug Discov*. 2012;6(2):130-6.
8. Olsen EA, Dunlap FE, Funicella T, Koperski JA, Swinehart JM, Tschen EH et al. A randomized clinical trial of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of androgenetic alopecia in men. *J Am Acad Dermatol*. 2002;47(3):377-85.
9. Ghonemy S, Alarawi A, Bessar H. Efficacy and safety of a new 10% topical minoxidil versus 5% topical minoxidil and placebo in the treatment of male androgenetic alopecia: a trichoscopic evaluation. *J Dermatolog Treat*. 2021;32(2):236-41.
10. Lee SW, Juhasz M, Mobasher P, Ekelem C, Mesinkovska NA. A Systematic Review of Topical Finasteride in the Treatment of Androgenetic Alopecia in Men and Women. *J Drugs Dermatol*. 2018;17(4):457-63.
11. Caserini M, Radicioni M, Leuratti C, Terragni E, Iorizzo M, Palmieri R. Effects of a novel finasteride 0.25% topical solution on scalp and serum dihydrotestosterone in healthy men with androgenetic alopecia. *Int J Clin Pharmacol Ther*. 2016;54(1):19-27.

12. Piraccini BM, Blume-Peytavi U, Scarci F, Jansat JM, Falqués M, Otero R et al. Efficacy and safety of topical finasteride spray solution for male androgenetic alopecia: a phase III, randomized, controlled clinical trial [published correction appears in J Eur Acad Dermatol Venereol. 2022 Dec 1;]. *J Eur Acad Dermatol Venereol*. 2022;36(2):286-94.
13. El-Esawy FM, Hussein MS, Ibrahim Mansour A. Serum biotin and zinc in male androgenetic alopecia. *J Cosmet Dermatol*. 2019;18(5):1546-9.
14. Buffoli B, Rinaldi F, Labanca M, Sorbellini E, Trink A, Guanziroli E et al. The human hair: from anatomy to physiology. *Int J Dermatol*. 2014;53(3):331-41.
15. Lee WS, Lee HJ, Choi GS, Cheong WK, Chow SK, Gabriel MT et al. Guidelines for management of androgenetic alopecia based on BASP classification--the Asian Consensus Committee guideline. *J Eur Acad Dermatol Venereol*. 2013;27(8):1026-34.
16. Mysore V, Parthasaradhi A, Kharkar RD, Ghoshal AK, Ganjoo A, Ravichandran G et al. Expert consensus on the management of Androgenetic Alopecia in India. *Int J Trichology*. 2019;11(3):101-6.
17. Cotton SG, Nixon JM, Carpenter RG, Evans DW. Factors discriminating men with coronary heart disease from healthy controls. *Br Heart J*. 1972;34(5):458-64.
18. Lotufo PA, Chae CU, Ajani UA, Hennekens CH, Manson JE. Male pattern baldness and coronary heart disease: the Physicians' Health Study. *Arch Intern Med*. 2000;160(2):165-71.
19. Oh BR, Kim SJ, Moon JD, Kim HN, Kwon DD, Won YH et al. Association of benign prostatic hyperplasia with male pattern baldness. *Urology*. 1998;51(5):744-8.
20. Hawk E, Breslow RA, Graubard BI. Male pattern baldness and clinical prostate cancer in the epidemiologic follow-up of the first National Health and Nutrition Examination Survey. *Cancer Epidemiol Biomarkers Prev*. 2000;9(5):523-7.
21. Vora RV, Kota RKSK, Singhal RR, Anjaneyan G. Clinical Profile of Androgenic Alopecia and Its Association with Cardiovascular Risk Factors. *Indian J Dermatol*. 2019;64(1):19-22.
22. Gordon KA, Tosti A. Alopecia: evaluation and treatment. *Clin Cosmet Investig Dermatol*. 2011;4:101-6.
23. Hu R, Xu F, Han Y, Sheng Y, Qi S, Miao Y et al. Trichoscopic findings of androgenetic alopecia and their association with disease severity. *J Dermatol*. 2015;42(6):602-7.

