"A ONE YEAR CROSS-SECTIONAL STUDY TO OBSERVE CORRELATION OF SERUM FERRITIN LEVELS, IN FEMALE PATIENTS WITH CHRONIC DIFFUSE HAIR LOSS, ATTENDING DERMATOLOGY CLINIC OF KLES Dr PRABHAKAR KORE HOSPITAL AND MEDICAL RESEARCH CENTRE, BELAGAVI"

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LIST OF ABBREVIATIONS USED

AA	Alopecia Areata
AGA	Androgenetic Alopecia
AV-I	Acne Vulgaris grade I
AV-II	Acne Vulgaris grade II
BASP	Basic and Specific Classification
BMI	Body Mass Index
CTE	Chronic Telogen Effluvium
DPN	Dermatosis PapulosaNigra
FAGA	Female Androgenetic Alopecia
FPA	Female Pattern Alopecia
FPHL	Female Pattern Hair Loss
GF	Growth Factor
GI	Gastro-Intestinal
Hb	Hemoglobin
HiCN	Cyanmethemoglobin
ID	Iron Deficiency
IDA	Iron Deficiency Anemia
IRS	Inner Root Sheath
ORS	Outer Root Sheath
PCOD	Poly Cystic Ovarian Disease
POH	Peri-Orbital Hyperpigmentation
SHBG	Sex Hormone Binding Globulin
Sr	Serum
TE	Telogen Effluvium
UAT	Unit Area Trichogram

ABSTRACT

Background: Hair forms the most important part of facial aesthetics. An average normal scalp has 1,00,000 hairs, with approximately 86% being in anagen, 1% in catagen, and 13% in telogen. With Telogen Effluvium (TE), the ratio shifts to 70% anagen and 30% telogen, with daily shedding of up to 300 hairs. Iron deficiency and thyroid disorders are common conditions often associated with diffuse hair loss, apart from other etiological causes. Iron is involved in many critical physiological processes within the hair follicle, suggesting that iron deficiency could disrupt hair synthesis. Iron-dependent genes in the hair follicle bulge region may be affected by iron deficiency. Serum ferritin is directly related to intracellular ferritin and thus to the total body iron stores. Hence low ferritin concentration is very specific for iron deficiency. The relationship between body iron status and different types of hair loss has been investigated in a number of studies, however with relatively discrepant findings. There has been controversy over the cut-off level of serum ferritin, below which it can be defined as nutritional deficiency, triggering hair loss. Therefore this study was undertaken to establish the truth regarding the correlation of serum ferritin levels, in patients with chronic diffuse hair loss.

Diffuse hair loss affecting the entire scalp, for which no obvious cause can be found, indicates Chronic Telogen Effluvium (CTE), while gradual diffuse hair loss with thinning of central scalp/widening of central parting line/ frontotemporal recession indicates Female Pattern Hair Loss (FPHL).

Aim: To evaluate whether chronic telogen effluvium and female pattern hair loss in patients, were associated with decreased tissue iron stores, as measured by serum ferritin and hemoglobin levels.

Materials and methods: All the female patients aged between 15 to 45 years, having CTE and FPHL were recruited in the study as per the inclusion and exclusion criteria. The inclusion criteria were, all consenting female subjects of age group 15-45 years, with chronic diffuse and patterned hair loss. The exclusion criteria were, the subjects who were on iron therapy, undergone GI/scalp surgeries, suffering from trichotillomania, hormonal abnormalities and subjects who were on regular medications for other systemic disorders. The sample size was 40 subjects with chronic diffuse hair loss. A detailed history along with systemic and dermatological examination was carried out. Diagnosis of CTE and FPHL was made by clinical examination, by performing hair pull test. Subjects of FPHL were graded as per Ludwig's grading scale. Serum ferritin and hemoglobin were estimated by standard tests. All the results were tabulated; analysis of results were made by using mean ± SD and Pearson's correlation coefficient, one sample't' test was used wherever applicable. The results were expressed as percentage and significance.

Results: In this study, the total sample size was 40 and number of subjects having CTE was more (35) as compared to the FPHL (5). Hair pull test was positive in all subjects having CTE and negative in subjects with FPHL. Severity of hair loss was assessed in all subjects of FPHL by Ludwig's scale (Type II grade: 80%, Type III grade: 20% subjects).

Mean age of all participants was 25.18 years; CTE was prevalent in the younger age group as compared to FPHL. Mean duration of hair loss was 20.28 months (range 6-60 months), which was less in CTE (18.37 months), as compared to FPHL (33.60 months).

Mean Hemoglobin (Hb) levels of all participants was 11.5gms/dL (range: 6.9-13.9 gms/dL), 45% of participants had hemoglobin less than 12 gms/dL (Normal range: 12-15gms/dL).Hb levels were less in subjects having CTE (11.33 gms/dL) as compared to

the subjects with FPHL(12.94 gms/dL). In CTE the percentage of subjects with Hb less than 12 gms/dL, was 51.42%.There was negative correlation between duration of hair loss and Hb.

Mean serum ferritin levels of all subjects was 18.6µg/L (range: 0-85.6 µg/L). 57.5% of the subjects had serum ferritin levels less than 12 µg/L indicating iron deficiency, 15% of the participants had serum ferritin levels ranging from 13-20 µg/L indicating iron depletion. 25% of the participants had serum ferritin levels ranging from 21-70 µg/L, indicating serum ferritin levels were lower than required for normal hair cycle. Only one participant had serum ferritin level more than 71 µg/L, indicating serum ferritin levels were within the normal range. 42.5% of the participants had serum ferritin levels less than 6 µg/L, which was definitely below the normal range as per specification of the test. Mean serum ferritin levels were low in subjects with CTE (16 µg/L), as compared to FPHL (36.64 µg/L) and the difference was statistically significant ('p'=0.0389*). As the range of serum ferritin is very wide, cut off levels of serum ferritin (≤ 15 µg/L, ≤ 40 µg/L and ≤ 70 µg/L) were adopted in this study. Majority of the subjects of this study had Iron Deficiency (ID), when cut off level of serum ferritin was used as ≤ 70 µg/L (FPHL=80% and CTE=100% of the subjects). When cut off level of serum ferritin was used as≤ 40 µg/L, 60% of the subjects in FPHL and 85.71% of the subjects in CTE, showed ID. Even when cut off level of serum ferritin was used as ≤ 20 µg/L, 60% of the subjects in FPHL and 74.28% of the subjects in CTE, showed ID. There was positive correlation between Hb and ferritin levels and negative correlation between duration of hair loss and serum ferritin levels. In the subjects with presence of stress (47.5%),the mean Hb was significantly low (10.76 gms/dL) and the mean serum ferritin in those subjects with stress was 11.34 µg/L, which was significantly less, when cut off level of serum ferritin was taken as 41µg/L.

Different etiological factors of hair loss like history of hair loss in the family was present in 7.5% of the subjects, 20% of the subjects were having seborrheic capitis/ dandruff, 62.5% of the subjects gave history of exposure to high external environmental changes and only 27.5% the subjects were using headscarf / helmet.

Conclusion: In this study, hemoglobin concentration was used to screen for iron deficiency and serum ferritin concentration was used to confirm iron deficiency. Subjects of this study had low serum ferritin levels at different definitions of ID and CTE, FPHL were associated with decreased iron stores. Hence this study confirms that chronic telogen effluvium and female pattern hair loss in patients are definitely associated with decreased tissue iron stores. As ferritin levels accurately reflect body iron stores, this study clearly demonstrated the association between low iron stores and chronic diffuse hair loss.

Keywords: Female Pattern Hair Loss, Chronic Telogen Effluvium, Hemoglobin concentration, Serum ferritin levels, Iron Deficiency, Hair loss.

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INTRODUCTION

Hair forms the most important part of facial aesthetics. Hair frames the face, the feature considered most important in terms of first impression. Following a person's smile, eyes and skin, their hair is often the next feature, people notice on first encounters. It is among the top three features, along with height and weight, used when describing others and one of the features most often recalled after a social interaction occurs.

Women place a greater emphasis than men on physical appearances and outward attractiveness. Societal norms dictate that hair is an essential part of a woman's sexuality, gender identity, and any hair loss generates feeling of low self-esteem and anxiety from a perception of diminished attractiveness. Women are more likely than men to have a lowered quality of life and to restrict social contacts, as a result of hair loss. Hair loss in women is associated with significant psychological morbidity. Fewer than 45% of women go through life with a full head of hair. An average normal scalp has 1,00,000 hairs, with approximately 86% being in anagen, 1% in catagen, and 13% in telogen.[1] With telogen effluvium, the ratio shifts to 70% anagen and 30% telogen, with daily shedding of up to 300 hairs.[2]

Chronic diffuse hair loss in female is broadly divided into two types, Telogen Effluvium (TE) & patterned hairloss,[3] TE is the most common cause of diffuse hair loss in adult females. TE, along with Female Pattern Hair Loss (FPHL) and chronic Telogen Effluvium (CTE), accounts for the majority of diffuse alopecia cases. Abrupt, rapid, generalized shedding of normal club hairs, 2-3 months after a triggering event like parturition, high fever, major surgery etc. indicates TE, while gradual diffuse hair

loss with thinning of central scalp/widening of central parting line/frontotemporal recession indicates FPHL. Excessive, alarming diffuse shedding, coming from a normal looking head with plenty of hairs and without an obvious cause is the hallmark of CTE.[4]

FPHL is more frequent than previously thought. FPHL may begin at any age following puberty and the prevalence increases during post-menopausal period with a possible hormonal influence.[5] In a community-based study from China, the prevalence of FPHL was 6% across all age groups ranging from 1.3% in the age group of 18-29 years, increasing steadily with age to 10.3% in the seventh decade and 11.8% thereafter; with a positive family history in 19.2%.[6] In Korean women, the prevalence of Androgenetic Alopecia (AGA) at all ages was 5.6%, 0.2% in the third, 3.8% in the fifth and 7.4% in the sixth decade. It was 11.7% and 24.7%, respectively, in the seventh decade and a positive family history was present in 45.2%.[7] In UK, 6% women aged less than 50 years were diagnosed as having FPHL, increasing to 38% in subjects aged 70 years and above.[8] In Maryborough (Australia), 64.4% of women above 20 years of age exhibited bitemporal hair loss. Overall, the prevalence of mid-frontal hair loss increased with age and affected 57% of women aged 80 years and above.[9] In another study comprising 1006 Caucasian women, FPHL was found to be common, beginning in the late twenties and reaching its peak after 50 years of age.[10] No concrete data is available from the Indian subcontinent. An estimated life-time prevalence of 1.7%; however, this figure is not a reliable estimate, as very few epidemiological studies have been published in this regard, owing partly to under-reporting.[11]

The common causes of chronic hair loss in females include thyroid disorders (hypothyroidism/hyperthyroidism), diabetes, PCOD, anaemia, hypoproteinemia. Other causes are stress, drugs, pregnancy, malignancies, chemotherapy etc. Among the various causes of chronic diffuse CTE, iron deficiency anemia, hypo/hyper thyroidism, malnutrition, acrodermatitis enteropathica, and acquired zinc deficiency has been cited as the most widely accepted ones. In most cases the diagnosis can be made clinically and the condition can be treated medically.[4]

Apart from complete blood count and routine urine examination, levels of serum ferritin and T3, T4, and TSH should be checked in all cases of diffuse hair loss without a discernable cause, as iron deficiency and thyroid disorders are the two common conditions often associated with diffuse hair loss and most of the time, there are no apparent clinical features to suggest them.

Iron deficiency is the world's most common nutritional deficiency in premenopausal women; the most common causes of iron deficiency are menstrual blood loss and pregnancy.[12] Hemoglobin concentration can be used to screen for iron deficiency, whereas serum ferritin concentration can be used to confirm iron deficiency.[12] A review of research shows that iron deficiency has a much closer link to hair loss, in fact, 72% of women with diffuse hair loss have iron deficiency.[13]

Currently, there is insufficient evidence to recommend universal screening for iron deficiency and to treat iron deficiency in patients of hair loss without anemia. Trost, Bergfeld and Calogeras[12] have published an excellent review on the relationship and management of iron deficiency and hair loss. They practice screening for iron deficiency even in patients of hair loss without anemia and believe that the

treatment of hair loss is enhanced when the serum ferritin concentration is raised to70 µg/ml.

The controversy regarding the association between nutritional factors and hair loss continues to persist.

Several studies have examined the relationship between iron deficiency and hair loss. Currently, there is insufficient evidence to recommend screening for iron deficiency in patients with hair loss on a routine basis and replacement of iron in the absence of iron deficiency anemia.

Authors who state that there is a link between iron deficiency and TE usually state that, in cases of low iron depots, hair follicles that have shed their hair at the end of telogen may temporarily fail to re-enter anagen, leading to a slow onset, diffuse hair loss .

Iron is involved in many critical physiological processes within the hair follicle, suggesting that iron deficiency could disrupt hair synthesis.[14] It is described that iron-dependent genes in the hair follicle bulge region may be affected by iron deficiency.[15] However, studies of iron as a cause for hair loss have produced conflicting results.

The association of low serum ferritin level and hair loss has been debated over the years. There has been controversy over the cut-off level of serum ferritin, below which it can be defined as nutritional deficiency, triggering hair loss. Serum ferritin is directly related to intracellular ferritin and thus to the total body iron stores. Only iron deficiency causes very low serum ferritin concentrations; therefore, a low ferritin concentration is very specific for iron deficiency.

In a study by Sinclair no direct relationship between low serum ferritin and hair loss was established and determination of serum ferritin as a routine investigation in women with chronic diffuse telogen hair loss was not found useful, doubting the role of iron supplementation in management of hair loss.[16]

Conflicting observational data have failed to determine whether an association exists between hair loss and iron deficiency in the patients. The relationship between body iron status and different types of hair loss has been investigated in a number of studies, however, with relatively discrepant findings. Therefore this study utilized an analytical cross-sectional methodology, to evaluate whether chronic telogen effluvium and female pattern hair loss in patients, were associated with decreased tissue iron stores, as measured by serum ferritin and hemoglobin levels. These findings have implications regarding understanding the triggers for hair loss, clinical trial design and therapeutics.

Some studies show that hair loss is associated with decreased serum ferritin levels, while other studies reveal that there is no co-relation between hair loss and serum ferritin levels. Hence the relationship between hair loss and iron deficiency continues to be a subject of debate. Therefore this study was undertaken to establish the truth regarding the correlation of serum ferritin levels, in patients with chronic diffuse hair loss.

OBJECTIVES OF THE STUDY

Primary objective:

To measure hemoglobin and serum ferritin levels in patients with hair loss, of the age group between 15 to 45 years, attending dermatology clinic at KLES Dr Prabhakar Kore Hospital & MRC, Belagavi.

Secondary objective:

To analyze correlation between serum ferritin and hemoglobin levels with hair loss by statistical methods.

REVIEW OF LITERATURE

INTRODUCTION

Hair is an appendage of skin that has attracted more attention for its cosmesis than for medical relevance. Hair follicular unit is composed of a hair follicle, sebaceous gland and arrector pili. Too much (e.g. hirsutism) and too little (e.g. alopecia) are both causes of immense psychological stress.

ANATOMY OF HAIR

A basic understanding of the differences in the physical, morphologic and histological hair properties of the different ethnic groups is important in the assessment, diagnosis and management of patients with hair loss. In addition, knowledge of the microscopic anatomy of the normal hair follicle is vital in understanding the disease of hair follicle.

Follicular morphogenesis: Hair structure development starts after 8 weeks of foetal life, with the appearance of placodes in the epidermal basal layer. Specialized mesenchymal cells organize in a small condensate, directly beneath the basement membrane, stimulating the overlying epithelial stem cells to invaginate and penetrate into the dermis forming an epidermal peg,[17] which continues to grow downward enclosing the dermal condensate forming the dermal papilla. The tip of the epidermal peg becomes the matrix portion of the hair bulb. Cells of the follicular matrix terminally differentiate into Inner Root Sheath (IRS) and the hair shaft that exits from the surface of the skin. Initial hair population is complete by 22 weeks. Fine lanugo hair develops in an advancing wave from the frontal to occipital scalp and it is shed

by 36 weeks. A second coat of lanugo hair appears and it is shed in a synchronized wave pattern at 3-4 month of life. [18]

Morphological variants of hair: There are two main types of hair present on the scalp

1. Terminal hairs are large with a diameter > 0.03 mm and length of 1cm, are often pigmented and medullated. They can be graded by hair shaft diameter as small, medium or large. Terminal hairs miniaturized to vellus hair proportions are described as vellus-like hairs, such as seen in alopecia areata or androgenetic alopecia. Terminal hairs are rooted in subcutaneous tissue or the deep dermis, while vellus hairs are rooted in the upper dermis. A normal scalp averages seven terminal hairs per vellus hair.
2. Vellus hairs are small with a diameter less than 0.03 mm, are often less than 1cm in length and lack in melanin and medulla.[19] True vellus hairs have thin, external root sheaths and short stelae in the upper dermis. Vellus like miniaturized hairs have thicker external root sheaths and long stelae extending into lower dermis or subcutaneous fat.[20]
3. Intermediate hairs are actually terminal hairs which also are thinner (0.03-0.06 mm) and hair bulbs of which are situated in the reticular dermis rather than in the subcutis.

Microscopic structure of hair

Anatomy of anagen hair: The anagen hair follicle is divided into four parts from deep to superficial viz: hair bulb, suprabulbar area, isthmus and infundibulum.

The infundibulum and isthmus are relatively constant structures, whereas the lower part of the hair follicle that is the hair bulb and the suprabulbar region undergoes repeated episodes of regression and regeneration during the hair cycle. On the scalp, hair follicles are arranged in groups of three or more follicles known as follicular units together with the sebaceous gland and arrector pili muscle, the hair follicle is part of the so called pilosabaceous unit.

Hair bulb, usually located in the subcutaneous fat, consists of hair matrix, the basophilic germinative layer which surrounds the dermal papilla, which is a flask shaped structure and is connected to the perifollicular dermal sheath by a narrow stalk. The dermal papilla contains a loop of capillary blood vessels and plays an essential role in the induction and maintenance of follicular epithelial differentiation.[21,22,23,24]

The size of hair shaft is determined by volume of the dermal papilla and it is the primary target for androgen action in the hair follicle in androgentic alopecia.

At the suprabulbar zone, the various layers of the anagen follicle begin to differentiate. Layers of the anagen follicle include: hair shaft, inner root sheath, glassy layer and fibrous root sheath.