24. Vidal CI. Overview of Alopecia: A Dermatopathologist's Perspective. *Mo Med*. 2015;112(4):308-12.
25. Whiting DA. Diagnostic and predictive value of horizontal sections of scalp biopsy specimens in male pattern androgenetic alopecia. *J Am Acad Dermatol*. 1993;28(5 Pt 1):755-63.
26. Nestor MS, Ablon G, Gade A, Han H, Fischer DL. Treatment options for androgenetic alopecia: Efficacy, side effects, compliance, financial considerations, and ethics. *J Cosmet Dermatol*. 2021;20(12):3759-81.
27. Suchonwanit P, Thammarucha S, Leerunyakul K. Minoxidil and its use in hair disorders: a review. *Drug Des Devel Ther*. 2019;13:2777-86.
28. Mazzarella GF, Loconsole GF, Cammisa GA, Mastrolonardo GM, Vena GA. Topical finasteride in the treatment of androgenic alopecia. Preliminary evaluations after a 16-month therapy course. *J. Dermatol. Treat* 1;8(3):189-92.
29. Suchonwanit P, Iamsumang W, Leerunyakul K. Topical finasteride for the treatment of male androgenetic alopecia and female pattern hair loss: a review of the current literature. *J Dermatolog Treat*. 2022;33(2):643-8.
30. Hajheydari Z, Akbari J, Saeedi M, Shokoohi L. Comparing the therapeutic effects of finasteride gel and tablet in treatment of the androgenetic alopecia. *Indian J Dermatol Venereol Leprol*. 2009;75(1):47-51.
31. Tanglertsampan C. Efficacy and safety of 3% minoxidil versus combined 3% minoxidil / 0.1% finasteride in male pattern hair loss: a randomized, double-blind, comparative study. *J Med Assoc Thai*. 2012;95(10):1312-6.
32. Patel DP, Swink SM, Castelo-Soccio L. A Review of the Use of Biotin for Hair Loss. *Skin Appendage Disord*. 2017;3(3):166-9.
33. Chandrashekar BS, Nandhini T, Vasanth V, Sriram R, Navale S. Topical minoxidil fortified with finasteride: An account of maintenance of hair density after replacing oral finasteride. *Indian Dermatol Online J*. 2015;6(1):17-20.
34. Sheikh S, Ahmad A, Ali SM, Ahmad MU, Paithankar M, Saptarshi D et al. A new topical formulation of minoxidil and finasteride improves hair growth in men with androgenetic alopecia. *Journal of Clinical & Experimental Dermatology Research*. 2015;6(1):1-6.
35. Rai PB, Khushwaha P, Jain N, Gupta S. Comparing the therapeutic efficacy of topical minoxidil and finasteride with topical minoxidil and oral finasteride in androgenetic alopecia: a randomized trial. *Int J Res Dermatol*. 2018;4(03):386-90.

36. Panchaprateep R, Lueangarun S. Efficacy and Safety of Oral Minoxidil 5 mg Once Daily in the Treatment of Male Patients with Androgenetic Alopecia: An Open-Label and Global Photographic Assessment. *Dermatol Ther*. 2020;10(6):1345-57.
37. Trüeb RM. Serum Biotin Levels in Women Complaining of Hair Loss. *Int J Trichology*. 2016;8(2):73-7.
38. Zubair Z, Kantamaneni K, Jalla K, Renzu M, Jena R, Jain R et al. Prevalence of Low Serum Vitamin D Levels in Patients Presenting with Androgenetic Alopecia: A Review. *Cureus*. 2021;13(12):e20431.
39. Gupta N. Clinical Evaluation of Intradermal Platelet Rich Plasma Administration along with Oral Biotin Supplement for the Management of Androgenetic Alopecia in Adult Males: A Randomised Clinical Trial. *Journal of Clinical & Diagnostic Research*. 2022;16(11).
40. Birnbaum MR, McLellan BN, Shapiro J, Ye K, Reid SD. Evaluation of Hair Density in Different Ethnicities in a Healthy American Population Using Quantitative Trichoscopic Analysis. *Skin Appendage Disord*. 2018;4(4):304-7.
41. Khandpur S, Suman M, Reddy BS. Comparative efficacy of various treatment regimens for androgenetic alopecia in men. *J Dermatol*. 2002;29(8):489-98.
42. Shah KB, Shah AN, Solanki RB, Raval RC. A Comparative Study of Microneedling with Platelet-rich Plasma Plus Topical Minoxidil (5%) and Topical Minoxidil (5%) Alone in Androgenetic Alopecia. *Int J Trichology*. 2017;9(1):14-8.
43. Paik JH, Yoon JB, Sim WY, Kim BS, Kim NI. The prevalence and types of androgenetic alopecia in Korean men and women. *Br J Dermatol*. 2001;145(1):95-9.
44. Krupa Shankar D, Chakravarthi M, Shilpakar R. Male androgenetic alopecia: population-based study in 1,005 subjects. *Int J Trichology*. 2009;1(2):131-3.
45. Guo EL, Katta R. Diet and hair loss: effects of nutrient deficiency and supplement use. *Dermatol Pract Concept*. 2017;7(1):1-10.
46. Prager N, Bickett K, French N, Marcovici G. A randomized, double-blind, placebo-controlled trial to determine the effectiveness of botanically derived inhibitors of 5-alpha-reductase in the treatment of androgenetic alopecia. *J Altern Complement Med N Y N*. 2002;8(2):143-52.

ANNEXURES

ANNEXURES

ANNEXURE I

INSTITUTIONAL ETHICS COMMITTEE CERTIFICATE

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2021/ 3456 **Date: 12/03/2021**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3291**

Project title: **"A three arm open label randomized control trial comparing minoxidil 5% solution and oral biotin VERSUS minoxidil 5% + finasteride 0.1% and oral placebo VERSUS minoxidil 5% and oral placebo in the management of male androgenic alopecia"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr. Yamini Sihag**
Guide:	**Dr. Saurabh Singh**
Co-Guide:	**Dr. Abhishek Bhardwaj, Dr. Anil Budania, Dr. Anupama Bains, Dr. Suman Patra & Dr. Shrimanjunath Sankanagoudar**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.
- In case of any issue related to compensation, the responsibility lies with the Investigator and Co-Investigators.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation. If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary
Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Basni Phase-2, Jodhpur, Rajasthan-342005; **Website:** www.aiimsjodhpur.edu.in; **Phone:** 0291-2740741 Extn. 3109
E-mail : ethicscommittee@aiimsjodhpur.edu.in; ethicscommitteeaiimsjdh@gmail.com

ANNEXURE II

CASE SHEET PROFORMA

"A THREE ARM OPEN LABEL RANDOMIZED CONTROLLED TRIAL COMPARING MINOXIDIL 5% SOLUTION AND ORAL BIOTIN VERSUS MINOXIDIL 5% + FINASTERIDE 0.1% AND ORAL PLACEBO VERSUS MINOXIDIL 5% AND ORAL PLACEBO IN THE MANAGEMENT OF MALE ANDROGENIC ALOPECIA"

Name: **ID:**

Age / Sex: **Address:**

1. Education status: : Illiterate Primary school Middle school

High school Intermediate Graduate

Professional

2. Occupation:

3. Marital status: Unmarried Married

4. Age of onset:

5. Total duration of illness:

6. H/O receding of hair line: Yes/No **Details:**

7. Pattern of hair loss: **specify pattern :**

8. H/O loss of hair from vertex: Yes/No **Details:**

9. H/O loss of hairs from occiput: Yes/No **Details:**

10. H/O scaling over scalp: Yes/No

Itching over scalp: Yes/No

- **Pain over scalp: Yes/No**
- **. H/O severe/uncontrolled scalp diseases- seborrheic dermatitis, scalp infection or scalp psoriasis.**

Yes/No **If,yes→Details:**

- **. H/O any other cutaneous illness: Yes/No** **If,yes→Details:**

11. H/O surgery/marked weight loss/extreme dieting/a stressful or major life event: Yes/No

If, yes→ Specify: Duration: Treatment taken:

12. H/O diabetes/ hypertension/ tuberculosis/ thyroid illness: Yes/No

If, yes→ Specify: Duration: Treatment taken:

13. H/O smoking/ Alcohol: Yes/No If, yes→ Duration:

15.Family history:

16. H/O cancer or chemotherapy taken: Yes/No If,yes→Details:

17. **Treatment History: H/O topical minoxidil/topical finesteride/oral supplement/oral herbal extract/any OTC medication for hair growth in the past: Yes/No**

If,yes→Details:

TYPE(TOPICAL/SYSTEMIC)	DRUG NAME	TREATMENT DETAILS	DURATION	RESPONSE

Examination:

General Examination:

Pallor-

Icterus-

Cyanosis-

Clubbing-

Lymphadenopathy-

Pedal Oedema-

Height: cm Weight: kg BMI:

Any significant finding:

Cutaneous Examination:

1. Pattern of alopecia: intact frontal hairline/receding frontal hair line/M pattern present/vertex involved

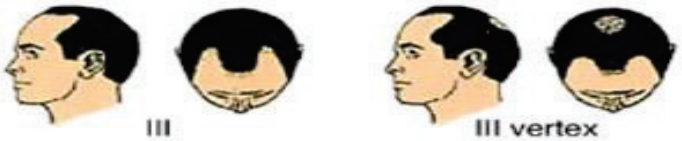
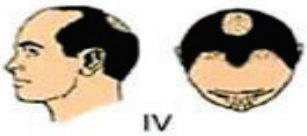
2. Scalp assessment:

- Skin of affected scalp
- Scalp scaling
- Scalp atrophy
- Any papule/pustule/crusts/ulceration/scarring/erythema

3. Examination of skin/nail/mucosa:

Any significant finding: present/absent

4. Norwood-Hamilton grading-

Norwood I shows a normal head of hair with no visible hair loss.	 I
Norwood II shows the hair receding in a wedge-shaped pattern.	 II
Norwood III shows the same receding pattern as Norwood II, except the hairline has receded deeper into the frontal area and the temporal area.	 III III vertex
Type IV on the Norwood Scale indicates a hairline that has receded more dramatically in the frontal region and temporal area. Additionally there is a balding area at the very top center of the head, but there is a bridge of hair remaining between that region and the front.	 IV
Type V on the Norwood Scale shows that very same bridge between the frontal region and the top center, also called the vertex, beginning to thin.	 V
Type VI on the Norwood Scale indicates that the bridge between the frontal region and the vertex has disappeared.	 VI
Type VII on the Norwood Scale shows hair receding all the way back to the base of the head and the sides just above the ears. Norwood patterns are determined genetically.	 VII

5. Dermoscopy : 3 points dermoscopy at baseline and after every 4 weeks

[A]- The subject's hair will be parted in the midline. Sites on the scalp were then measured at 12 from glabella

[B] 24 cm from glabella in midline

[C] 30 cm from glabella in midline

	Baseline	1st visit	2nd visit	3rd visit	4th visit	5thvisit	6th visit
Point A • **HSTH** • **BPPS** • **WPPS** • **Yellow dots** • **Focal atrichia** • **SHCP**							
Point B • **HSTH** • **BPPS** • **WPPS** • **Yellow dots** • **Focal atrichia** • **SHCP**							
Point C • **HSTH** • **BPPS** • **WPPS** • **Yellow dots** • **Focal atrichia** • **SHCP**							

1. **Non vellus target area hair count and non-vellus target area hair width:** point on the receding hair line at a fixed distance from supraorbital foramen

	Baseline	**End of 12 weeks**	**End of 24 weeks**
NON VELLUS TAHC			
NON VELLUS TAHW			

2. Hair pull test: positive/ negative

3. Side effects-

	1st follow up visit	2nd follow up visit	3rd follow up visit	4th follow up visit	5th follow up visit	6th follow up visit
Hypertrichosis						
Headache						
Stinging/burning						
Itching						
Dryness/scaling						
Palpitations/chest pain/hypotension						
Gynaecomastia						
Sexual ADR						
Other						

4. **Visual analogue scale :** based on 100 mm vas scale in which
   0-negative
   50-neutral
   100-positive

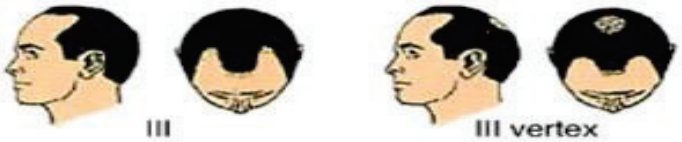
	BASELINE	END OF 12 WEEKS	END OF 24 WEEKS
End points Quality of life Effect of hair loss condition on life Effect of hair loss condition on social life Degree of self-confidence Effect of treatment of hair loss condition on first impressions in social situations Effect of hair loss condition on job Effect of treatment of hair loss condition on first impressions made in job Global benefit Feeling about present hair loss condition Feeling of having control over hair loss† Whether patient's expectations were met Hair styling Description of hair thickness Satisfaction with amount of hair styling products used Satisfaction with amount of time spent daily on styling hair			