The hair shaft medulla may be continuous, discontinuous or absent. Hair shaft cortex contains alpha keratin-intermediate filaments.

The cuticle acts as a barrier to physical and chemical insults and also maintains the integrity of the hair shaft. Wear and tear leads to gradual loss with breaking and lifting of the free margins of cuticular cells. If weathering is severe, it may lead to exposure of cortex and fracture of the hair shaft.

The infundibulum extends from the skin surface, where it merges with epidermis, to the opening of the sebaceous duct.

The isthmus is a transitional zone of follicular keratinization. In mid portion of the isthmus, the inner root sheath desquamates resulting in separation between the hair shaft and the follicular wall. At this point, the cells of the outer root sheath begin to cornify without formation of granular cell layer, called as trichilemmal keratinization. There is no inner root sheath here. Hair follicle skin cells reside in the lower part of the isthmus close to the insertion of the arrector muscle.[25] Hair follicle skin cells show distinctive biochemical properties, they are slow cycling and proliferate only during the onset of anagen. Daughter cells known as transient amplifying cells, input into the outer root sheath of the lower part of the hair follicle, where they migrate in a downward direction.

Anatomy of catagen hair: At the end of anagen, the hair matrix disappears and epithelium of the lower follicle undergoes disintegration by apoptosis. The vitreous layer and also fibrous sheath markedly thicken. As the catagen phase progress over a 2-3 week period, the hair papilla follows the disintegrating epithelial column upwards into the dermis. A collapsed fibrous sheath is left behind as the epithelial column moves upwards. This collapsed structure is called the ‘stela’.

Anatomy of telogen hair: At the end of catagen, the dermal papilla is a condensed ball of spindle shaped nuclei within a scanty stroma. The papilla lies just below epithelium called the secondary hair germ. When sectioned transversely, the secondary hair germ appears like an asterisk.

HAIR FOLLICLE INNERVATION

Several different types of nerve endings are found around human hair follicles, including free nerve endings, pilo-ruffini nerve endings and Merkel nerve endings.[26]

ULTRASTRUCTURE OF HAIR

Hair is formed of hard keratin with high sulfur content that is responsible for its extraordinary tensile strength.[27] The hair cortex is made up of a low sulphur fibrillar component tightly packed in a sulfur-rich matrix. The fibrillar component consists of macrofibrils. The macrofibrils comprise of smaller microfibrils and protofibrils. X-ray diffraction study of the hair reveals that the fibrillar component of the cortex consists of alpha-keratin chains arranged in the form of three to seven intertwined helices.[18] Thus, with hair stretching, the diffraction pattern changes owing to straightening of the helices.

PHYSIOLOGY OF HAIR

The Hair Cycle: Hair follicles undergo repetitive sequence of growth and rest known as the hair cycle. Five phases of the growth cycle are recognized.

1. Growth phase (anagen I-VI): It is an active growing period and lasts weeks to years depending upon size and site of the hair. Anagen hair on the scalp lasts between 2 to 7 years. The duration of this phase is responsible for determining the final length of the hair. Under normal circumstances, 80% to 90% of hair follicles on the human scalp are in anagen at any one time.
2. Regression phase (catagen): It is a brief transitional phase between anagen and telogen and lasts about 2-3 weeks. About 1% of the scalp hair is in catagen phase.

3. Resting phase (telogen): The period between the completion of follicular regression and the onset of the next anagen phase is termed as telogen or club hair.
4. Shedding phase (exogen): The club hair is eventually shed through an active process termed as exogen or teloptosis.[28]
5. Lag phase (kenogen): It is phase in the hair cycle where the follicle is empty after hair shedding and persists in the stage for a variable duration.

HAIR SHEDDING

The first ever hair shedding starts in utero and occurs as a wave, which begins in the anterior frontal line and progresses backwards. Follicles in the occipital area are the last to enter hair shedding and occur 8-12 weeks postnatally; this gives rise to neonatal alopecia. The second is the synchronous hair shedding affecting all hairs. Rarely, synchronous shedding can persists for years.[29] Slight variation in telogen/anagen ratio can occur seasonally.

MECHANISM OF HAIR CYCLING

Various theories have been proposed. One of the most widely accepted one is *bulge activation hypothesis.* It proposes that the stem cells of the follicle reside not in the bulb matrix but in the bulge region, this being the lowermost part of the follicle.[30] These cells divide at the end of telogen to give rise to the transient amplifying cells that multiply to form the matrix cells with a fixed life span. After this, the bulb disappears and the follicle returns to telogen, only to restart the cycle. The dermal papilla, which moves upwards during telogen, may interact with the bulge cells to start their activation. The variation in the proliferative capacity of the transient

amplifying cells may be responsible for the variation in the length of the anagen. The keratinocytes of the bulge area differ from the surrounding keratinocytes by expressing a different set of keratins, namely, keratin 19, high expression of A3 B1 integrin and slow cycling nature.[31,32] This signifies their special role in hair development.

CONTROL OF HAIR GROWTH

The periodic change of this organ to a quiescent state and its orderly transformation from the vellus to the terminal type or vice versa is controlled in various ways.[33]

1. Hemocrine (endocrine) method: Occurs via the blood stream, as with androgens or thyroid hormones.
2. Paracrine method: Secretion of ILGF-1 by follicular papillary cells mediates the effects of androgens. Hence, binding of androgens to androgen receptors over papillary cells leads to release of ILGF-1 that stimulate germinative/matrix cells to multiply and cause hair growth.
3. Juxtacrine method: Close juxtaposition of the epithelial and mesenchymal cells involved in follicle regrowth (telogen to anagen switch) is necessary for this to occur.
4. Autocrine method: In this, secretion of growth factors by the same cells that are stimulated by it.
5. Intracrine method: This is by intracellular synthesis of proteins that stimulate cellular proliferation and thereby stimulate hair growth.

The last 2 mechanisms amplify the actions of the first 3 mechanisms and are responsible for differences in hair growth, which is affected by cytokines and GFs.

Hormonal control of hair growth

Various hormones (androgens, thyroid hormones, insulin, cortisol, oestrogens, prolactin and growth hormones) affect hair growth.[34] They act in concert with numerous growth factors (IGF-1) and cell receptors (Vitamin D receptor).

Androgens: They are the most important of all hormones for hair development. Those follicles that respond to changes in androgen metabolism are grouped as androgen-dependent hair. In addition, growth hormone has a synergistic effect on the growth of androgen-dependent hair.[35]

In 5α-reductase deficiency which results in low levels of the active metabolite of testosterone, the beard hair growth is retarded and patterned baldness does not occur.[36] In women with virilizing syndromes, facial hair grows in male pattern and at times even the scalp may show male pattern alopecia. Administration of an antiandrogen reverses these effects.

Androgens cause loss of hair at one site (female pattern alopecia in hirsutism) and overgrowth of hair at other site (hypertrichosis in beard, moustache and chest hairs in hirsutism). This variable response of hair follicles from different body regions to androgens could be due to difference in the sensitivity or the number of androgen receptors or a difference in androgen metabolism of the follicles.[37]

Nonandrogenic Hormones: By inhibiting the onset of follicular activity, estradiol retains the club hair in the follicle.[35] This is probably responsible for the postpartum alopecia resulting from the loss of retained telogen hair as the estrogen levels fall. Prolactin increases adrenal androgen synthesis and suppresses estrogen levels, leading to fall in Sex Hormone Binding Globulin (SHBG) levels.

Neural control on hair growth

Various neurotrophins and their receptors play an important role in hair morphogenesis and regeneration. Neurotrophin-3 (NT-3) promotes hair morphogenesis and induces the catagen phase. Glia-derived neurotrophic factors, neurturin and brain-derived neurotrophic factors also affect the hair cycle.[38]

Immunological control on hair growth

The immune system affects and is affected by the hair cycle. The various cytokines secreted by the perifollicular macrophages and mast cells adversely affect the hair cycle. IL-1, TNF-α and FGF-5 secreted by macrophages induce catagen. Perifollicular mast cell degranulation has been observed at the onset of anagen-catagen and telogen-anagen transformation.[38] It has been shown that there is loss of this immune privilege in alopecia areata.

RATE OF HAIR GROWTH

On an average, scalp hairs grow 0.45 mm/day; beard, 0.35 mm/day; and extremities, 0.25 mm/day, whereas the vellus hairs on the forehead grow only 0.03 mm/ day, shaving or cutting of hair does not alter its caliber or rate of growth.[35]

HAIR DISTRIBUTION PATTERNS

There are approximately 5 million hair follicles on the body and around 1,00,000 to 1,50,000 scalp hairs present on a normal scalp. The scalp possesses the greater density of follicles ranging from 118-350 hair follicles/sq.cm.[39] The hair density is maximum at birth and gradually decreases as the age advances.

HAIR SLOPE PATTERNS/TRICHOGLYPHICS:[40]

The study of hair slope patterns is termed as trichoglyphics. Various physical and local chemical factors acting during hair morphogenesis probably contribute to their development. Interrelationship between the sloping patterns gives rise to specific patterns like a whorl or an interlocking cross. Hair whorls are most obvious on the scalp and generally, there is a single parietal clockwise whorl and the rest of the hair slope away from it.

Abnormal trichoglyphics have been associated with neurological abnormalities. A recent research indicates that a single gene may control both handedness and hair whorl direction.[41] Smith and Gong have detailed associations of various abnormal hair slope patterns with specific malformations and dysmorphic syndromes.

AGING AND HAIR

Effects of aging are observed on all the components of the hair follicle. Thus, the follicle decreases in size, the shaft diameter reduces and the pigment is gradually diminished and finally lost. The terminal follicle turns into an indeterminate one and then a vellus follicle before involuting totally and becoming lost permanently. The papilla becomes thickened with the deposition of glycosaminoglycans.

FUNCTIONS OF HAIR

They help In tactile perception by means of its rich nerve network,[38] protection of scalp from sunlight and trauma, protection of eyes from foreign bodies, sunlight and sweat, screening nasal passages, reduce friction in intertriginous areas and give apocrine odour as a sex signal.

ALOPECIA

Loss of hair is one of the commonest complaints that baffle dermatologists. Various classification schemes for alopecia exist, but all are imperfect. Based on whether permanent damage has occurred to the follicles, the alopecias are divided into scarring or cicatricial alopecia and nonscarring or noncicatricial alopecia. In scarring alopecia hair follicles are permanently lost. In contrast, nonscarring alopecia is potentially reversible. It is generally agreed upon that barring a few exceptions follicles cannot develop anew after birth. Therefore, patches of scarring alopecia cannot regrow even when the initiating pathology has ceased to be active.

In non-scarring alopecias, follicles appear preserved, although they can sometimes be difficult to appreciate when miniaturized. The three most common forms of non-scarring alopecias are androgenetic alopecia (AGA), telogen effluvium, and alopecia areata.

The commonest non-scarring alopecia is androgenetic alopecia (AGA). Alopecia may be diffuse or patchy. Conditions with diffuse alopecia include telogen effluvium, postpartum alopecia, neonatal alopecia, anagen effluvium, drug-induced alopecia, alopecia associated with systemic diseases, hair shaft defects, and occasionally alopecia areata.

Patchy alopecia occurs in alopecia areata, fungal, bacterial and viral infections of the scalp, trichotillomania, traction alopecia, and tick bite alopecia.

However, certain hair diseases demonstrate a biphasic pattern, where non-scarring hair is seen early in the course of the disease, and permanent hair loss be common apparent in the later stages of disease. Examples of diseases demonstrating biphasic pattern include androgenetic alopecia, alopecia areata and traction alopecia.

INVESTIGATIONS FOR HAIR LOSS

Various methods are available for evaluation of a patient presenting with hair loss. Most of them do not fit the "ideal" requirements to suit the needs of the clinician, researcher or the patient. These evaluation methods have their own merits and demerits.

HAIR EVALUATION METHODS

Clinical tests :Hair pull test, daily hair counts, standardized wash test, 60-s hair count, hair weight estimation, contrasting felt examination, hair feathering, Wood's lamp examination, trichometry, trichtillometry and hair growth window

Microscopic: Trichogram/hair pluck test examination of hair, unit area trichogram (UAT), phototrichogram, global photography, skin surface microscopy/ dermoscopy, trichoscan, light microscopy of hair, polarizing microscopy of hair, electron microscopy of hair and confocal microscopy of hair

Scalp biopsy and miscellaneous tests

Daily Hair Counts: This can be useful to quantify how much the patient is losing. It is said that it is normal to lose up to 100 hairs per day. Patients are instructed to collect hair shed in one day, count and place them in plastic bags. Daily hair counts for 7 days are maintained. It is expected to lose more hairs on shampoo days.[42,43]

If the patient is losing more than 100 hairs per day, the hair should be examined microscopically to detect the pathology in hair bulb and hair shaft abnormalities. Appearance of the hair bulb can distinguish between telogen effluvium, anagen effluvium and active diffuse alopecia areata.[44]

Standardised Wash Test: In this test, the patient refrains from shampooing for 5 days and then she shampoos and rinses the hair in the basin with the hole covered by gauze. The hairs remaining in the water and the gauze are collected and sent for examination. Hairs must be counted and divided into ≤ 3 cm and ≥ 5 cm in length. This is an important technique to differentiate telogen effluvium from female pattern hair loss

60-Second Hair Count:[45] Before shampooing, comb the hair for 60 seconds over a sheet of contrasting colour, starting with the comb at the back top of the scalp moving comb forward to the front of the scalp. Repeat the procedure before three consecutive shampooing and always use the same comb or brush. Count number of hairs in the comb or brush and on the sheet after each hair count and record. Patient is asked to repeat the above procedure monthly and bring the results.

Hair pull Test : It is also known as the traction test or Sabouraud's sign or the pull-out sign.[46] Approximately 20-60 hairs are grasped between the thumb, index and middle fingers from the base of the hairs near the scalp and firmly, but not forcefully, tugged away from the scalp. If more than 10% hairs are pulled away from the scalp, this constitutes a positive pull test and implies active hair shedding. The patient must not shampoo for at least a day prior to the pull test (Figure 1).

Clinical correlation: This test is based on the concept of "gentle" pulling of the hair to bring about shedding of telogen hairs.[47] It helps to assess the severity and location of hair loss. The test is positive in cases of telogen effluvium, anagen effluvium, loose anagen syndrome, early cases of patterned alopecia and at the advancing edge of alopecia areata.

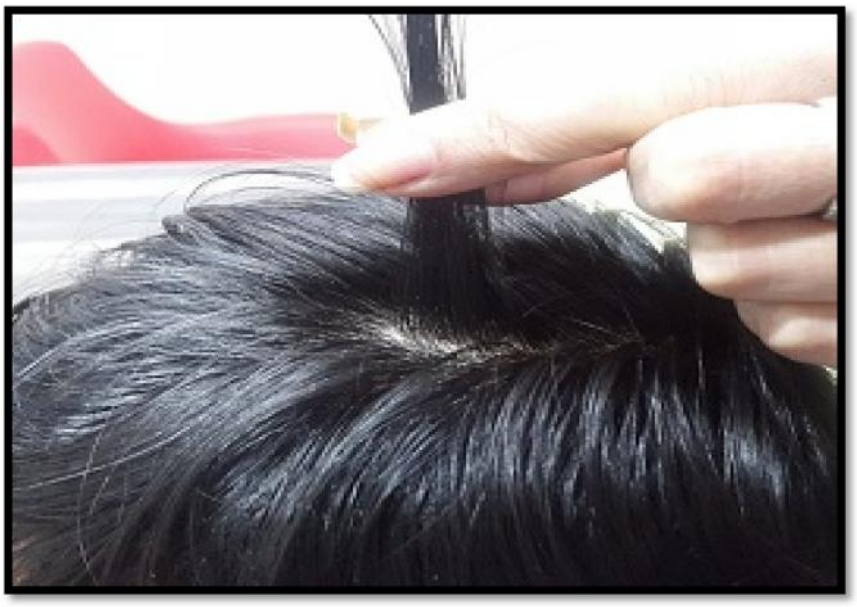


Figure 1 : Hair Pull Test – Approximately 60 hairs are grasped from the proximal portion of the scalp and tugged from the proximal to distal end.

Hair weight*:* Hairs in a given target area be clipped close to the scalp at baseline, the hairs are allowed to grow for a fixed period of time and then the target area hairs clipped again close to the scalp, collected and subsequently weighed. This method is difficult to practice and has been used only in clinical trials.[48]

Contrasting felt examination: This test is used to see short, miniature hairs of the scalp. An index card with black felt glued on one side and white felt on the opposite side is used. After making a parting in the hair, the index card is held along the scalp. Fine short hairs with broken or tapered distal tips project up along the edges of the felt. These miniature hairs can be seen in the androgen-dependent areas of women presenting with androgenetic alopecia. In a regrowing telogen effluvium, a classic short frontal fringe is seen.[44]

Global Photography: The Canfield technique has recently been validated. Use of a stereotactic positioning device on which the patient's chin and forehead are fixed, and on which a given camera and flash device are mounted, assures that the view,

magnification and lighting are the same at consecutive study visits. It is important to ask the patients to keep the same hair style and colour and the coordinators attempt to duplicate baseline hair parting and combing in subsequent visits. Four standard views are (vertex, midline, frontal and temporal) are advocated.[49] Paired comparison of global photographs (before and after treatment) is a precise appreciation of hair growth after drug treatment.[48]

Dermoscopy and Videodermoscopy (Trichoscopy): It helps in demonstration of features and hence enhances patient compliance in treatment.[50,51] Unlike the conventional handheld dermoscope, videodermoscopy permits rapid, high-resolution viewing at several magnifications, together with the ability to capture the viewed images digitally and to store them for later use.[52] It has also been used in the measurement of hair growth parameters such as hair diameter, hair length, and their modification with medications. Dermoscopy and videodermoscopy have a role in the diagnostic assessment of scalp and hair disorders.

Merits and Demerits: Dermatoscopy offers fast and highly instructive clues to the diagnosis and prognosis of hair and scalp disorders, including primary cicatricial alopecias. Videodermoscopy also serves as a step prior to performing a biopsy. It can help the clinician to find the right place to take the sample, thereby avoiding unnecessary biopsies.

Hair Pluck Test/Trichogram*:* Hairs are taken from specified sites on the fifth day after the last shampoo, because the number of telogen hair reduced by a hair washes. Around 60-80 hairs are grasped, plucked, twisting and lifting the hair shafts rapidly in the direction of emergence from the scalp. Hair shafts are then cut off 1 cm above the root sheaths and roots are arranged side by side on a slide and then taped.[53] The hair

roots are examined under the microscope and the hairs are classified into anagen, telogen, catagen, and dystrophic.

Traumatized anagen hairs lack an ORS or sometimes both root sheaths but have an angulated bulb. Severely traumatized anagen hairs lack both IRS and ORS and even a bulb, which is represented by an irregular tapering lower end. Both these varieties of hairs are common in normal persons and are caused by the trauma of plucking and have been previously confusingly termed as dysplastic or dystrophic hair. True dystrophic hairs occur sometimes in individuals who have a severe disturbance of hair keratinization, for example, AA and drug-induced anagen effluvium. The normal values for anagen, telogen and catagen are 80%-88%, 10%-20%, and 1%-2%, respectively. Trichograms may be classified according to the predominant pattern into telogen (telogen effluvium), dystrophic (anagen effluvium), and mixed dystrophic-telogen (AA).[54] Shriveled and atrophic roots are also a feature of protein calorie malnutrition.[55]

Unit Area Trichogram (*UAT****):*** It refers to the proportion of the various types of hair counted after epilating all hair in a small marked out area (30 sq.mm).[56,57] A fixed area is marked on the scalp with a fibre-tip pen. All hairs within and on the scribe line were epilated individually with forceps in the direction of the hair growth to minimize damage to the hair bulb.

Merits and demerits of phototrichogram and unit area trichogram: UAT is more accurate than the regular trichogram, as it takes into account not only anagen/telogen ratios but also hair density and diameter. The plucking procedure causes hair damage, leading to dystrophic hairs, which are difficult to interpret. Also, early anagen and vellus hairs canbe easily missed because of their small size.[48]

Phototrichogram: It is a non-invasive technique that allows in vivo study of physiology of the hair cycle and measurement of various hair growth variables.[58,59] The variables are calculated on a specified area on the scalp, usually 1 cm^2, over a specified time period, usually 2 days. Thus, with this procedure, the exact number of hairs per centimeter square, hair density, rate of hair growth, growing (anagen) and non-growing (telogen) hairs, anagen:telogen ratio and hair diameter, can be derived and used to monitor the effect of treatment.

For hair diameter measurement, the clipped hairs are spread on a glass slide and dry mounted with a transparent adhesive tape to measure their diameter under the microscope using 40X magnification. A calibrated micrometer scale having a least measurement of 0.01 mm is used. The diameter of hair is measured close to their bases using the measuring eyepiece.

The marked area is then photographed using a digital camera, under fixed light conditions, from a fixed distance. The patient is advised not to wash her hair for the next 2 days and then, exactly after 48 hours, the second photograph is taken in a similar manner from the specified site and the above variables can be assessed.

Contrast-Enhanced Phototrichogram: It involves colouring hair with black-coloured dye immediately before starting the procedure. These temporarily coloured hairs give a better contrast temporarily on the white scalp, making this method more sensitive for less-pigmented and thin hairs. This contrast enhancement is not required in the Indian setting as we have usually darkly pigmented hairs thus making the procedure still simpler for us to, carry out.[60]

Trichoscan: It combines standard epiluminescence microscopy with automatic digital image analysis for the measurement of human hair. The software quantifies the number of hairs and the anagen-telogen ratio within one operation. The use of Trichoscan initially involves shaving a scalp area (approximately 1.8 sq cm). After 3 days, hairs in the shaven area are dyed and a digital photograph is taken at 20-fold magnification and saved. The trichoscan software works on the basis that telogen hairs do not grow. The software uses this as a basis for calculation of the anagen:telogen ratio. Thus, the basic procedure is quite similar to that of the classical phototrichogram.[61,62]

Trichometry : It is the measurement of hair diameter in an objective way of assessing patients with hirsutism, hypertrichosis or alopecia. Measurements of hair diameter above the ORS are more constant, and it is preferable to measure it in the proximal 40 mm of hair. Disparities in diameter measurements are common because of the oval cross-sectional area of the hair shaft and because of swelling of shafts due to moisture. Dermoscopy and laser beam diffraction can be used for trichometry. A rough indication of hair diameter can be obtained by calculating the vellus hair index (vellus/terminal hair number) from shavings obtained from a standard sized area.[63]

Trichtillometry: The mean force required for epilation can be measured by a trichtillometer. The normal epilation force of more than 36 g is reduced to less than 19 g in kwashiorkor. Easy pluckability of hair is observed in malnutrition and loose anagen hair syndrome.[63]

Light Microscopic Examination*:* This is the cheapest, most widely available, and commonest hair investigation and has a resolution of about 0.2 picometer. Hairs are plucked from the area of interest, and the proximal 1-2 cm of hair is mounted on a

slide and examined by routine light microscopy. Hairs can be mounted onto the slide by adding a drop of water/saline or by firmly fixing it with a cellotape. The hair shafts should be examined for fractures, irregularities, coiling and twisting or other hair shaft disorders. The free ends should be checked to see whether they are tapered, cut, fractured or weathered. If fungal infection is suspected, hairs should be placed on a glass slide in 20% KOH to demonstrate fungal hyphae and spores.

Polarizing Microscopy of Hair: Polarized light is the light in which all the rays vibrate in one plane. A polarizing microscope has two disc accessories and the placement of the discs is such that they allow light vibration in planes perpendicular to each other. Through the eyepieces, only a dark background is seen unless a doubly refractile object is placed in the path of polarized light, in which case the doubly refractile object appears illuminated against a dark background. Alternating dark and light regions give a "tiger tail" appearance and are suggestive trichothiodystrophy.

Electron Microscopy of Hair*:* This provides better resolution but require an expensive setup. A resolution of about 2 nm can be achieved with transmission microscopy. Hairs being amorphous, staining them with heavy metals improves the visibility of their structure. Silver stains are avidly taken up by cysteine present in cortical and cuticular structures.

Confocal Microscopy of Hair: This is a non-invasive technique that projects high quality three-dimensional optical images of a transverse section of the skin and hair in real time. This instrument has been mainly explored by researchers in the study of hair morphogenesis[64] and assessing the effects of various hair cosmetics.[65] Better resolution has led to demonstration of hair shaft breakages in intact hairs in trichothiodystrophy.[66]

Scalp Biopsy: Scalp biopsies are indicated in all cases of cicatricial alopecia and undiagnosed cases of non-cicatricial alopecia. The biopsies for non-cicatrizing alopecia are performed with transverse/horizontal sectioning, which allows a greater number of hair follicles to be examined. According to Sinclair *et al*., the application of the diagnostic criteria achieved accurate diagnostic definition in 98% of women with triple horizontal biopsies vs. 79% with single horizontal biopsy. The biopsy is performed under local anaesthesia and with a skin biopsy punch of at least 4 mm diameter, which gives an effective diameter of 12.6 sq mm. The biopsy must be deep and should include the entire follicular unit, including some subcutaneous fat. Normally, a scalp biopsy has 35-40 hairs at the upper level of papillary dermis and at the reticular dermis level, the number is reduced to around 35 and at the subcutaneous fat level, the numbers are 30. The upper level contains telogen, anagen as well as terminal, vellus and vellus-like miniaturized hairs. The deeper level contains anagen terminal hairs only. The vellus follicles are defined as follicles containing hairs in which the diameter of the hair shaft is equal to or less than the thickness of the inner sheath of the same follicle and the diameter is ≤ 0.03 mm. The anagen hairs are identified in transverse sections by the presence of a normal-appearing inner root sheath and the absence of individual cell necrosis. The catagen hair is a brief resting stage showing loss of matrix and thin epithelium of dermal papilla. The catagen hairs are counted along with telogen hairs. The telogen hairs can be recognized below the level of the sebaceous duct by loss of inner root sheath. There is an irregular stellate configuration to the keratotic elements forming the remnants of the inner root sheath. Cornifying club having serrated rim, which inter-digitates with surrounding outer root sheath, characterizes an early telogen hair bulb. Late telogen hair follicle shows completely cornified club. The end stage of telogen or telogen germinal unit is seen as

an irregular basaloid star-shaped island of cells marked by a peripheral nuclear palisade. The scalp biopsy gives the actual number of hair follicles in the specified area, and their stage in the hair cycle can be assessed. It is also diagnostic in AGA and CTE. Also, this is the only technique to diagnose cases of inflammatory alopecia with scarring.

X-Ray Diffraction Studies of Hair: It have been used for ultrastructural studies of normal hair and effects of extraneous factors like hair dyes on structural integrity of hair.[67]

***Autoradiography*:**[68] This test is for the measurement of hair growth. The patient is injected 0.05 mL of saline containing 185 Bq L-cystineintradermally to form a wheal of about 6 mm diameter. A radioactive band is visualized on auto-radiographs. This procedure is repeated again after 3-4 weeks. The inter-radioactive distance gives the hair growth rate.

Conclusion: The global photography and questionnaire are of greater significance to the individual hair clinician whereas the phototrichogram is most suitable for clinical trials. Although scalp biopsy is diagnostic for female pattern hair loss, it is currently underutilized. While the results of fully automated computerized image analysis have largely been disappointing, there is a large scope of refinement in this field.[48]

ANDROGENETIC ALOPECIA (AGA)

(Syn.: Common Baldness, pattern alopecia, male pattern alopecia, female pattern hair loss, patterned or premature Baldness)

It is the most common cause of hair loss. Although it is medically benign condition, it can have a significant psychosocial impact for patients.

Aetiology of Female Androgenetic Alopecia:

AGA involves both genetic and hormonal factors. Genetics determine both the density and the location of androgen-sensitive hair follicles on site-specific areas of the scalp. After puberty, androgens trigger a series of events within these genetically programmed hair follicles, predominantly of the fronto-parietal scalp, that transform terminal to miniaturized follicle.[69]

Etiology of Female Pattern Hair Loss:

Genetics: Different genes may be involved in women in the development of FPHL. androgen receptor genes, aromatase genes and estrogen receptor gene (ESR2)[71] have been identified.

Estrogen receptor gene is (ESR2) predominant receptor within the hair follicle and thought to be the principal mediator of estrogen effects on hair follicle. The recent research on estrogen receptor beta gene, suggested that the hair follicle becomes more rather than less sensitive to estrogen, and the heightened sensitivity of the hair follicles to estrogen causes baldness in women with this particular gene.[71] In situations where women are taking either the contraceptive pill or hormone replacement therapy and they experience hair loss.

Systemic Hormones Effect: While the role of androgens is well established in the pathogenesis of male pattern hair loss, their role in female pattern hair loss has been recently questioned. There was no response in women without signs of androgen excess to cyproteroneacetate.[72] Even though scalp hair loss and hirsutism are essential features of hyperandrogenism in women, several investigations failed to demonstrate raised androgen levels in women. In all studies there is a variable proportion of

women with hair loss who do not show clinical or biochemical signs of androgen excess.[73]

Androgen-independent mechanisms too, are likely to be involved in genetically susceptible women. Another hypothesis is that in the presence of genetic susceptibility, it is the estrogen to androgen ratio (as represented by the ratio of free testosterone) that could trigger FPHL.[74]

Local hormones effect, Hair cycle dynamics, and Hair follicle miniaturisation: Whatever the etiology, the follicular changes in male AGA and FPHL appear identical, that is, there is a final common pathway of follicular miniaturisation with a progressive transformation of terminal hair follicles into vellus-like follicles and shortening of anagen phase with increase in kenogen phase.

Explanation for differences in pattern hair loss among men and women:[75]

1. Women have 3.0-3.5 times less 5α-reductase (I and II) respectively than frontal hair follicle in men.
2. Lower levels of circulating androgen, as estrogens play a protective role by lowering free androgen levels by increasing SHBG.
3. The total androgen receptor concentration is 40% less in women than male frontal hair follicle.
4. Aromatase is 6 times high in the frontal follicles and 4 times higher in the occipital follicles of women than in those of men.

Contact with arrector pili: In female pattern hair loss, uniform loss of contact was not demonstrated with arrector pili muscle. The follicle closest to the insertion of

the arrector pili appears to be the most resistant to miniaturisation. This explains the heterogenecity in miniaturisation in individual follicular units in FPHL.[76]

FEMALE PATTERN HAIR LOSS

Female pattern hair loss (FPHL) as a distinctive entity was first described about 30 years ago. The first chosen term androgenetic alopecia (AGA), which has been known for many years, is debatable because hair loss does not necessarily only occur in women with hyperandrogenemia. AGA in women has been termed as "female pattern hair loss" because the androgen dependence of hair loss in all women with patterned alopecia, has not been sufficiently demonstrated.[77]

Onset : True prevalence of female pattern hair loss is difficult to determine, given the most authors either have not clearly stated the diagnostic criteria used or have chosen to focus on only one pattern of FPHL. There are notable differences in the age of onset of pattern hair loss in men and women. In women, there are 2 main peaks of onset of pattern hair loss; the third decade and the second peak is from the age of about 40 through menopause.[77] Those with an earlier age of onset have more severe hair loss. Notably, Tosti *et al* has reported androgenetic alopecia in 20 children below age group 6-10 years.[78]

Clinical Features: Women may present with either an episodic or continuous increase in hair shedding without any noticeable reduction in hair volume or diffuse thinning over the crown with loss of hair volume with no history of hair shedding.[79] Women with AGA-related increased hair shedding often present prior to the development of reduction in hair volume over the crown. Women with androgenetic alopecia share the same area of potential hair loss as men, that is, the top of the scalp. However, phenotypically they do not usually have the same patterns of loss as

delineated in the Hamilton-Norwood. Majority of women are diagnosed as FPHL by thinning over the midline part with preservation of frontal hair margin, the pattern of loss that is only apparent when one performs a midline part. On closer examination with a lens, the hair unit number is reduced from the usual three hairs per unit to two or even single hair per unit. Moreover, small eraser like areas (4-6 mm) of partial or complete alopecia occur in areas or diffuse thinning.[77,80]

The "Christmas tree" pattern of hair loss i.e., increasing hair loss towards the front of the scalp, is another useful clinical clue to diagnose androgenetic alopecia in women.[81]

However, a majority of women do not present with the above clinical features and may go undiagnosed.[82] In a subset of women, hair loss occurs over the parietal, temporal and occipital scalp with or without vertex thinning, thus posing a diagnostic challenge to the clinician.

Patterns of hair loss in women:

- Diffuse central thinning with preservation of frontal margin (commonest).[83]
- Frontal accentuation (Christmas tree) that is, breach in frontal line.[77]
- Diffuse thinning of hair over entire scalp, often more noticeable thinning towards the back of scalp.[84]
- Diffuse thinning of hair over the parietal region.[85,86]
- Male pattern (frontoparietal).[87]
- Bitemporal thinning is commonly associated with, but not necessarily indicative of female pattern loss.[88]
- There is no any recession of the frontal hairline although the hair on the frontal margin is miniaturized that is, finer and shorter.

Demonstrable hyperandrogenism was noted in 10% of women with moderate to severe diffuse AGA without hirsutism or menstrual distrubances.[89] In females who presented with AGA, polycystic ovaries were observed in 28%-67%, hirsuitism in 21%, and acne in 43%.[90]

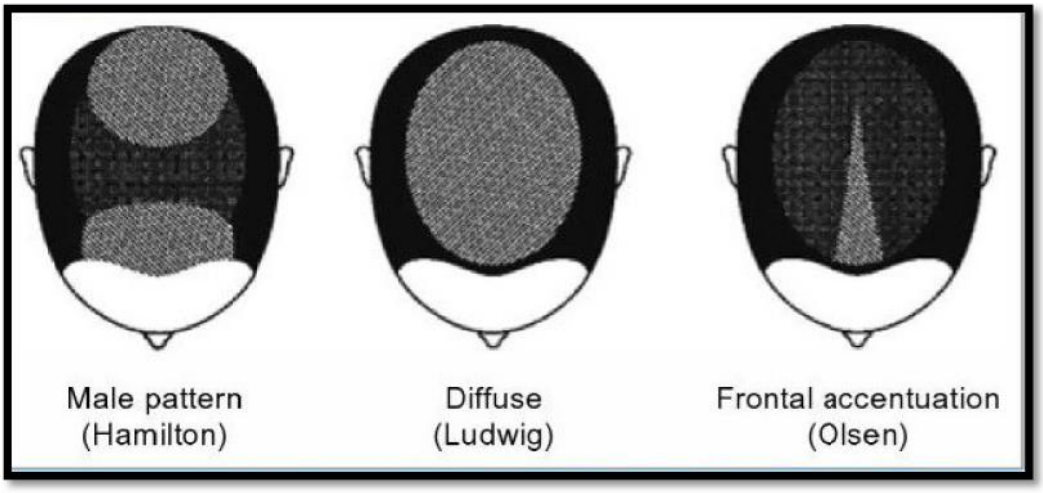


Figure 2 : Classification of androgenetic alopecia in females.
Hamilton's Male Pattern Type;
Ludwig's Diffuse Vertical Thinning Type;
Olsen's Christmas Tree Type of Hair Loss

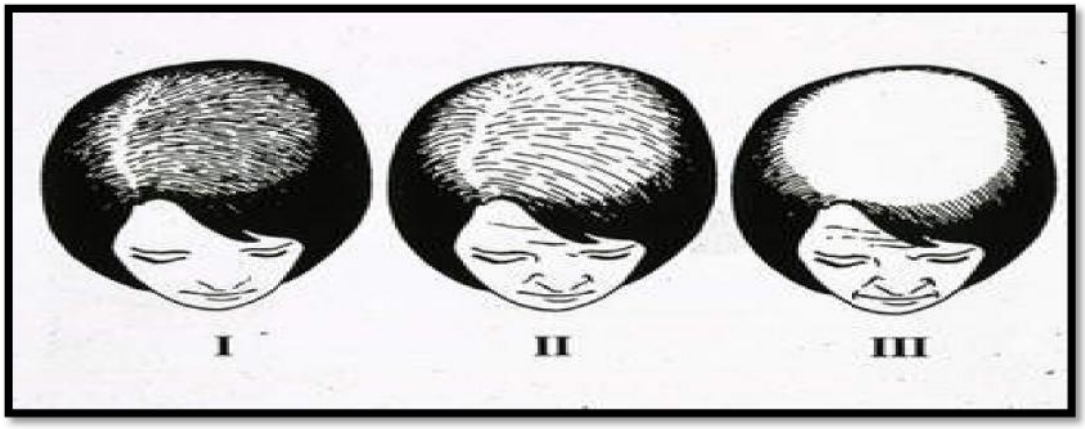


Figure 3 : Ludwig's classification for female pattern hair loss.

Grade I: Perceptible thinning of the hair on the crown, limited in the front by a line situated 1–3 cm behind the frontal hair line.