5. Laboratory assessment

Markers	Pre-treatment
SERUM BIOTIN	

ANNEXURE III

NORWOOD-HAMILTON GRADING

Norwood I shows a normal head of hair with no visible hair loss.	 I
Norwood II shows the hair receding in a wedge-shaped pattern.	 II
Norwood III shows the same receding pattern as Norwood II, except the hairline has receded deeper into the frontal area and the temporal area.	 III III vertex
Type IV on the Norwood Scale indicates a hairline that has receded more dramatically in the frontal region and temporal area. Additionally there is a balding area at the very top center of the head, but there is a bridge of hair remaining between that region and the front.	 IV
Type V on the Norwood Scale shows that very same bridge between the frontal region and the top center, also called the vertex, beginning to thin.	 V
Type VI on the Norwood Scale indicates that the bridge between the frontal region and the vertex has disappeared.	 VI
Type VII on the Norwood Scale shows hair receding all the way back to the base of the head and the sides just above the ears. Norwood patterns are determined genetically.	 VII

ANNEXURE IV
VISUAL ANALOGUE SCALE

Based on 100 mm vas scale in which
0-negative
50-neutral
100-positive

	BASELINE	END OF 12 WEEKS	END OF 24 WEEKS
End points Quality of life Effect of hair loss condition on life Effect of hair loss condition on social life Degree of self-confidence Effect of treatment of hair loss condition on first impressions in social situations Effect of hair loss condition on job Effect of treatment of hair loss condition on first impressions made in job Global benefit Feeling about present hair loss condition Feeling of having control over hair loss† Whether patient's expectations were met† Hair styling Description of hair thickness Satisfaction with amount of hair styling products used Satisfaction with amount of time spent daily on styling hair			

ANNEXURE V

INFORMED CONSENT FORM (ENGLISH)

ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR

Title of Thesis/Dissertation: "A THREE ARM OPEN LABEL RANDOMIZED CONTROLLED TRIAL COMPARING MINOXIDIL 5% SOLUTION AND ORAL BIOTIN VERSUS MINOXIDIL 5% + FINASTERIDE 0.1% AND ORAL PLACEBO VERSUS MINOXIDIL 5% AND ORAL PLACEBO IN THE MANAGEMENT OF MALE ANDROGENIC ALOPECIA"

Name of PG Student: **Dr. Yamini Sihag** Mobile No. **9557108612**

Patient/ Volunteer Identification No.: _____

I, _____ S/o or D/o _____

R/o _____

give my full, free, voluntary consent to be a part of the study ""A three arm open label randomized controlled trial comparing minoxidil 5% solution and oral biotin versus minoxidil 5% + finasteride 0.1% and oral placebo versus minoxidil 5% and oral placebo in the management of male Androgenic Alopecia"

The procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary and I am aware of my right to opt out of the study at any time without giving any reason.

I understand that the information collected about me and any of my medical records may be looked at by responsible individuals or from regulatory authorities. I give permission for these individuals to have access to my records.

Date: _____ _____

Place: _____ Signature/Left thumb impression

(If minor, Parent/Guardian signature)

This to certify that the above consent has been obtained in my presence.

Date: _____ _____

Place: _____ Signature of PG Student

1. Witness 1

   _____
   Signature
   Name: _____
   Address: _____

2. Witness 2

   _____
   Signature
   Name: _____
   Address: _____

ANNEXURE VI

INFORMED CONSENT FORM (HINDI)

अखिल भारतीय आयुर्विज्ञान विज्ञान संस्थान

जोधपुर, राजस्थान

सूचित सहमति पत्र

शोध का शीर्षक: : "**मिनोक्सिडिल** 5% **घोल प्लस बायोटिन बनाम मिनोक्सिडिल** 5% **घोल और** 0.10% **फिनास्टेराइड प्लस प्लेसबो की तुलना में, मिनोक्सिडिल** 5% **घोल के साथ प्लेसबो, एंड्रोजेनिक खालित्य के साथ पुरुष रोगी में बाल विकास पर -एक खुला लेबल यादृच्छिक नियंत्रण परीक्षण** "

पीजी छात्र का नाम:_____

मोबाइल नंबर:

रोगी / स्वयं सेवी पहचान संख्या: _____

मैं,_____**पुत्र/पुत्री**_____

_____**का निवासी**_____

उपरोक्त अध्ययन का एक हिस्सा बनने के लिए मेरी पूर्ण, स्वतंत्र, स्वैच्छिक सहमति देता हूँ।

जिस प्रक्रिया और प्रकृति को मुझे अपनी पूरी संतुष्टि के लिए अपनी भाषा में समझाया गया है मैं पुष्टि करता हूं कि मुझे प्रश्न पूछने का अवसर मिला है।

मैं समझता हूं कि मेरी भागीदारी स्वैच्छिक है और मुझे किसीभी कारण दिए बिना किसी भी समय अध्ययन से बाहर निकलने का मेरा अधिकार है।