Grade II: Pronounced rarefaction of the hair on the crown within the area seen in Grade I.

Grade III: Full baldness (total denudation) within the area seen in Grades I and II.

Grading Scales for FPHL

They include; Ludwig scale[83], Olsen scale[77], Sinclair scale/5 point grading scale (Visual analogue scale)[92], Savin Scale[93] and BASP scale.[94]

Sinclair 5-Point Visual Analogue Scale which assesses the degree of hair loss using the midline part. This is a simplification of the widely accepted Savin Density Scale.

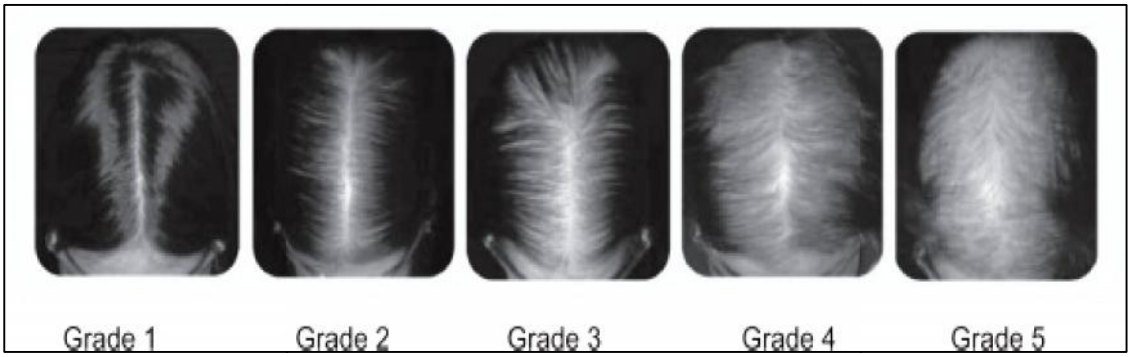


Figure 4 : Sinclair's 5 point visual analogue scale for FPHL

Grade 1: is normal. This pattern is found in all girls prior to puberty but in only forty-five percent of women aged eighty or over.

Grade 2: shows a widening of the central part.

Grade 3: shows a widening of the central part and thinning of the hair on either side of the central part.

Grade 4: reveals the emergence of a diffuse hair loss over the top of the scalp.

Grade 5: indicates advanced hair loss

Diagnosis: Evaluation of an adult woman presenting with diffuse, non-scarring hair loss of the scalp is rather arduous, considering the various causes of hair loss in women. In most cases, the anxiety and emotional distress of the patient may seem rather disproportionate to the degree of hair thinning.

Diagnosis of FPHL is mainly by clinical and trichoscopic methods and histopathological evaluation is necessary in difficult cases. Along with the scalp hair assessment, clinical examination for features of androgenism should be undertaken. Women with menstrual cycle disturbances or those exhibiting marked acne, hirsutism, or both, should be investigated fully.[77]

Women with mid scalp widening are straightforward case of FPHL. When women present with increased hair shedding, but little or no reduction in hair volume over the mid-frontal scalp, various differential diagnoses should be considered, in particular acute and chronic telogen effluvium and diffuse alopecia areata.

Other causes for diffuse hair shedding in women includes[79] drug-induced hair loss telogen effluvium, telogen gravidarum, chronic telogen effluvium (CTE), early androgenetic alopecia, diffuse alopecia areata, scarring alopecia, radiotherapy, iron deficiency, starvation/malabsorption/crash diet, hypothyroidism and hyperthyroidism, chronic renal failure, hepatic failure, syphilis, acute lupus erythematosis and advanced malignancy.

Acute and chronic telogen effluvium occur after a triggering event or repeated insults respectively, with a positive hair pull test from various areas of scalp and with no hair diversity in affected areas. CTE is an idiopathic entity showing no miniaturization of hair follicles on trichoscopy or biopsy even after long term follow-up.[95] Terminal to vellus ratio (T:V) < 4:1 on histopathology, differentiates FPHL from chronic telogen effluvium where T:V ratio is > 8:1.

In diffuse alopecia areata, the rapidity and volume of hair loss presenting in various areas; typical mixture of telogen, distorted anagen, and broken hairs on hair pull test and characteristic trichoscopic findings differentiate it from FPHL.

Trichotillomania may be localized to the top of the scalp as in female pattern hair loss but is distinguished by a negative hair pull and a confluence of short hairs of variable lengths in the affected area on trichoscopy and presence of trichomalacia on histopathology.

The rare loose anagen syndrome can be distinguished by a markedly positive painless hair pull test showing more than 80% anagen hairs devoid of sheaths.[95]

Cicatricial alopecia clinically appears with glossy underlying skin, perifollicular erythema, miniaturized hairs and a characteristic histopathology.

Trichoscopy plays an important role in diagnosis of early or Grade 1 and 2 of Sinclair classification when there is no apparent thinning or widening over the mid-scalp in women presenting with chronic diffuse hair loss.

Hair diameter diversity more than 20% is considered as criterion for diagnosis of FPHL by Tosti *et al.*[96]

DiagnosticTrichoscopic Criteria for FPHL (by Adriana Rakowskai[97])

Major Criteria: Ratio of;

1. More than four yellow dots in four images (70-fold magnification) in the frontal area.
2. Lower average hair thickness in the frontal area compared to the occiput.
3. More than 10% of thin hairs (below 0.03 mm) in the frontal area.

Minor criteria: Increased frontal to occipital ratio of;

1. Single-hair pilosebaceous units
2. Vellus hairs
3. Perifollicular discoloration

Fulfillment of two major criteria or one major and two minor allows to diagnose FAGA based on trichoscopy with a 98% specificity. However negative hair diameter diversity (less out 20%) on dermatoscopy does not rule out underlying FPHL.

Management of Female Pattern Hair Loss

Minoxidil: Topical minoxidil has been shown to arrest hair loss or to induce mild to moderate hair regrowth in approximately 60% of women with FPHL.[98] Topical minoxidil appears to be a safe therapy with side effects only of local irritation and hypertrichosis and there is a low incidence of contact dermatitis.

Antiandrogens: Oral antiandrogen therapy with cyproterone acetate, spironolactone or flutamide is widely used in the treatment of FPHL although there is a dearth of clinical trial data. Spironolactone is a synthetic steroid, acts by competitively blocking androgen receptors. It also weakly inhibits androgen biosynthesis. Flutamide is a potent orally administered anti-androgen that competitively blocks the binding of androgen to its receptor.[99] Flutamide can improve hair growth after only 6 months of treatment, and offers long-term stability in FPHL.[99] Finasteride has not shown the same efficacy in FPHL as seen in male AGA. In postmenopausal women, a 1-year course of finasteride 1 mg daily failed to improve hair loss over placebo.[100]

Hair Transplantation: It is emerging as an important option for patients with FPHL who do not have success with medical therapies. A new trend in hair transplants is the adjuvant use of platelet-rich plasma.

Camouflage: In recent years, topical sprays, powders, keratin fibers, hair extensions and wigs have become widely accepted among women as tools for improving hair appearance.

Light Therapy: A variety of laser and light sources have been tried for treatment of hair loss, with varied success.

DIFFUSE ALOPECIA

Diffuse hair loss is one of those complaints in dermatology in which the patient's symptoms are usually out of proportion with clinical signs. This is because at least 25% of the 100,000 scalp hairs need to be lost to produce any noticeable hair thinning.[101] Loss of more than 100 hairs/day is considered abnormal. Considering the average number of hair lost and the reduction in hair density that starts in the third decade, if there is significant hair loss, one should first rule out AGA. This is especially so in women in whom the pattern of hair loss is diffuse frontovertical thinning rather than the sharply defined bitemporal balding in men. Telogen effluvium should be considered next. It is readily diagnosed by a careful history and examination of lost hair and a trichogram if needed. Endocrine imbalances such as hypothyroidism, hyperthyroidism, or hypopituitarism should then be sought. Detailed and repeated queries about drug and chemical usage are needed to exclude this aetiology. Nutritional deficiencies due to malnutrition, malabsorption, or metabolic defects must be ruled out. Lastly, chronic liver dysfunction, malignancies, debilitating

diseases, and collagen vascular diseases can also induce the syndrome of chronic diffuse alopecia.

TELOGEN HAIR LOSS

Hair loss predominantly of the telogen type occurs in telogen effluvium. While it is clinically useful to determine whether hair loss is of the telogen or anagen type, it may not always be helpful for diagnosis. There are conditions where both telogen and anagen types of hair loss may occur, for example, cytotoxic drugs at lower dosages, which on long-term use can cause telogen hair loss and can cause anagen hair loss when administered at higher dosages. Similarly, AA in the acute phase shows more anagen hair loss than telogen, although it is often cited commonly as an example for telogen hair loss.

Conditions with telogen hair loss includes; telogen effluvium, AGA, postpartum alopecia, alopecia areata and neonatal alopecia.

Uncommon causes include hypothyroidism, medications (antithyroid drugs), withdrawal of OCPs, ionizing radiation and cytotoxic drugs.

TELOGEN EFFLUVIUM

Etiopathogenesis: It is the loss of telogen hair that occurs 2-4 months after an acute systemic stressful episode. The hair matrix, being one of the fastest multiplying tissues of the body, is highly susceptible to a variety of endogenous insults. The typical reaction pattern of the follicles is to "go into a shell" by reverting back to their inactive stage, that is, telogen. The situations under which this phenomenon occurs were collectively termed as "telogen effluvium" by Kligman.[101] However, many times a diffuse loss of telogen hair occurs because of hereditary and endocrine (AGA in

females) or autoimmune (AA) factors. Occasionally, more than one etiological factor may be operative (multifactorial alopecia). In iron deficiency anemia, follicles fail to regenerate following a normal telogen, leading to diffuse hair thinning in the absence any obvious hair shedding.[18,102]

Mechanisms of Telogen Effluvium[103]

Early anagen release: As occurs with stressful events, telogen is precipitated prematurely.

Delayed anagen release: As occurs with postpartum alopecia, hairs maintained in anagenfor an unduly long period are suddenly precipitated into telogen when the stimulus (estrogen and progestogen released from the placenta) is withdrawn. Neonatal alopecia and that following withdrawal of oral contraceptives may also follow this mechanism.

Short anagen cycle: This is proposed to because of the so-called chronic telogen effluvium wherein persistent shedding of telogen hair occurs due to shortened anagen. However, some authorities have doubted the existence of this entity as similar shortening of anagen phase occurs in AGA in women similar to that in men. However, in men, because of the easily recognizable patterning of the alopecia, differentiation from telogen effluvium is easier.

The stressful circumstances that can result in telogen effluvium include; febrile states, difficult labor, major accident or surgical trauma, hemorrhage, severe emotional stress, starvation, crash dieting, leukemia, lymphoma, and severe liver or kidney dysfunction. Telogen effluvium has also been observed occurring after allergic contact dermatitis of scalp to hair dyes.[104] It has been proposed that the anagen

follicles are adversely affected because of exposure to acute inflammation and hence the follicles enter the telogen phase to protect themselves, thus leading to "acute anagen release" and giving rise to telogen effluvium.

The febrile state is usually malaria, typhoid, or tuberculosis and the fever is more often high and recurrent.[18] Telogen effluvium never results in total alopecia and is always diffuse.

Diagnosis: On trichogram or phototrichogram, more than 20%, and at times more than 50%, of hairs are found to be of the telogen type. On histopathology the number of telogen hair follicles is substantially increased. The normal terminal:vellus ratio of 7:1 is preserved in telogen effluvium (cf. AGA in which the terminal: vellus ratio is <4:1).[105] Telogen effluvium needs to be differentiated from AGA in females, postpartum alopecia, neonatal alopecia, drug-induced alopecia, diffuse AA and alopecias caused by collagen vascular disorders, thyroid disorders, and pituitary disorders.

There is no specific therapy as the condition is reversible. The hairs regrow within 6 months, but in severe cases, regrowth may be incomplete.[106]

CHRONIC TELOGEN EFFLUVIUM (CTE)

It is characterized usually by an abrupt onset of diffuse hair loss of scalp persisting for more than 6 months and having a fluctuant course for up to 6-7 years. This condition predominantly affects healthy women in the fourth to fifth decade of life. Hair loss is so severe that on occasion up to 400-500 hairs can be lost per day. This severe hair thinning is often associated with marked bitemporal recession of the hairline.[107] A trichogram confirms telogen hair loss. A scalp biopsy reveals near

normal features except in early stages, when telogen follicles are significantly more than the anagen type. Absence of any obvious cause for telogen hair loss such as hormonal imbalance and deficiencies of macro and micronutrients is a prerequisite for the diagnosis of CTE. This condition needs to be differentiated from FPA. Patients need to be reassured that they will never go bald completely and will regain all hairs except when patients have underlying AGA.

POSTPARTUM ALOPECIA

Hair loss occurs 1-3 months after parturition in most women. This is a loss of telogen hairs that are retained during pregnancy because of the influence of high circulating estrogens that are "withdrawn" after delivery.

ALOPECIA CAUSED BY ENDOCRINE DISEASES

Diffuse hair loss is a frequent finding and at times is a presenting symptom of hypothyroidism.[108] The telogen count is raised as in telogen effluvium.[109] The alopecia is reversible after treatment but in severe and prolonged cases, follicular atrophy may occur.[110] In hyperthyroidism, alopecia is common but mild and reversible. Hypoparathyroidism and pseudo-hypoparathyroidism may also cause patchy alopecia.[106] Hypopituitarism may be associated with sparse hair.

DRUG-INDUCED ALOPECIA

Hormones: Antithyroid drugs such as propyl and methyl thiouracils and carbimazole can induce a hypothyroid state and resultant hair loss. Oral contraceptive pills may cause a temporary increase in the proportion of telogen hair that is reversible in 6 months.[18] Increased shedding of telogen hair 2-12 weeks after the stoppage of oral contraceptives, observed in some women, is similar to postpartum

alopecia. In genetically predisposed women, a high progesterone containing pill may induce AGA.[18] Cimetidine and danazol cause hair loss probably through a similar mechanism.[111] Bromocriptine, tamoxifen, and octreotide have also caused alopecia.[112]

Drugs Affecting Keratinization: Clofibrate, nicotinic acid, and other newer cholesterol-lowering agents such as atorvastatin and simvastatin can induce diffuse alopecia along with ichthyosis because of a disturbance of keratinisation.[113,114] Antipsychotics belonging to the butyrophenone group may also induce alopecia and ichthyosis possibly through a reduction of cholesterol levels.[111] Dixyrazine, a phenothiazine antipsychotic, has also caused hair loss along with ichthyosis.[115] Acute hypervitaminosis A results in generalized exfoliation and loss of hair, whereas a chronic vitamin A overdose leads to dryness of the skin and sparse, coarse, and brittle hair over the scalp and other regions. Retinoids, viz. isotretinoin and acitretin, when given in adequate doses can produce hair loss probably by progressively reducing the anagen duration and inducing a telogen anchorage defect.[116]

Antihypertensives: Propranolol[117] and other beta blockers[118] may cause diffuse alopecia in very few cases. Captopril chelates zinc ions that may lead to an acquired zinc deficiency state manifesting as alopecia that responds to zinc therapy.[111] Ramipril also produces alopecia by an unknown mechanism.[114]

Antiepileptics: Carbamazepine, valproate, trimethadione and gabapentin[119] have occasionally caused alopecia

NSAIDs: Chronic high-dose aspirin ingestion can cause iron deficiency anaemia, which may result in hair loss.[35]

Immunomodulators: Sulfasalazine, mesalazine, immunoglobulin infusions and IFN-α have occasionally induced alopecia.[117,120]

Other Drugs : Mepacrine and para amino salicylic acid cause a lichenoid eruption that can lead to cicatricial alopecia.[121] Allopurinol, amphetamine, amiodarone, bromocriptine, itraconazole, fluconazole,[122] terfenadine, loratadine, fluoxetine, gentamicin, lithium, tamoxifen,[123] tenoxicam, theophylline, omeprazole,[124] iodine, carbamide, metyrapone and pyridostigmine are uncommon causes of alopecia.

Newer Drugs: Amongst the relatively newer drugs, celecoxib, granisetron, leuprolide, tacrolimus, terbinafine and triptorelin[125] have been reported to cause alopecia[114]

Chemicals in the Environment: Rook and Dawber emphasize the importance of considering the possibility of accidental and occupational exposure to chemicals in the differential diagnosis of unexplained alopecia.[18] Occupational exposure to sodium borate, boric acid in washing powders, or use of boric acid containing mouthwashes has led to hair loss. Exposure to chloroprene, used in the manufacture of synthetic rubber,[18] and mercury can result in alopecia. Thallium is used in pesticides and rat poisons can be accidentally consumed, also can lead to hair loss.

Nutritional Alopecia: In protein-energy malnutrition, the hair growth slows, the shaft diameter reduces, and the hairs lighten in colour. In severe cases, the hair is brittle tending to alopecia. Alternate periods of better and worse nutrition may result in alternate bands of darker and lighter hairs commonly referred to as the flag sign. Essential fatty acid deficiency, developing in patients on long-term total parenteral nutrition, manifests as dry scaly skin, associated with diffuse scalp and eyebrow thinning. In acrodermatitis enteropathica and acquired zinc deficiency, alopecia develops later in the course of the disease following the gastrointestinal, central

nervous system, and cutaneous changes. Topical safflower oil, which contains 60%-70% of linoleic acid, is equally effective.[126] Biotin deficiency can cause hair loss, but is quite rare since intestinal bacteria synthesize the vitamin. Raw eggs contain avidin, which binds biotin thereby preventing its absorption. Extensive small bowel resection without parenteral supplementation may lead to biotin deficiency.[127] Recovery after biotin supplementation is dramatic. Malabsorption syndromes and pancreatic diseases are accompanied by alopecia, the mechanism of which is not understood.

Hair in HIV Disease: The hairs become thinner, lighter in colour, and sparser. Individuals with curly hair, loose their curls and the hair grows straighter.[128] However, curling of straight hair after administration of antiretroviral therapy has been reported.[129] Many of these changes are similar to those seen with nutritional deficiencies and may be because of the "wasting" caused by HIV infection. Alopecia may occur in the HIV infected patients, secondary to various medications such as zidovudine, lamivudine, indinavir, cidofovir and fluconazole. Although HIV causes immunosuppression and AA is associated with cell-mediated immunity, there are reports of AA and alopecia universalis occurring in HIV-infected patients.[130]

DIFFUSE NONSCARRING ALOPECIA OF OTHER CAUSES

Secondary and tertiary syphilis can be accompanied by moth-eaten alopecia, diffuse alopecia as well as loss of lateral eyebrows. Diffuse alopecia, may be one of the diverse manifestations of systemic lupus erythematosus. Similar alopecia may be observed in dermatomyositis, systemic sclerosis, mixed connective tissue disease and Sjogren's syndrome. Sparse, dry, thin and short hairs in the frontal region (lupus hair) are encountered in systemic lupus erythematosus and sometimes in dermatomyositis and Sjogren's syndrome. Cirrhosis of liver, renal failure and malignancies may be

associated with alopecia. Tumors involving the hypothalamus or post encephalitic damage to the mid brain have led to total permanent alopecia in some patients. Reversible hair loss may follow head injuries.

IRON[131]

The average daily intake of iron in Indian adult is 20 mg, but amount absorbed is equal only to losses. Thus the amount of iron absorbed is normally about 3-6% of the amount ingested.[131] Various dietary factors affect the availability of iron for absorption; for example, the phytic acid found in cereals react with iron to form insoluble compounds in the intestine, as do phosphates and oxalates. Most of the iron in the diet is in the ferric (Fe^{+++}) form, whereas it is the ferrous (Fe^{++}) form that is absorbed. Ferric reductase activity is associated with the iron transporter in the brush borders of enterocytes. Gastric secretions dissolve the iron and permit it to form soluble complexes with ascorbic acid and other substances that aid its reduction to the ferrous form. Almost all iron absorption occurs in duodenum. Most of the iron is bound to an iron binding protein apoferritin to form ferritin, which is the principle storage form of iron in intestine and many other tissues. Ferritin molecule can contain as many as 4500 atoms of iron. Hence it is main storage form of iron in tissues. In the plasma ferrous is converted to ferric and bound to the iron transport protein transferrin. Normally transferrin is about 35% saturated with iron and the normal plasma iron level is about 110µg/dL, in women. Of total iron (4-5 grams) in body, 70% is in haemoglobin; 3% in myoglobin and remaining 27% in ferritin. Ferritin is also found in plasma and ferritin-iron is in equilibrium with plasma-iron. In iron deficiency, the amount of circulating transferrin is increased and its percent saturation with iron is decreased; therefore, more iron moves from the intracellular iron carrier

to transferrin and less bind to apoferritin. Intestinal absorption of iron is regulated by- dietary intake, state of iron stores and state of erythropoiesis. The amount of iron lost from body in female is 1.2 to 1.5 mg/day. Iron deficiency occurs due to insufficient dietary intake. Common causes of iron deficiency are due to increased demand of iron for haematopoiesis, rapid growth in infancy/adolescence, pregnancy, lactation and erythropoietin therapy. Causes for iron deficiency due to iron loss include; blood loss during haemorrhage, childbirth, surgery, gastrointestinal tract loss, genitourinary tract loss, respiratory tract loss, menstruation, phlebotomy and blood donation. Iron deficiency also due to decreased absorption from GIT as in case of sprue, Crohn's disease, surgery and inflammation.

SERUM FERRITIN[132,133]

Ferritin is a protein complex that plays an important role in iron storage and is recognized as the main iron-binding protein in nonerythroid cells. Intracellular ferritin is synthesized by the smooth endoplasmic reticulum. Serum ferritin is synthesized by the rough endoplasmic reticulum and glycosylated by the Golgi apparatus before being secreted. Ferritin is a protein that stores iron, releasing it when the body needs it. Ferritin usually present in body's cells, with a little circulating in the blood and it contains 20 percent iron. The greatest concentrations of ferritin are in the cells of the liver and immune system. Transferrin is a protein that combines with ferritin to transport it to where new red blood cells are made. Generally, serum ferritin is directly related to intracellular ferritin and thus total body iron stores. Iron deficiency cause very low serum ferritin concentrations; therefore a low serum ferritin concentration is very specific for iron deficiency. Normal serum ferritin level ranges from 24to 300 µg/ L. According to some the normal range is 11 to 307 ng/ml in

women. Normal serum ferritin levels vary between laboratories but generally concentrations >300 µg/L in men and postmenopausal women and >200 µg/L in premenopausal women are regarded as elevated. Although many laboratories use serum ferritin concentrations of 10 to 15 ng/mL as the lower limits of normal based on reference sample groups, this only gives a sensitivity of 59% and a specificity of 99% for diagnosing iron deficiency. In women of childbearing age, using a cut-off of 10 to 15 ng/mL yields a sensitivity of 75% and specificity of 98%. A cut-off of 30 ng/mL yields a sensitivity of 92% and a specificity of 98%, while a cut-off of 41 ng/mL yields a sensitivity of 98% and a specificity of 98%.[134] Investigators consider serum ferritin to be the most powerful screening tool for iron deficiency. One large review concluded that serum ferritin had a greater predictive value than other tests of iron status, such as transferrin saturation and erythrocyte zinc protoporphyrin.[135] Ferritin is also an acute phase reactant and is elevated in anaemia of chronic disease.

IRON AND HAIR LOSS

Like other organs of body, hair also needs adequate nutrition for its proper growth and development, and hair is affected in various nutritional deficiencies. Besides protein-energy malnutrition, various micronutrients have also been studied as etiological factors of hair loss. Iron deficiency is the most common deficiency disorder in the world, and at the present time, is the one nutritional deficiency disorder, that still persists in developed countries.[12] Iron is involved in many critical physiological processes within the hair follicle, suggesting that iron deficiency could disrupt hair synthesis.[14] It is described that iron-dependent genes in the hair follicle bulge region may be affected by iron deficiency.[15] However, studies of iron as a cause for hair loss have produced conflicting results.

Ferritin is present in plasma in trace amounts, and the serum concentration correlates well with the amount of iron stores. Iron deficiency in the aetiology of hair loss has also been studied for more than 45 years. Ever since Hard in 1963 demonstrated the importance of non-anemic iron deficiency as an etiologic factor in diffuse loss of scalp hair in women, there have been many contradictory reports regarding the association of decreased iron stores with alopecia.[136]

The overall nutritional status of the people in this part of the world is poor and the prevalence of overt iron deficiency is high in our population, more so in women. It seems likely that the iron reserves even in non-anemic individuals may be low. Besides, consumption of a lot of tea further decreases the iron reserves in the body of individuals with borderline iron reserves.

For detection of iron deficiency (ID), serum ferritin level can be used as a very early marker. It is a main iron-binding protein in non-erythroid cells reflecting total body iron stores. It decreases from very early stage of iron deficiency as iron reserves go down. Because only ID can cause very low serum ferritin concentration (FC), a ferritin concentration is very specific for ID .

Haemoglobin and haematocrit[137]

Haemoglobin is a conjugated protein present in red cells, consisting of heme and globin. Adult haemoglobin are of two types; Hb A and Hb A2. Normal value of Hb in adult female is 12 to 15 gms/dL. Haemoglobin plays a major role in binding to oxygen within circulating red blood cells. The concentration of iron-containing haemoglobin in circulating red blood cells can be measured easily. Haematocrit is the percentage of blood that is occupied by red blood cells.

Haemoglobin concentration and haematocrit are frequently used to screen for iron deficiency because of their low cost and wide standard availability. However, haemoglobin concentration and haematocrit are only decreased in full-blown Iron Deficiency Anaemia (IDA), not in just iron deficiency. In addition, decreased haemoglobin concentration or haematocrit does not indicate the cause of anaemia. Reduced haemoglobin concentration and haematocrit can be found in many other conditions, such as folate deficiency, vitamin B_{12} deficiency, thalassemia, sickle cell disease, anaemia of chronic disease, and chronic renal failure. As the prevalence of iron deficiency has declined since the 1970s, anaemia has become a less effective predictor of iron deficiency. When anaemia is used in women of childbearing age to diagnose iron deficiency, it yields a sensitivity of 37% and a specificity of 93%.

FERRITIN AND HAIR LOSS

The relationship between body iron status and different types of hair loss has been investigated in a number of studies, however, with relatively discrepant findings.

Rasheed H. *et al*[138] in their study, found serum ferritin levels in telogen effluvium (14.7 ± 22.1µg/l) and female pattern hair loss (23.9 ± 38.5 µg/l) cases (n=80), were significantly lower than in controls (n=40).

Sinclair R[16] conducted a study to evaluate the relationship between low serum ferritin (≤20 µg/L) and chronic diffuse telogen hair loss in 194 women. His results, suggest that there is no clear association between low serum ferritin and chronic diffuse telogen hair loss.

Moeinvaziri M *et al*[139] in his case-control study found the mean ferritin level was statistically significantly lower in patients with diffuse telogen hair loss (n=30), than in subjects without hair loss (16.3 ± 12.6 vs. 60.3 ± 50.1 ng/mL).

Olsen EA *et al*[140] suggest by their study that iron deficiency is common in women but not increased in patients with female pattern hair loss or chronic telogen effluvium as compared to control subjects.

Esfandiarpour I *et al*[141]in their case control study, found a higher mean level of serum iron and ferritin in patients with hair loss as compared to control subjects.

Bregy A and Trueb RM[142] found no association between serum ferritin levels >10 microg/L, and hair loss activity in women.

Kantor *et al.*[143] showed that the mean ferritin levels in women with androgenetic alopecia and alopecia areata were significantly lower than those without alopecia, while as there was no difference in the mean ferritin levels of telogen effluvium, alopecia totalis and alopecia universalis patients, when compared with those of controls.

Reduced serum ferritin levels were also seen in patients with diffuse alopecia in studies conducted by Rushton *et al.*[136] and Van Neste and Rushton White *et al*, demonstrated similar findings in patients with alopecia areata. Positive association of low serum ferritin and alopecia in women was also reported by Kantor *et al.*[143] and Headington. Sinclair[16] has contradicted the above observation and is of the view that there is no clear association between low serum ferritin levels and alopecia. However, the lower limit of serum ferritin level was taken as 20ng/ml whereas in other studies it was taken from 40-70ng/ml. In a recent retrospective study conducted by Olsen *et al*,

they took 15ng/ml as a cut-off level and found that the incidence of iron deficiency is not more in patients with female pattern hair loss and chronic telogen effluvium. This lower limit of ferritin is a matter of hot debate for the present day trichologists all over the world.

In another case-controlled study by Aydingoz *et al*, the authors compared 10 female subjects with FPHL and 46 healthy controls. There was no difference in the prevalence of depleted iron stores or iron deficiency anaemia in both the groups.[144]

Rushton and Ramsay[13] demonstrated that women with androgenetic alopecia responded best to treatment with antiandrogen cyproterone acetate and ethinylestradiol when their serum ferritin level was above 40ng/ml.

In another study conducted by Rushton,[152] it was found that the optimal hair growth potential is considered to exist when specific parameters for biochemical variables are operating. These include red blood cell and serum folate concentrations within normal range, serum vitamin B12 levels between 300 and 1000ng/l, haemoglobin levels greater than 13gm/dl and serum ferritin concentration of 70ng/ml or greater.

It was also argued by Rushton *et al*.[136] that the reference range used by Sinclairfor serum ferritin has been derived from a population containing a high proportion of iron deficient women.

This difference in the results obtained as regards to chronic telogen effluvium patients while taking the lower limit of serum ferritin as 20ng/ml can be explained on the basis of the “threshold hypothesis” as proposed by Kantor *et al*. which states that the decreased iron stores, lower the threshold for developing different types of

alopecia. They proposed that in patients with a very strong genetic predisposition to developing alopecia, it is possible that low body iron stores are not important in triggering these disorders. In comparison, in those individuals with a mild hereditary predisposition or with the presence of other triggering factors, low iron stores may lower their threshold to the point where they develop alopecia. Theoretically it will be this subgroup of patients who are best candidates for iron therapy. Lastly, in those individuals without a hereditary predisposition or without other triggering factors, low iron stores would not cause alopecia.[136]

Since non-anemic iron deficiency was first suggested as an etiologic factor for diffuse hair loss in women in 1963, low iron stores have been considered a possible contributing factor in hair loss.[134] Assessment of serum ferritin levels is therefore generally recommended as part of the routine investigation, and dermatologists commonly prescribe iron supplementation in women under the assumption that low iron stores may be causing hair loss. However, contradictory data have so far failed to support this practice. Various observational studies have evaluated the association between decreased ferritin levels and hair loss and have resulted in opposing conclusions. As ferritin is also an acute-phase reactant, it is often elevated in the course of various diseases; in this case, a normal C-reactive protein (CRP) can be used to exclude elevated ferritin caused by acute-phase reactions.

In a prospective double-blind, placebo-controlled study in women with chronic telogen effluvium, subjects receiving 72 mg of iron and 1.5 g of L-lysine daily for 6 months showed a serum ferritin level increase, from 41.3 to 68.9 ng/mL and a 31% reduction in the amount of hair shed. The deleterious effects of iron deficiency are partly due to impaired oxygen delivery to the tissues and to a

deficiency of iron-containing compounds. From a biologic point of view, hair follicle matrix cells as the most rapidly proliferating cells in the body, appear to have lower levels of ferritin and higher levels of free iron. Another likely mechanism for the possible effect of iron on hair growth stems from its requirement as a cofactor for ribonucleotide reductase, the rate-limiting enzyme for DNA synthesis. Iron depletion could prevent proper function of this enzyme, resulting in inhibition of proliferation.

Low serum ferritin and vitamin D2 are associated with hair loss in females with TE and FPHL. Screening to establish these levels in cases of hair loss and supplementing with them, when they are deficient may be beneficial in the treatment of disease.

Some studies shows that hair loss is associated with decreased serum ferritin levels, while other studies reveal that there is no co-relation between hair loss and serum ferritin levels. Hence the relationship between hair loss and iron deficiency continues to be a subject of debate. More detailed cohort and interventional studies are necessary to assess the role of iron in diffuse hair loss, especially in women during childbearing years and to establish the truth regarding the cut-off level of serum ferritin, in patients with chronic diffuse hair loss. This is because there has been controversy over the cut-off level of serum ferritin, below which it can be defined as nutritional deficiency, triggering hair loss. Hence more studies are required in these directions.

MATERIALS AND METHODS

A cross-sectional design was adopted for this study as it was best suited for the purpose. The details of the study methodology are described below:

- ***Study Source:*** The study was conducted in the Department of Dermatology, Venereology and Leprosy, KLES Dr Prabhakar Kore Hospital and Medical Research Centre, Belagavi.
- ***Study Duration:*** This study was conducted between January 2015 to December 2015.
- ***Ethical Clearance:*** It was granted by the JNMC Institutional Ethics Committee of Human Subjects Research.
- ***Study Design***: Cross-sectional study.
- ***Sample size:*** The study was a non randomized observational study. This study was conducted on female patients having chronic diffuse hair loss, who attended the out-patient Department of Dermatology, Venereology and Leprosy during the study period. A sample size of 40 was selected based on the formula $n= Z\alpha^2 pq/d^2$ where $Z\alpha = 1.96$, $p= 63.8$, $q= 36.2$ and $d = 15$.
- ***Sample selection criteria:*** All the female patients aged between 15 to 45 years, having chronic telogen effluvium and female pattern hair loss, attending dermatology clinic of KLES Dr Prabhakar Kore Hospital and MRC, Belagavi, were recruited as per the inclusion and exclusion criteria. The study protocol was briefed and those subjects who came forward voluntarily to participate in the study were included in the study. None of these participants were taking treatment for hair loss.

- ***Inclusion criteria :*** All the consenting female subjects of the age group 15-45 years, with chronic diffuse hair loss, subjects having diffuse thinning of hair, subjects with patterned hair loss, attending out-patient department of Dermatology, Venereology and Leprosy between January 2015 to December 2015.
- ***Exclusion criteria:*** Subjects who did not give consent, subjects who were on iron therapy, undergone GI/scalp surgeries, suffering from trichotillomania, hormonal abnormalities and subjects who were on any regular medications for other systemic disorders.
- ***Data collection:*** Before starting the actual study, an informed and written consent was obtained from each participant, the format of which is shown in Annexure No. 1. A thorough physical and clinical examination of each participant was carried out, after eliciting a detailed history and only those who fulfilled the inclusion criteria were included in the study. All the participants were non-alcoholics, non-smokers and took no drugs. They all had almost similar diet, lifestyle, social background and activity. No physical abnormalities were detected in any of the participants. All the subjects had normal vision and hearing. They were sound physically, mentally and emotionally. A detailed history regarding the age, marital status, number of children, occupation, stress, duration of hair loss, family history, personal habits and history of previous treatment/surgery was taken. Dermatological and systemic examination was carried out. Details of hair, skin conditions and presence of pallor were noted. Clinical photograph of the conditions and tests were taken. The physical characteristics like name, address, occupation, age, Body Mass Index (BMI), duration of hair loss, marital status, number of

pregnancies, presence of stress, type of diet, exercise, past history of surgeries, diabetes, hypertension, exposure to abnormal environment, greying of hair, similar complaints in the family, usage of scarf/helmet, hair oil, shampoo, hair dye, mehendi, hair washing and combing methods were enquired and enlisted in Annexure No 2. Diagnosis of chronic telogen effluvium and female pattern hair loss was made on clinical examination and by performing hair pull test. The female pattern hair loss was graded by using Ludwig's scale. The data was noted in a pre-tested and pre-designed proforma. Serum ferritin and haemoglobin levels of the study subjects were measured by standardized method in Hi Tech laboratory of KLE's Dr Prabhakar Kore hospital & MRC, Belgaum.

- ***Advantages of the investigation:*** Hemoglobin concentration is frequently used to screen for iron deficiency because of its low cost and wide standard availability. However, hemoglobin concentration is only decreased in full-blown iron deficiency anemia, not just in iron deficiency. Generally, serum ferritin is directly related to intracellular ferritin and thus total body iron stores. Only iron deficiency causes very low serum ferritin concentrations; therefore a low serum ferritin concentration is very specific for iron deficiency. Further many investigators consider, serum ferritin to be the most powerful screening tool for iron deficiency. One large review concluded that serum ferritin had a greater predictive value than other tests of iron status, such as transferrin saturation and erythrocyte zinc protoporphyrin.[135] Hence these two methods (Serum ferritin and hemoglobin levels) were used to detect iron deficiency in patients with hair loss in the present study.

- ***Statistical method for data analysis:*** Analysis of result was made by using mean ± SD and Pearson's correlation coefficient, one sample 't' test was used wherever applicable. The results were expressed as percentage and significance.