मैं समझता हूं कि मेरे और मेरे मेडिकल रिकॉर्ड के बारे में एकत्रित की गई जानकारी को या विनियामक प्राधिकरणों से जिम्मेदार व्यक्ति द्वारा देखा जा सकता है।मैं इन लोगों के लिए मेरे रिकॉर्डोंतक पहुंचकी अनुमति देता हूं।

तारीख : _____ _____

जगह: _____ **हस्ताक्षर / बाएं अंगूठे का छाप**

(नाबालिग कि, माता-पिता / अभिभावक हस्ताक्षर)

यह प्रमाणित करने के लिए कि मेरी उपस्थिति में उपरोक्त सहमति प्राप्त की गई है

तारीख : _____ _____

जगह: _____ **पीजी छात्र के हस्ताक्षर**

1. **गवाह** 1 2. **साक्षी** 2

_____ _____

हस्ताक्षर **हस्ताक्षर**

नाम:_____ **नाम**: _____

पता: _____ **पता**: _____

ANNEXURE VII

PATIENT INFORMATION SHEET (ENGLISH)

ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR

This document has been given to provide more information about male androgenetic alopecia and this research related to treatment of male androgenetic alopecia.

The current research project is titled: **"A three arm open label randomized controlled trial comparing minoxidil 5% solution and oral biotin versus minoxidil 5% + finasteride 0.1% and oral placebo versus minoxidil 5% and oral placebo in the management of male Androgenic Alopecia"**

Male androgenetic alopecia is the most common type of hair loss in men affecting 50%-60% of male by the age of 50 years. Onset of alopecia may be at any age following puberty and there is an increasing frequency with age. The etiology is considered as multifactorial, involving a complex interplay between several genes, androgens and environmental factors. Hair loss in male androgenetic alopecia usually follow a pattern which is recession of frontal hair line, mainly in a triangular pattern, latter followed by a vertex thinning with progression until the top of scalp is completely bald. Currently approved treatment for male androgenetic alopecia are Minoxidil solution and oral Finasteride but apart from approved treatment oral nutritional supplement in the form of biotin is also commonly prescribed. In this study we try to find the efficacy of oral biotin for hair regrowth in male androgenetic alopecia patient .

At first visit you will be clinically evaluated and will be asked to fill visual analogue scale questionnaire. You will receive either of the treatment option- (A) Minoxidil 5% solution 1 ml BD plus oral biotin 5 mg tablet OD, (B) Minoxidil 5% with Finasteride 0.10% solution 1 ml BD plus oral placebo OD, (C) Minoxidil 5% solution 1 ml BD plus oral placebo OD. You will be called for follow up 4 weekly preferably in physical OPD, but teleconsultation can be done if you are not able to come due to COVID 19 pandemic. Treatment will be given for a total duration of 6 months. In case of treatment failure (no hair growth at the end of 3 months, confirmed by clinical examination/dermoscopy) you will be started on oral Finasteride. All drugs prescribed in this study are relatively safe and side effects will be monitored at each 4 weekly follow up visit.

The following research is done to compare minoxidil 5% solution plus biotin versus minoxidil 5% solution and finasteride 0.10% plus placebo in comparison with minoxidil 5% solution plus placebo on hair growth in male patient with androgenetic alopecia.

You are also informed that all the information given by you will be kept confidential. You also reserve the right that during this research can withdraw the consent & can be out of this research without explaining the reasons.

Principle investigator: Dr.Yamini Sihag Contact number: 9557108612

ANNEXURE VIII

PATIENT INFORMATION SHEET (HINDI)

अखिल भारतीय आयुर्विज्ञान संस्थान जोधपुर, राजस्थान

रोगी सूचना पत्रक (पीआईएस)

यह दस्तावेज़ पुरुष एंड्रोजेनिक खालित्य के बारे में अधिक जानकारी प्रदान करने के लिए दिया गया है और यह शोध पुरुष एंड्रोजेनिक खालित्य के उपचार से संबंधित है।

वर्तमान शोध परियोजना का शीर्षक है: "मिनोक्सिडिल 5% घोल प्लस बायोटिन बनाम मिनोक्सिडिल 5% घोल और 0.10% फिनास्टेराइड प्लस प्लेसबो की तुलना में, मिनोक्सिडिल 5% घोल के साथ प्लेसबो, एंड्रोजेनिक खालित्य के साथ पुरुष रोगी में बाल विकास पर -एक खुला लेबल यादृच्छिक नियंत्रण परीक्षण "