- ***Tests used for diagnosis:***

  Hair Pull Test: It is also known as the traction test or Sabouraud's sign or the pull-out sign.[52] Approximately 20-60 hairs are grasped between the thumb, index and middle fingers from the base of the hairs near the scalp and firmly, but not forcefully, tugged away from the scalp. If more than 10% hairs are pulled away from the scalp, this constitutes a positive hair pull test and implies active hair shedding. The patient must not shampoo for at least a day prior to the pull test (Figure 5).

  Clinical correlation: This test is based on the concept of gentle pulling of the hair to bring about shedding of telogen hairs.[53] It helps to assess the severity and location of hair loss. The test is positive in cases of telogen effluvium, anagen effluvium, Loose anagen syndrome, early cases of patterned alopecia and at the advancing edge of alopecia areata.

Figure 5 :Traction test (Sabouraud's sign or the pull-out sign).

Merits and Demerits:

In hair pull test, the pulling force is distributed uniformly all over the whole bundle, which creates variation in the pulling force from one hair to another. Negative tests also do not rule out the diagnosis. However, these tests give clinician, an estimate of the amount of hair shedding.

- **The Ludwig Scale:** It uses 3 different classifications/grades/types, to diagnose the severity of female pattern hair loss. The grades are as shown in Figure 6.

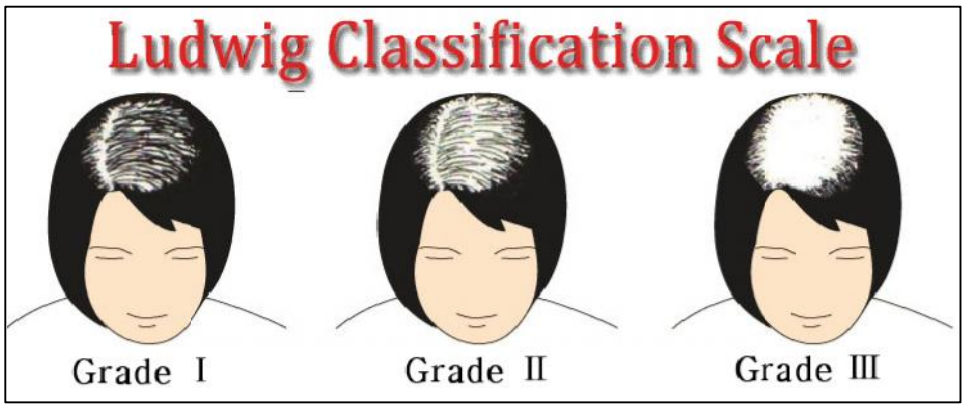


Figure 6 : Ludwig's grading for FPHL

Grade I: In this stage, hair loss is considered to be mild. Most women may have difficulty in noticing that hair loss has occurred, as the frontal hairline remains relatively unaffected. Hair loss may occur on the top and front of the scalp, however such hair loss may be noticeable when the hair is parted down the center of the scalp, as more and more scalp will become visible over time.

Grade II: This type of hair loss is considered as moderate. In this stage, women may notice each of the following: thinning, shedding, general decrease in volume, and a center part that continues to widen over time.

Grade III: It is the final and most extreme classification of female pattern hair loss. In this stage, hair is so thin that it has difficulty camouflaging the scalp,

rendering it visible to the naked eye. This may be worsened by a number of factors including hair miniaturization, progressive thinning and extensive loss.

- **Laboratory tests:**

  Hemoglobin estimation: In this study, EDTA blood was used for quantitative determination of hemoglobin concentration of blood samples.

  Principle: In a reagent solution the ferrous ions (Fe^{++}) of hemoglobin are oxidized to the ferric (Fe^{+++}) state by potassium ferricyanide to form methemoglobin, which subsequently reacts with the cyanide ions provided by potassium cyanide to form cyanmethemoglobin. The amount of cyanmethemoglobin can be measured spectrophotometrically at a wavelength of 540 nm and compared to known hemoglobin standards in order to determine the hemoglobin concentration of the unknown sample. Cyanmetheamoglobin method is the internationally recommended method for determining hemoglobin

  Cyanmetheamoglobin method: After the WBC dilution is lysed, the system shines a beam of white light through the WBC aperture bath and then through an optical filter. This transmittance of light, through a standard path length of hemoglobin solution is compared to the transmittance of such light in the same way through a reagent blank system and convert this ratio to absorbance. It then converts absorbance to hemoglobin values in gms/dL using calibration factor.

  Procedure: Sampling, reagent delivery, mixing, and processing and printing of results are automatically performed by the LH 500/780 series systems.

  Sample size: 185 µl for automatic mode, 125 µl manual mode.

  Reagent Volume: Diluent volume- 53 ml

Test Temperature: 18-29^{0}C

Reference range: Men: 13-16 gms/dL, Women: 12-15 gms/dL.

Calibration: Performed once in a year.

Serum ferritin estimation: By ADVIA Centaur System: The ADVIA Centaur Ferritin assay is a two-site sandwich immunoassay using direct chemiluminometric technology, which uses constant amounts of two anti-ferritin antibodies. The first antibody, in the Lite Reagent, is a polyclonal goat anti-ferritin antibody labeled with acridinium ester. The second antibody, in the solid phase, is a monoclonal mouse anti-ferritin antibody, which is covalently coupled.

The system automatically performs the following steps: It dispenses 25 µL of sample into a cuvette and dispenses 100 µL of Lite Reagent and 450 µL of solid phase and incubates for 7.5 minutes at 37°C. It separates, aspirates, and washes the cuvettes with reagent water. It dispenses 300 µL each of acid reagent and base reagent to initiate the chemiluminescent reaction and reports results according to the selected option.

Sample: Serum or heparinized plasma are the recommended sample types. This assay requires 25 µL of sample for a single determination. The serum sample should be allowed to clot adequately before centrifugation. The samples should be free of fibrin or other particulate matter. The sample should be tightly covered and refrigerated at 2 to 8°C if the assay is not completed within 48 hours. The two-point calibration is performed every 28 days. Normal Value: 6-160 µg/L.

RESULTS

A one year cross sectional study, to observe correlation of serum ferritin levels, in female patients with chronic diffuse hair loss, attending dermatology clinic of KLES Dr.Prabhakar Kore Hospital & MRC, Belagavi was conducted on 40 subjects from January 2015 to December 2015, to evaluate whether chronic telogen effluvium (CTE) and female pattern hair loss (FPHL) in patients, are associated with decreased tissue iron stores, as measured by serum ferritin and hemoglobin levels.

Primary objective was to measure serum ferritin and hemoglobin levels in patients with hair loss of the age group between 15 to 45 years, attending dermatology clinic at KLES Dr.Prabhakar Kore Hospital & MRC, Belagavi.

Secondary objective was to analyze correlation between serum ferritin and hemoglobin levels with hair loss by statistical methods.

Serum ferritin and hemoglobin levels of the study subjects were measured by standardized method in Hi Tech lab of KLES Dr.Prabhakar Kore Hospital & MRC, Belagavi.

The patients included in the study, underwent blood tests like estimation of serum ferritin and hemoglobin levels, which required drawing of blood. Hence, this intervention was stated in the informed and written consent and also was carried out after institutional ethical clearance.

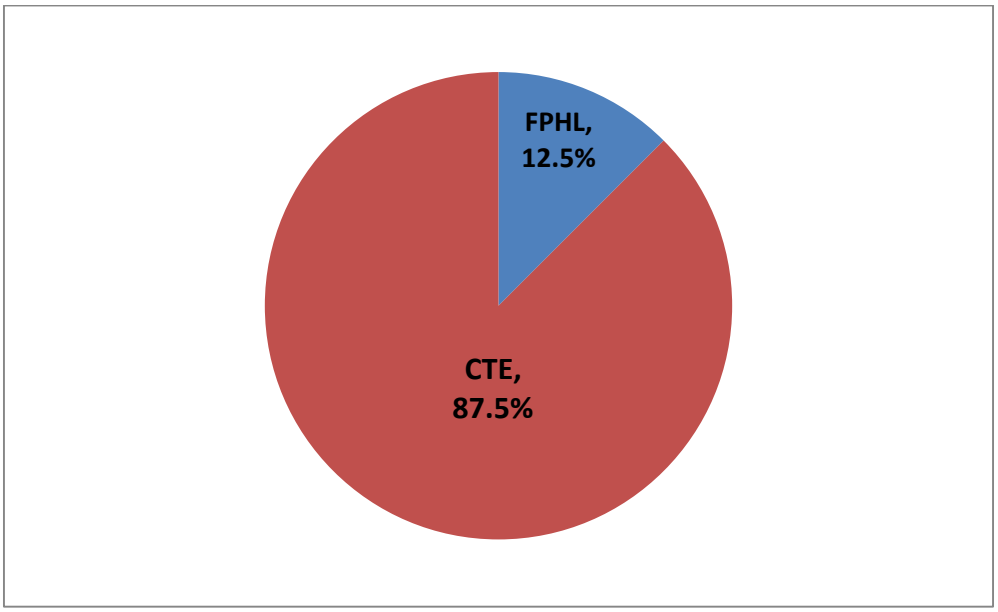
DISTRIBUTION OF TYPES OF HAIR LOSS:

Forty subjects having hair loss were included in the study as per inclusion and exclusion criteria; out of which 5 subjects were diagnosed as having FPHL (12.5%) and 35 subjects were having CTE (87.5%).

Table No 1: Distribution of subjects having different types of hair loss

Type of hair loss	Total no of subjects	Percentage
FPHL	5	12.5%
CTE	35	87.5%

Graph 1: Percentage of subjects having different types of hair loss



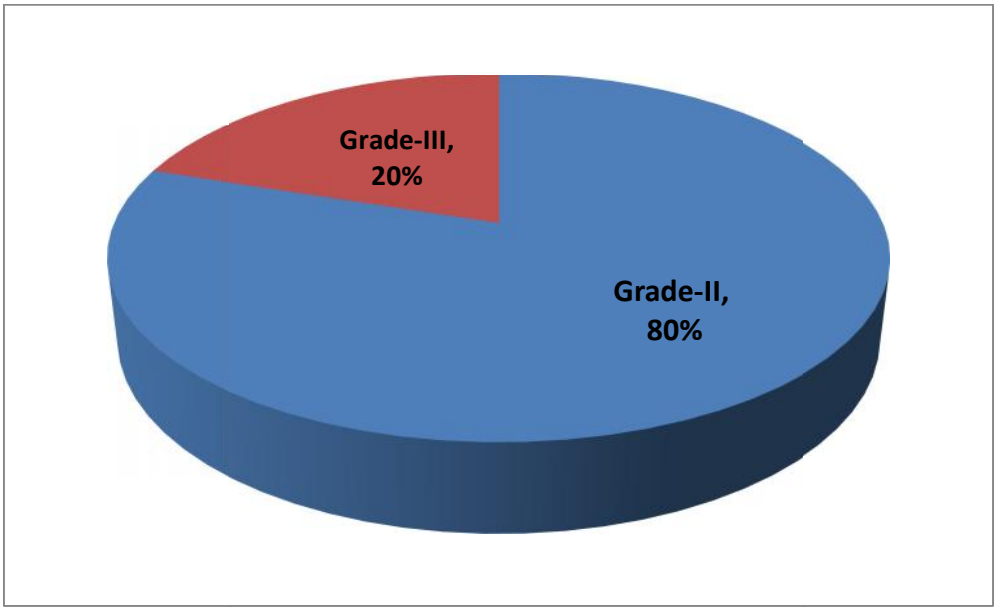
Hair Pull Test[46] was positive in all subjects having CTE and negative in subjects having FPHL.

Severity of hair loss was assessed in all 5 subjects of FPHL by Ludwig scale,[83] in which 4 subjects had grade II (80%) and one subject had grade III (20%) hair loss.

Table No 2 : Distribution of severity of FPHL (grading).

Grading (type) of hair loss	No of subjects	Percentage
Grade-I	0	0%
Grade-II	4	80%
Grade-III	1	20%

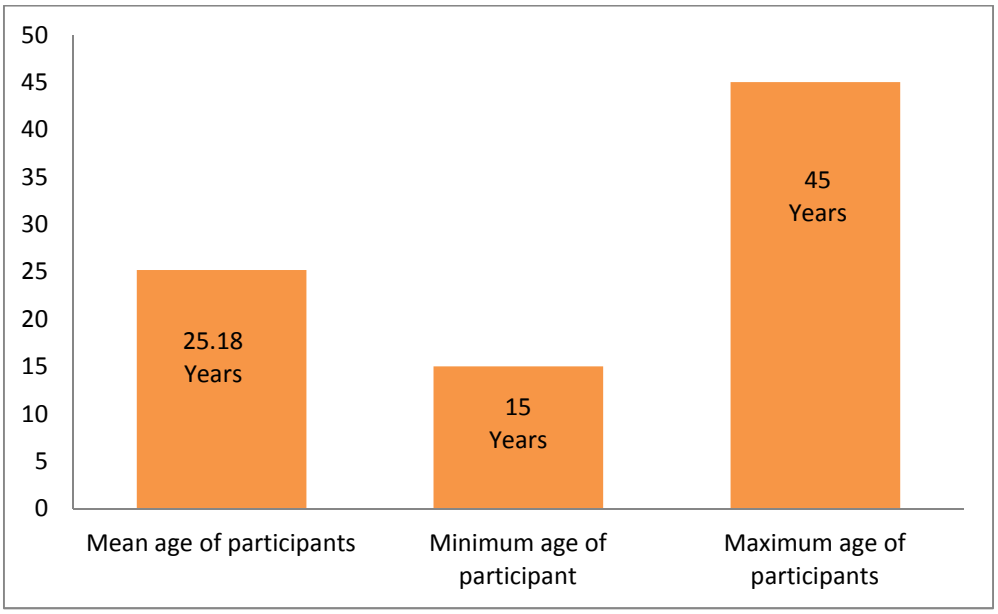
Graph 2 : Distribution of severity of FPHL (grading).



AGE DISTRIBUTION:

Mean age ± standard deviation of all participants was 25.18±8.94 years, with minimum and maximum age being 15 and 45 years respectively. So the age range was 15 to 45 years and median being 21.5 years.

Graph 3 : Age distribution of participants in years.



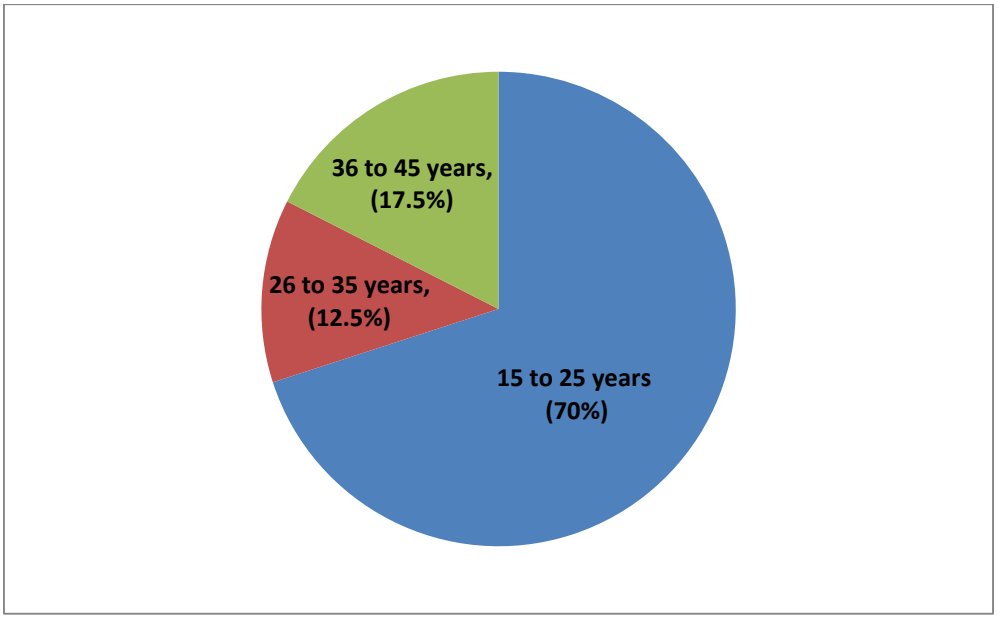
The number of subjects in the age group of 15 to 25 years was 28 (70%). Five subjects were in the age group of 26 to 35 years, which amounts to 12.5%. The number of subjects in the age group of 36 to 45 years was 7 (17.5%). Hence maximum number of subjects having hair loss were in the younger age group.

All the five subjects (12.5%) having FPHL were in the age group of 36 to 45 years, indicating this type of hair loss was prevalent in aged women. In case of females having CTE, only 2 subjects (5%) were in the age group of 36 to 45 years, 28 subjects (70%) were in the age group of 15 to 25 years and 5 subjects (12.50%) were in the age group of 26 to 35 years.

Table No 3: Distribution pattern of age in different types of hair loss.

	CTE		FPHL		All subjects	
Age group	No of subjects	%	No of subjects	%	No of subjects	%
15 to 25 years	28	70.00	0	0.00	28	70.00
26 to 35 years	5	12.50	0	0.00	5	12.50
36 to 45 years	2	5.00	5	12.50	7	17.50
Total	35	87.50	5	12.50	40	100.00
Mean age in years	22.63		43.00		25.18	
SD of age in years	6.11		2.92		8.94	

Graph 4 : Age distribution pattern of all subjects.



DISTRIBUTION OF DURATION OF HAIR LOSS

Mean duration ± standard deviation of hair loss was 20.28±16.84 months, which ranges from 6 to 60 months. Median duration of hair loss was 12 months, first quartile being 8.00 months and third quartile was 33.00 months. Duration of hair loss was less than 12 months in 23 (57.5%) subjects.

Table No 4 : Duration of hair loss of subjects in months.

Mean	SD	Median	Range
20.28	16.84	12	6 to 60

Graph 5 : Duration of hair loss in months.