50 वर्ष की आयु तक 50% -60% पुरुषों को प्रभावित करने वाला एंड्रोजेनिक खालित्य बालों के झड़ने का सबसे आम प्रकार है। खालित्य की शुरुआत यौवन के बाद किसी भी उम्र में हो सकती है और उम्र के साथ बढ़ती आवृत्ति है। इसका कारण बहुक्रिया के रूप में माना जाता है, जिसमें कई जीन, एण्ड्रोजन और पर्यावरणीय कारकों के बीच एक जटिल परस्पर क्रिया शामिल है। पुरुष एंड्रोजेनिक खालित्य में बालों का झड़ना आमतौर पर एक पैटर्न का पालन करते हैं जो ललाट के बालों की रेखा की मंदी है, मुख्य रूप से एक त्रिकोणीय पैटर्न में, बाद में शीर्ष के बालों का झड़ना जब तक खोपड़ी का शीर्ष पूरी तरह से गंजा नहीं होता है। वर्तमान में पुरुष एंड्रोजेनिक खालित्य के लिए अनुमोदित उपचार मिनोक्सीडिल घोल और मौखिक फिनास्टेराइड हैं लेकिन बायोटिन के रूप में अनुमोदित उपचार मौखिक पोषण के पूरक के रूप में भी आमतौर पर निर्धारित किया जाता है। इस अध्ययन में हम पुरुष एंड्रोजेनिक खालित्य में बालों की बढ़वार के लिए मौखिक बायोटिन की प्रभावकारिता खोजने की कोशिश करते हैं।

पहली बार में आप का चिकित्सकीय मूल्यांकन किया जाएगा और आप को विज़ुअल एनालॉग स्केल प्रश्नावली भरने के लिए कहा जाएगा। आप को कोई भी एक उपचार मिलेगा- (ए) मिनॉक्सीडिल 5% घोल 1 मिली दिन में दो बार प्लस मौखिक बायोटिन 5 मिग्रा टैबलेट दिन में एक बार, (ब) मिनोक्सिडिल 5% , 0.10% फिनास्टेराइड के साथ घोल 1 मिली दिन में दो बार प्लस मौखिक प्लेसबोदिन में एक बार (सी) मिनोक्सिडिल 5 % घोल 1 मिली दिन में दो बार प्लस प्लेसीबो दिन में एक बार । शारीरिक ओपीडी में अधिमानतः 4 साप्ताहिक का पालन करने के लिए आप को बुलाया जाएगा, लेकिन अगर कोविड 19 महामारी के कारण आप नहीं आ पा रहा है, तो टेलीफोन परामर्श किया जा सकता है। 6 महीने की कुल अवधि के लिए उपचार दिया जाएगा। उपचार की विफलता के मामले में (3 महीने के अंत में कोई बाल विकास नहीं, नैदानिक परीक्षा / डर्मोस्कोपी द्वारा पुष्टि की गई) आप को मौखिक फ़ाइनेस्टराइड पर शुरू किया जाएगा। इस अध्ययन में निर्धारित सभी दवाएं अपेक्षाकृत सुरक्षित हैं और प्रत्येक 4 साप्ताहिक अनुवर्ती दौरे पर दुष्प्रभावों की निगरानी की जाएगी।

निम्न शोध को मिनोक्सिडिल 5% घोल प्लस बायोटिन बनाम मिनोक्सिडिल 5% घोल और फिनास्टेराइड 0.10% प्लस प्लेसबो की तुलना में मिनोक्सिडिल 5% घोल प्लस प्लेसबो के साथ पुरुष मरीज में एंड्रोजेनिक खालित्य के साथ बाल विकास पर किया जाता है।

आप को यह भी सूचित किया जाता है कि उसके द्वारा दी गई सभी जानकारी गोपनीय रखी जाएगी। आप को यह अधिकार भी है कि इस शोध के दौरान, आप बिना कारण बताए सहमति को वापस ले सकता है और इस शोध से बाहर हो सकता है।

सिद्धांत अन्वेषक: डॉ यामिनी सिहाग **संपर्क नंबर**: 9557108612

ANNEXURE IX

MASTER CHART KEYS

SR.NO	VARIABLES	CODE
1.	Group	1= Group A 2= Group B 3= Group C
2.	Age	In years
3.	Gender	1= Male
4.	Address	1 = Jodhpur 2 = Outside jodhpur
5.	Education	1= Illiterate 2= Primary school 3= middle school 4 = high school 5 = Intermediate 6 = Graduate 7 = Professional
6.	Occupation	1 = Student 2 = Professional
7.	Marital status	1 = Unmarried 2 = Married
8.	Age of onset of disease	In years
9.	Total duration of disease	In years
10.	Frontal hair line recession	1 = Yes 2 = No
11.	Pattern of hair loss	1 = Yes 2 = No
12.	Vertex involved	1 = Yes 2 = No
13.	Occiput involved	1 = Yes 2 = No
14.	Scaling over scalp	1 = Yes 2 = No
15.	Itching over scalp	1 = Yes 2 = No
16.	Pain over scalp	1 = Yes 2 = No
17.	Other cutaneous diseases	1 = Yes 2 = No 3 = Tinea 4 = Acne 5 = Vitiligo 6 = Pityriasis Versicolor 7 = Melasma 8 = Hidradenitis suppurativa

18.	Trigger present at baseline	1 = Surgery 2 = Weight loss 3 = Dieting 4 = Stress 5 = None
19.	Systemic illness present	1 = Epilepsy 2 = Hypertension 3 = Tuberculosis 4 = Thyroid disorders 5 = None
20.	History of smoking	1 = Yes 2 = No
21.	History of alcohol consumption	1 = Yes 2 = No
22.	Family history of AGA	1 = Yes 2 = No
23.	History of cancer/chemotherapy	1 = Yes 2 = No
24.	Past treatment history (oral supplement or topical)	1 = Yes 2 = No
25.	Baseline Hamilton Norwood grades	1 = Grade I 2 = Grade II 3 = Grade III 4 = Grade III Vertex 5 = Grade IV
26.	Global photographic assessment	1 = -3 2 = -2 3 = -1 4 = 0 5 = + 1 6 = + 2 7 = + 3
27.	Global photographic assessment by investigator 1 and 2	0 = Worsening and no change Percentage As numbers = Improvement
28.	Hair shaft thickness heterogeneity over frontal scalp	1 = Yes 2 = No
29.	Brown peripilar sign over frontal scalp	1 = Yes 2 = No
30.	White peripilar sign over frontal scalp	1 = Yes 2 = No
31.	Yellow dots over frontal scalp	1 = Yes 2 = No
32.	Focal atrichia over frontal scalp	1 = Yes 2 = No
33.	Scalp honey coomb pigmentation over frontal scalp	1 = Yes 2 = No
34.	Hair shaft thickness heterogeneity over vertex scalp	1 = Yes 2 = No

35.	Brown peripilar sign over vertex scalp	1 = Yes 2 = No
36.	White peripilar sign over vertex scalp	1 = Yes 2 = No
37.	Yellow dots over vertex scalp	1 = Yes 2 = No
38.	Focal atrichia over vertex scalp	1 = Yes 2 = No
39.	Scalp honey coomb pigmentation over vertex scalp	1 = Yes 2 = No
40.	TAHC over frontal scalp at baseline	Number of hairs per square centimetre
41.	TAHC over frontal scalp at visit 6	Number of hairs per square centimetre
42.	TAHC over vertex scalp at baseline	Number of hairs per square centimetre
43.	TAHC over vertex scalp at visit 6	Number of hairs per square centimetre
44.	TAHW over frontal scalp at baseline	In micrometre
45.	TAHW over frontal scalp at visit 6	In micrometre
46.	TAHW over vertex scalp at baseline	In micrometre
47.	TAHW over vertex scalp at visit 6	In micrometre
48.	Hair pull test at baseline	1 = Yes 2 = No
	Adverse drug reactions	
49.	Hypertrichosis	1 = Yes 2 = No
50.	Headache	1 = Yes 2 = No
51.	Burning sensation/stinging over scalp	1 = Yes 2 = No
52.	Itching over scalp	1 = Yes 2 = No
53.	Dryness or scaling over scalp	1 = Yes 2 = No
54.	Palpitations or chest pain or tachycardia	1 = Yes 2 = No
55.	Gynaecomastia	1 = Yes 2 = No
56.	Sexual adverse effects	1 = Yes 2 = No
57.	Papules and pustules over scalp and forehead	1 = Yes 2 = No
58.	Periorbital itching and edema	1 = Yes 2 = No
59.	VAS at baseline	Number
60.	VAS at visit 6	Number
61.	Baseline serum biotin concentration	Picogram per millilitre

ANNEXURE X

MASTER CHART