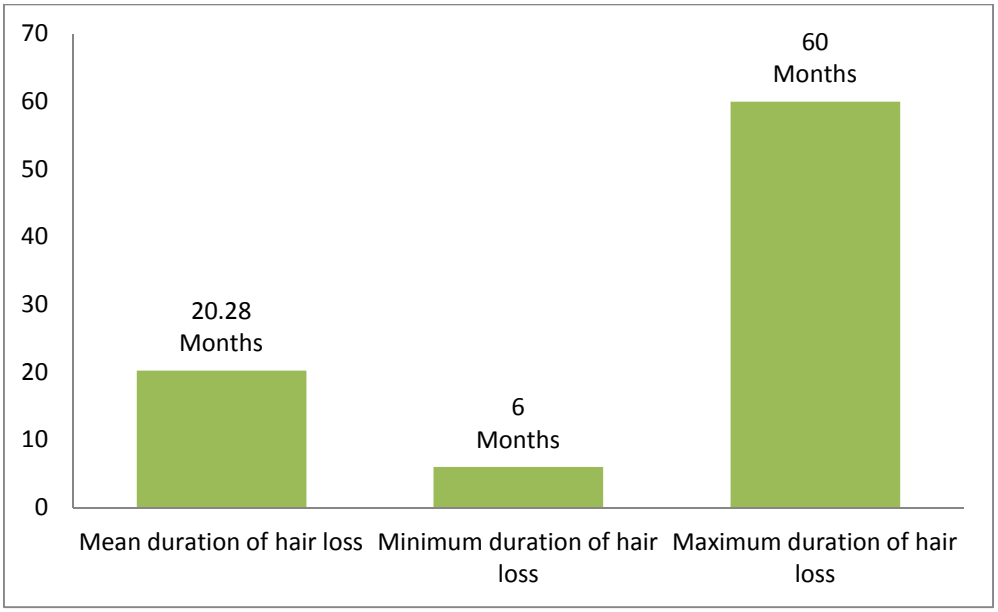
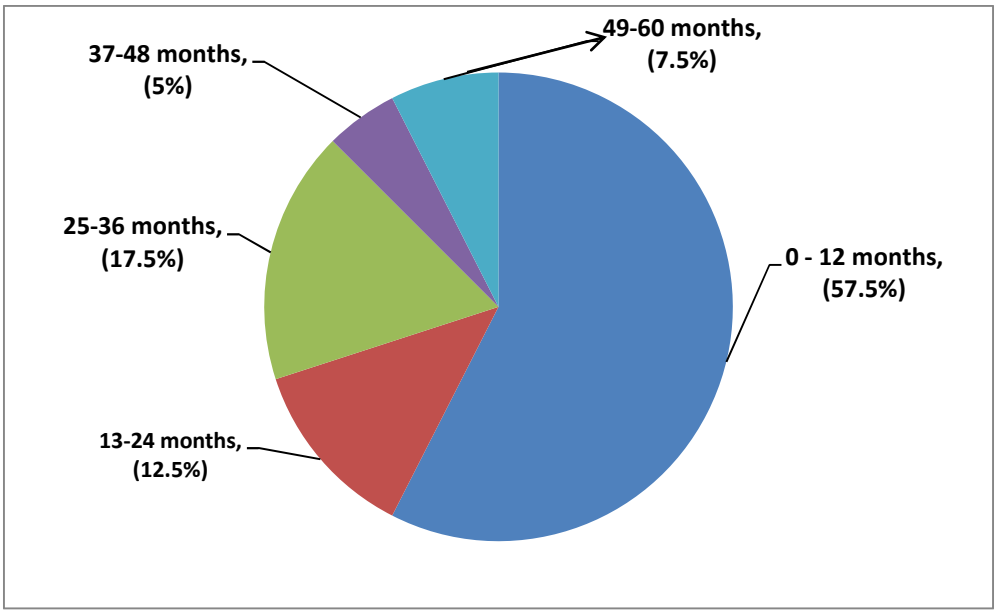


Table No 5 : Distribution of duration of hair loss in subjects.

Duration of hair loss	No of subjects	%
≤ 12 months	23	57.5
13 to 24 months	5	12.5
25 to 36 months	7	17.5
37 to 48 months	2	5
49 to 60 months	3	7.5

Graph 6 : Distribution of duration of hair loss.

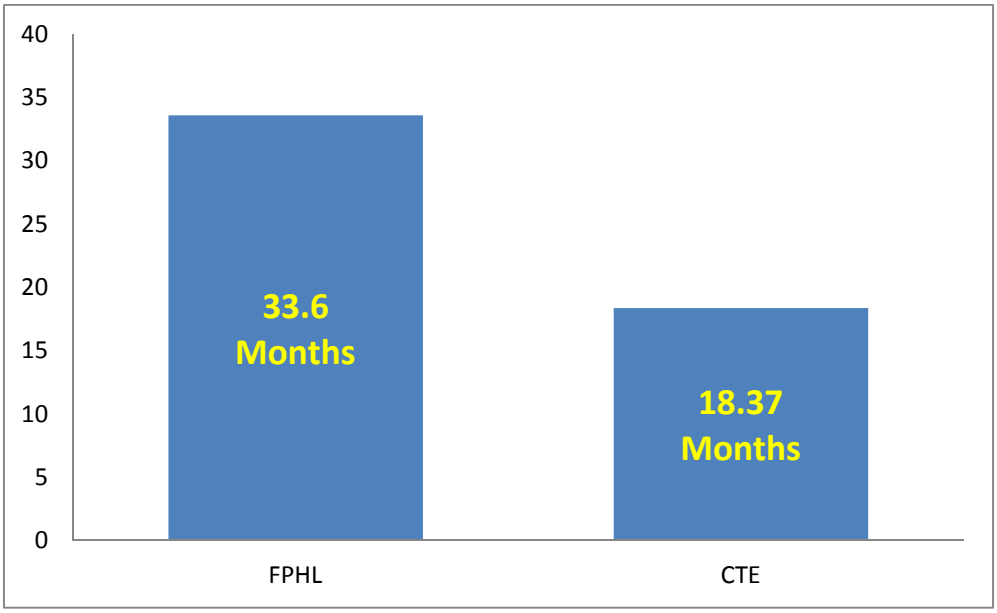


The mean duration of hair loss in FPHL was 33.60 months and 18.37 months in CTE; indicating duration of hair loss was more in FPHL as compared to CTE.

Table No 6 : Mean duration of hair loss in FPHL and CTE.

Type of hair loss	No of subjects	Mean duration of hair loss
FPHL	5	33.60 months
CTE	35	18.37 months

Graph 7 : Mean duration of hair loss in FPHL and CTE.



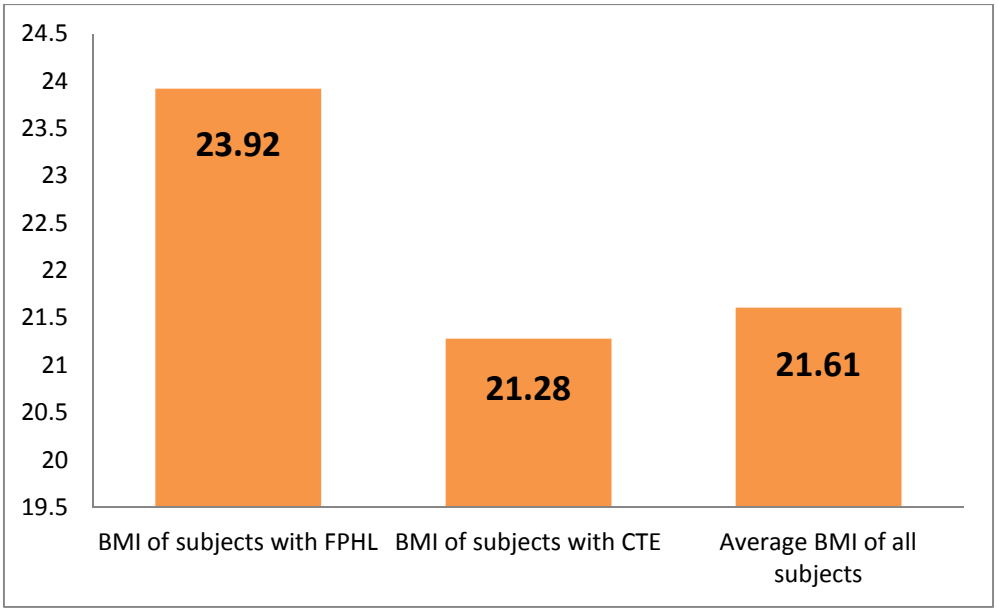
DISTRIBUTION OF BMI

The average BMI of all subjects was 21.61, which ranges from 16.4 to 26.42. The average BMI of subjects with FPHL was 23.92 and that of CTE was 21.28, indicating BMI of subjects with CTE was less than that of subjects with FPHL. The BMI of all the subjects were in the normal range.

Table No 7 : Distribution of BMI in subjects.

BMI of subjects with FPHL	BMI of subjects with CTE	Average BMI of all subjects
23.92	21.28	21.61

Graph 8 : Average BMI of all subjects and subjects with FPHL, CTE.



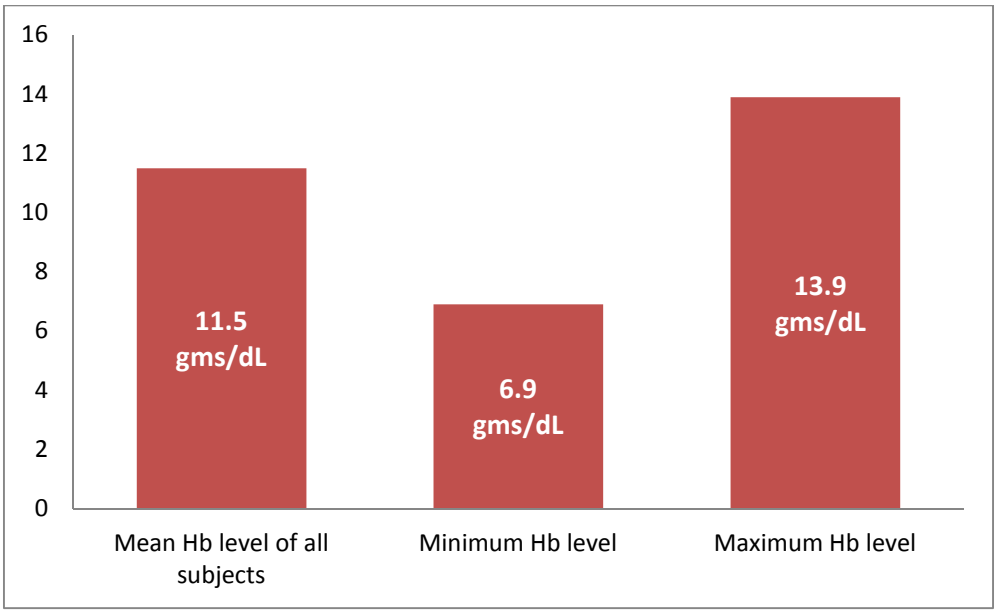
DISTRIBUTION OF HEMOGLOBIN LEVELS:

Mean hemoglobin (Hb) levels ± standard deviation was 11.5±1.76 gms/dL, which ranges from 6.9 to 13.9 gms/dL and median was 12 gms/dL. In this study, there was no significant difference between the mean Hb levels of all subjects as compared to normal minimum value of Hb (t=1.639, p= 0.109) by one sample 't' test. 18 out of 40 participants (45%) had Hemoglobin level less than 12 gms/dL, which was below the normal range. 22 out of 40 participants (55%) had Hemoglobin level more than 12 gms/dL.

Table No 8: Hemoglobin levels of subjects in gms/dL.

Mean	SD	Median	Range
11.5	1.76	12	6.9 to 13.9
Subjects having Hemoglobin less than 12 gms/dL		**Subjects having Hemoglobin more than 12 gms/dL**	
No of subjects	%	No of subjects	%
18	45	22	55

Graph 9 : Hemoglobin levels of subjects in gms/dL.



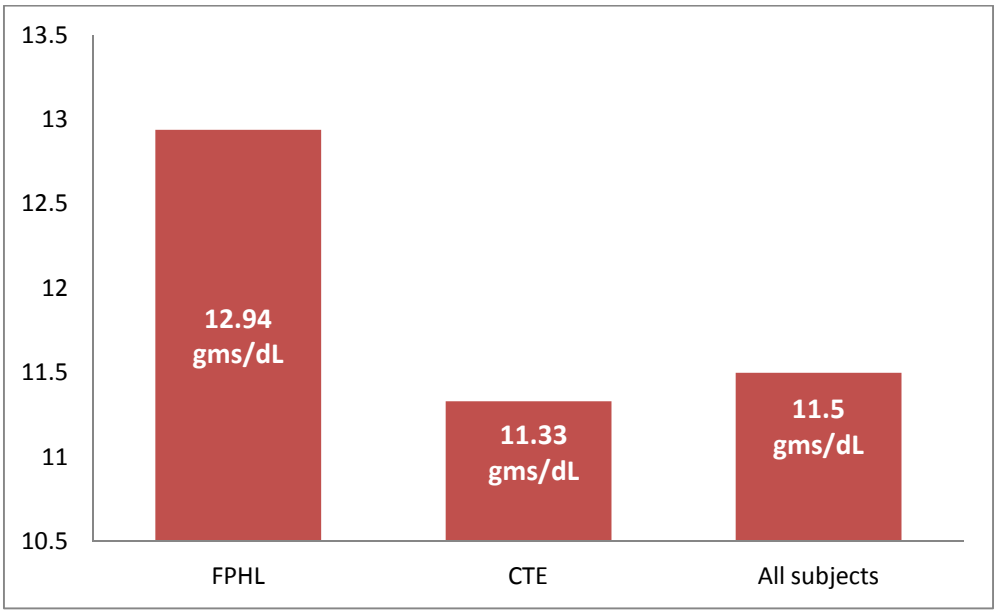
Distribution of Hb levels in different types of hair loss:

The mean Hb levels in FPHL was 12.94 gms/dL and in case of subjects with CTE was 11.33 gms/dL. There was statistically significant difference between the mean Hb levels of subjects with CTE as compared to normal minimum value of Hb (t= 2.168, p= 0.037*) by one sample 't' test.

Table No 9 : Distribution of Hb levels in different types of hair loss.

Mean Hb levels in FPHL	Mean Hb levels in CTE	Mean Hb levels of all subjects
12.94 gms/dL	11.33 gms/dL	11.5 gms/dL

Graph 10 : Distribution of Hb levels in different types of hair loss



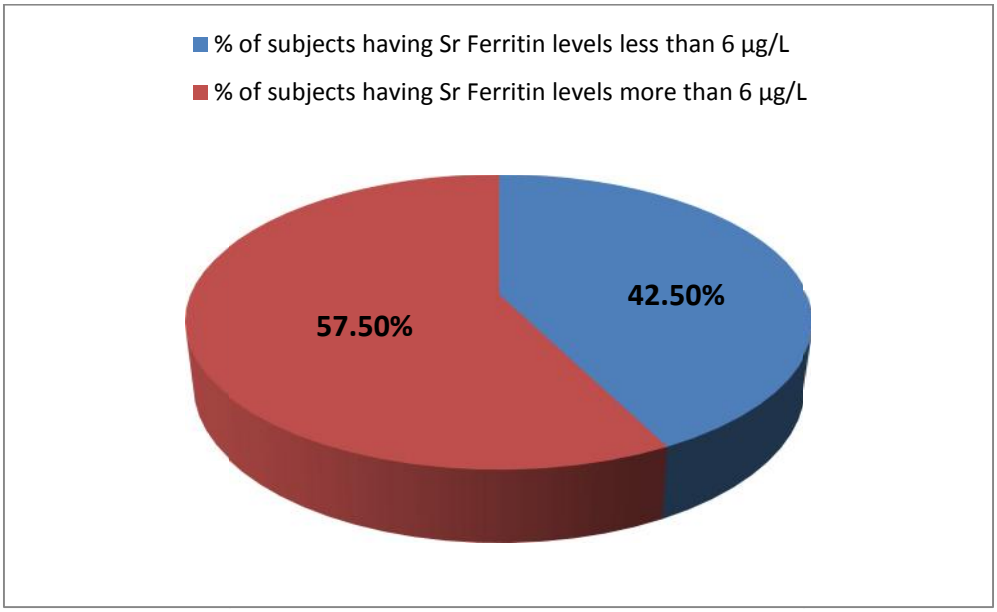
DISTRIBUTION OF SERUM FERRITIN LEVELS:

All the values of serum ferritin levels were converted from ng/ml to µg/L. Mean serum ferritin levels ± standard deviation was 18.6±21.08 µg/L, which ranges from 0 to 85.6 µg/L. Median serum ferritin levels was 8.9 µg/L, first quartile being 4.92 µg/L and third quartile was 36.4 µg/L. 17 subjects (42.5%) had serum ferritin levels less than 6 µg/L, which is below the normal value. There was statistically significant difference between the mean serum ferritin levels of subjects as compared to normal minimum value of serum ferritin (t= 5.163, p= 0.0001*) by one sample 't' test.

Table No 10 : Serum ferritin levels of all subjects in µg/L.

Mean	SD	Median	Range
18.6	21.08	8.9	0 to 85.6
Subjects having serum ferritin levels less than 6 µg/L		**Subjects having serum ferritin levels more than 6 µg/L**	
No of subjects	%	No of subjects	%
17	42.5	23	57.5

Graph 11: Percentage of subjects with serum ferritin levels <&>than 6 µg/L.



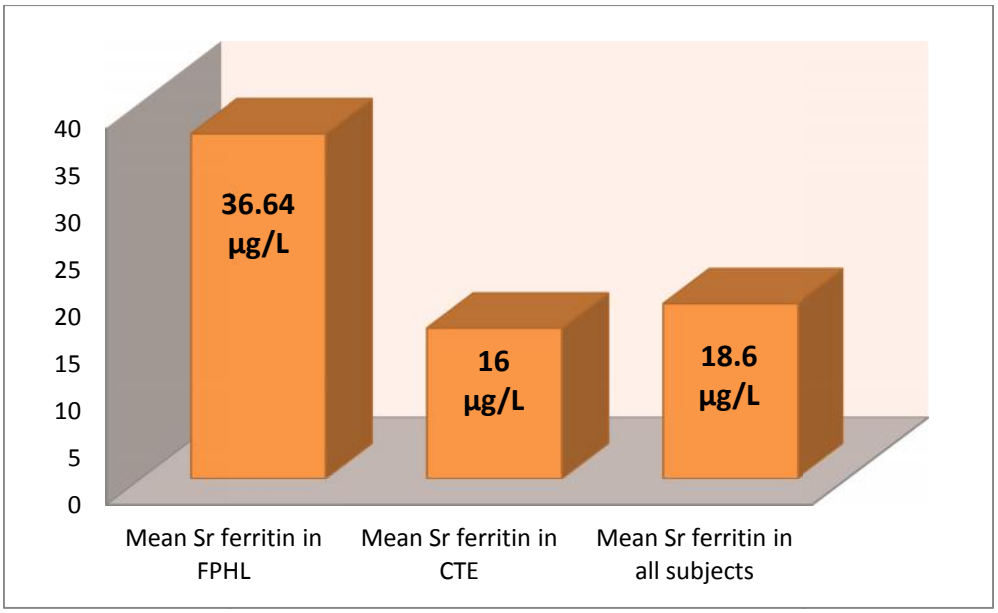
Distribution of Serum Ferritin levels in different types of hair loss:

The mean serum ferritin levels in FPHL was 36.64 µg/L and in case of subjects with CTE was 16 µg/L, indicating serum ferritin levels were low in subjects having CTE as compared to subjects with FPHL. There was statistically significant difference in the serum ferritin levels of FPHL and CTE types (‘t’=2.138, ‘p’=0.0389*)

Table No 11 :Distribution of serum ferritin levels in different types of hair loss.

Mean ferum ferritin levels in FPHL	Mean serum ferritin levels in CTE	Mean serum ferritin levels of all subjects
36.64 µg/L	16 µg/L	18.6 µg/L

Graph 12 : Distribution of mean serum ferritin levels in different types of hair loss.



23 participants (57.5%) had serum ferritin levels less than 12 µg/L indicating iron deficiency, 6 participants (15%) had serum ferritin levels ranging from 13-20 µg/L, indicating iron depletion. 10 participants (25%) had serum ferritin levels ranging from 21-70 µg/L, indicating serum ferritin levels are lower than required for normal hair cycle. Only one participant (2.5%) had serum ferritin level more than 71 µg/L indicating serum ferritin levels are within the normal range. 17 participants (42.5%) had serum ferritin levels less than 6 µg/L, which was definitely below the normal range as per specification enumerated in the test. 2 participants (5%) had serum ferritin levels as zero µg/L.

There is wide range of serum ferritin level (6-160 µg/L) and many laboratories use lower limit as 6-12 µg/L, which gives low specificity for diagnosis of iron deficiency. But as per the literature, a cut off level of 41 µg/L yields specificity of 98% and sensitivity of 98%. Hence the participants are also evaluated for the same. 30 participants (85.7%) of CTE had serum ferritin levels less than 41 µg/L and 3 participants(60%) of FPHL had serum ferritin levels less than 41 µg/L. In total, 33 participants (82.5%) had serum ferritin levels less than 41 µg/L.

When cut off level of serum ferritin was taken as 41µg/L, there was statistically significant difference between the mean serum ferritin levels of all subjects as compared to the cut off level of serum ferritin value of 41µg/L (t= 6.722, p= 0.0001*, by one sample ‘t’ test).

When cut off level of serum ferritin was taken as 41µg/L, there was statistically significant difference between the mean serum ferritin levels of subjects with CTE as compared to the cut off level of serum ferritin value of 41µg/L (t= 7.924, p= 0.0001*, by one sample ‘t’ test).

Table No 12 : Distribution of serum ferritin levels in study participants.

Serum ferritin levels	No of subjects	%
Less than 6 µg/L	17	42.5
Between 6-12 µg/L	6	15
Between 13-20 µg/L	6	15
Between 21-70 µg/L	10	25
More than 71 µg/L	1	2.5

Graph 13 : Distribution of serum ferritin levels in all study participants.

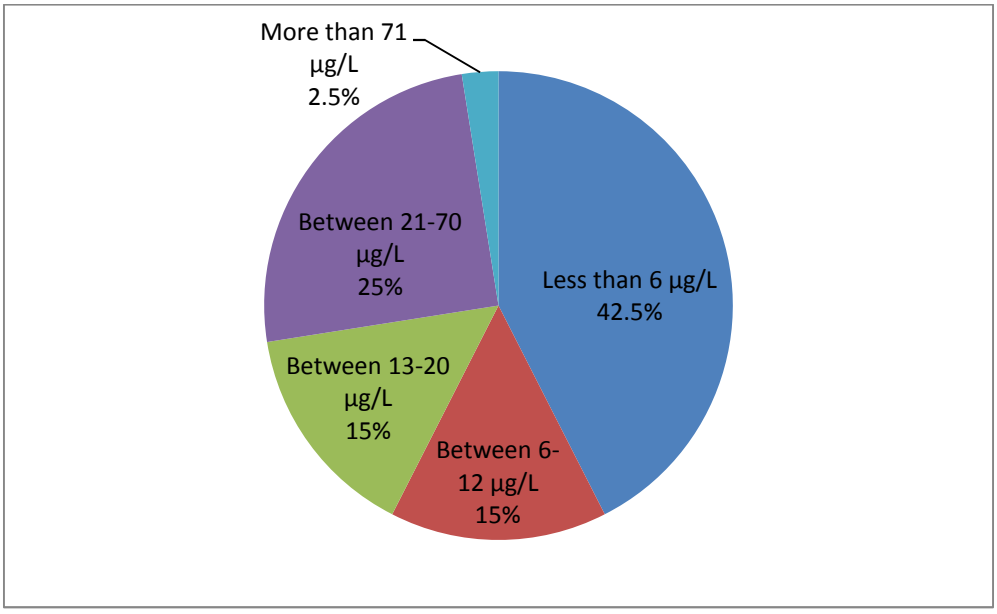
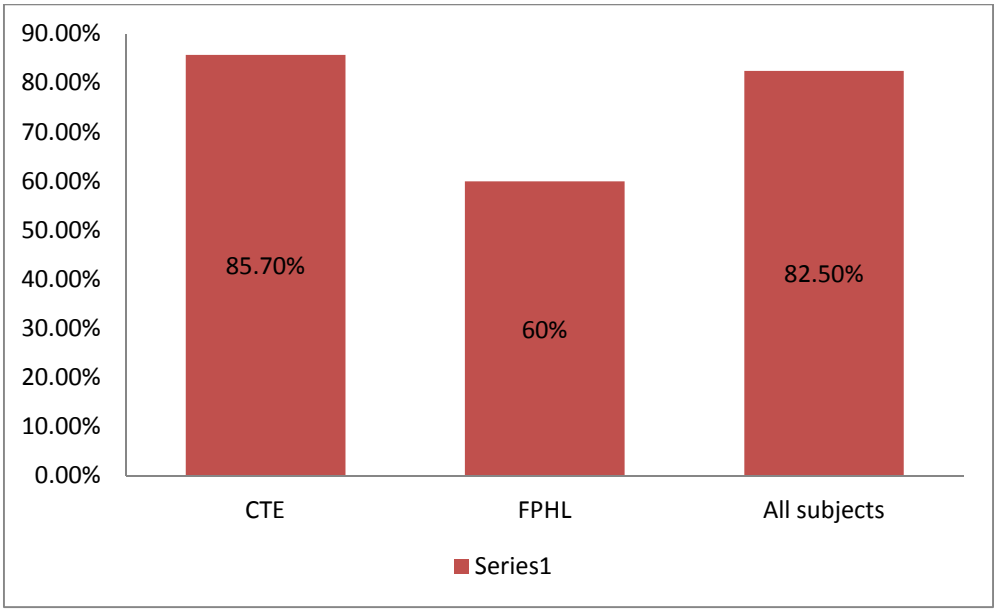


Table No 13 : Percentage of subjects having serum ferritin levels less than 41 µg/L.

Subjects	No of subjects	Percentage
CTE	30	85.7%
FPHL	3	60%
All subjects with hair loss	33	82.5%

Graph 14 : Percentage of subjects having serum ferritin level less than 41 µg/L.



DISTRIBUTION OF PALLOR:

The clinical pallor was present in 16 subjects (40%) and absent in 24 subjects (60%), out of the total 40 study participants. The clinical pallor was absent in all 5 subjects (100%) of FPHL and 19 subjects of CTE (54.2%). In case of subjects with

presence of clinical pallor, the mean Hb in those subjects was 9.92 gms/dL, which was significantly less than the normal lower range of Hb ('t'=5.0030, 'p'=0.0001* by one sample 't' test).

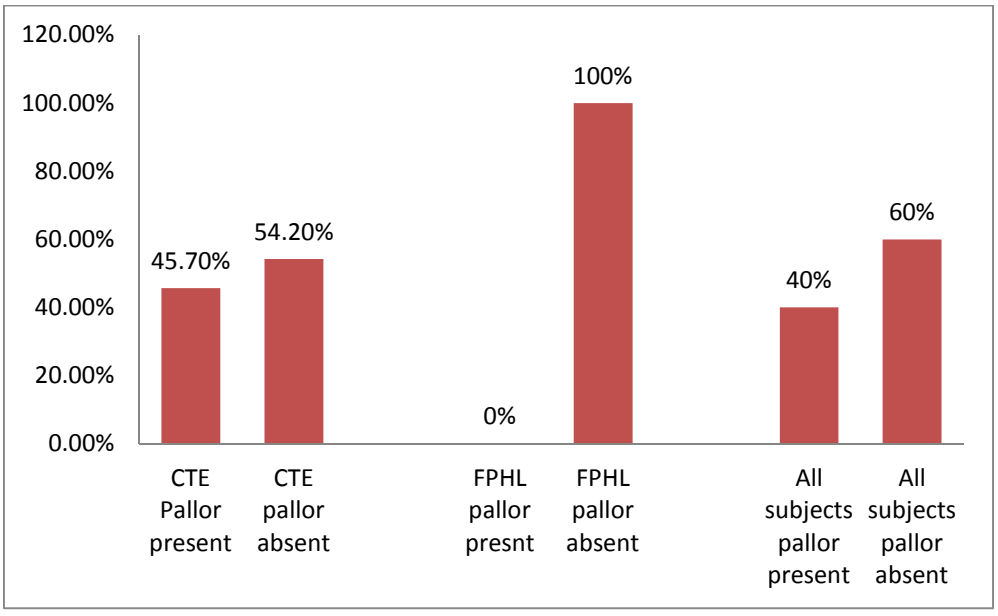
In case of subjects with presence of clinical pallor, the mean serum ferritin in those subjects was 3.89 µg/L, which was significantly less than the normal lower range of serum ferritin ('t'=3.0358, 'p'=0.0090* by one sample 't' test).

In case of subjects with presence of clinical pallor, the mean serum ferritin in those subjects was 3.89 µg/L, which was significantly less when cut off of serum ferritin level was taken as 41 µg/L ('t'=53.2560, 'p'=0.0001* by one sample 't' test).

Table No 14 : Distribution of clinical pallor in subjects.

Subjects	Clinical pallor present		Clinical pallor absent	
	No of subjects	%	No of subjects	%
CTE	16	45.7%	19	54.2%
FPHL	0	0%	5	100%
All subjects with hair loss	16	40%	24	60%

Graph 15 : Distribution of clinical pallor in subjects.



STRESS DISTRIBUTION PATTERN:

Out of total 40 subjects with hair loss, 19 subjects gave history of having stress (47.5%) in life and 21 subjects (52.5%) were devoid of stress.

Stress was present in 2 subjects (40%) and absent in 3 subjects (60%) of FPHL.

Stress was present in 17 subjects (48.5%) and absent in 18 subjects (51.5%) of CTE.

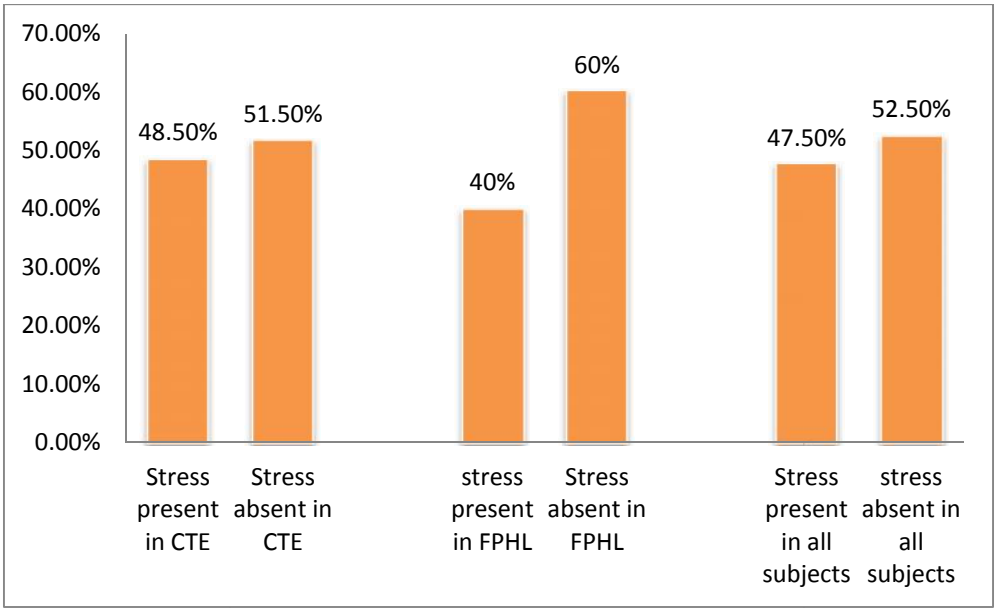
In case of subjects with presence of stress, the mean Hb in those subjects was 10.76 gms/dL, which was significantly less than the normal lower range of Hb ('t'=2.8420, 'p'=0.0111*by one sample 't' test).

In case of subjects with presence of stress, the mean serum ferritin in those subjects was 11.34 µg/L, which was not significantly less than the normal lower range of serum ferritin ('t'=1.1860, 'p'=0.2510 by one sample 't' test). In case of subjects with presence of stress, the mean serum ferritin in those subjects was 11.34 µg/L, which was significantly less when cut off level of serum ferritin was taken as 41 µg/L ('t'=6.5824, 'p'=0.0001* by one sample 't' test).

Table No 15 : Distribution of stress in study subjects.

Subjects	Stress present		Stress absent	
	No of subjects	%	No of subjects	%
CTE	17	48.5%	18	51.5%
FPHL	2	40%	3	60%
All subjects with hair loss	19	47.5%	21	52.5%

Graph 16 : Presence and absence of stress in study subjects.



CORRELATION OF AGE OF THE PARTICIPANTS WITH DURATION OF HAIR LOSS, HEMOGLOBIN LEVELS AND SERUM FERRITIN LEVELS:

Age of the participants was compared with duration of hair loss, which showed no association between the two ('r'=0.179, 'p'= 0.268).

Age of the participants was compared with hemoglobin levels, which showed positive correlation ('r'=0.314, 'p'= 0.048*).

Age of the participants was compared with serum ferritin levels, which showed positive correlation. ('r'=0.313, 'p'= 0.049*).

Table No 16: Correlation of age with duration of hair loss, hemoglobin levels and serum ferritin levels

Parameters	'r'	'p'	Correlation
Age with duration of hair loss	0.179	0.268	No correlation
Age with hemoglobin levels	0.314	0.048*	positive correlation
Age with serum ferritin levels	0.313	0.049*	positive correlation

CORRELATION OF DURATION OF HAIR LOSS WITH HEMOGLOBIN AND SERUM FERRITIN LEVELS:

Correlation between duration of hair loss and Hb was analyzed statistically which showed negative correlation (r = -0.020 and p= 0.903).

Correlation between duration of hair loss and ferritin was studied statistically, which showed negative correlation (r= -0.202 and p= 0.211).

Correlation between Hb and ferritin was studied statistically, which showed positive correlation (r=0.396 and p= 0.012*).

Table No 17 : Correlation of duration of hair loss with Hb and serum ferritin levels. Correlation between Hb and serum ferritin levels.

Parameters	'r'	'p'	Correlation
Correlation between duration of hair loss and Hb	-0.020	0.903	negative correlation
Correlation between duration of hair loss and ferritin	-0.202	0.211	negative correlation
Correlation between Hb and ferritin levels	0.396	0.012*	positive correlation

DISTRIBUTION OF SOCIOECONOMIC FACTORS:

Of 40 participants 13 (32.5%) were married and 27 (67.5%) were unmarried. 11(84.6%) out of 13 married women had children ranging from 1 to 4 and more number of married women had 2 children (n= 7). In 4 (36.3%) subjects, delivery was by LSCS.

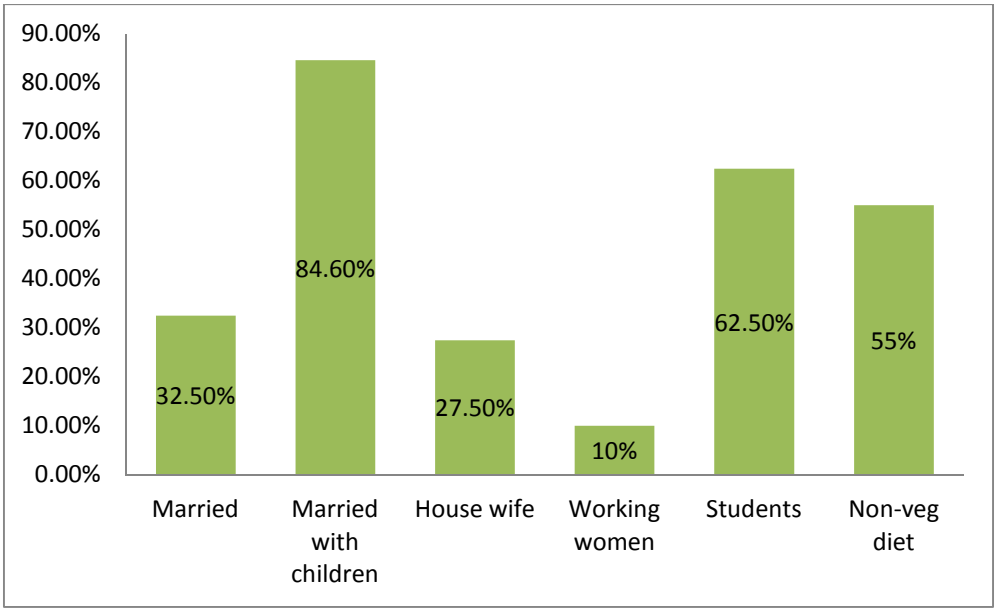
Of 40 participants 11(27.5%) were house wives, 4(10%) were working women and 25 (62.5%) were students. All the participants of FPHL were housewives. In subjects with CTE, 6 (17.14%) were housewives, 4(11.42%) were working women and 25 (71.42%) were students.

Of 40 participants 22 (55%) participants were taking non vegetarian diet and 18 (45%) participants were vegetarians.

Table No 18 : **Distribution of socioeconomic factors among all study participants.**

Status	No of participants	%
Unmarried	27	67.5%
Married	13	32.5%
Married with children	11	84.6%
Occupation: House wife	11	27.5%
Occupation: working	4	10%
Occupation: Students	25	62.5%
Diet: Non-veg	22	55%

Graph 17 : Distribution of socioeconomic factors in the study subjects.



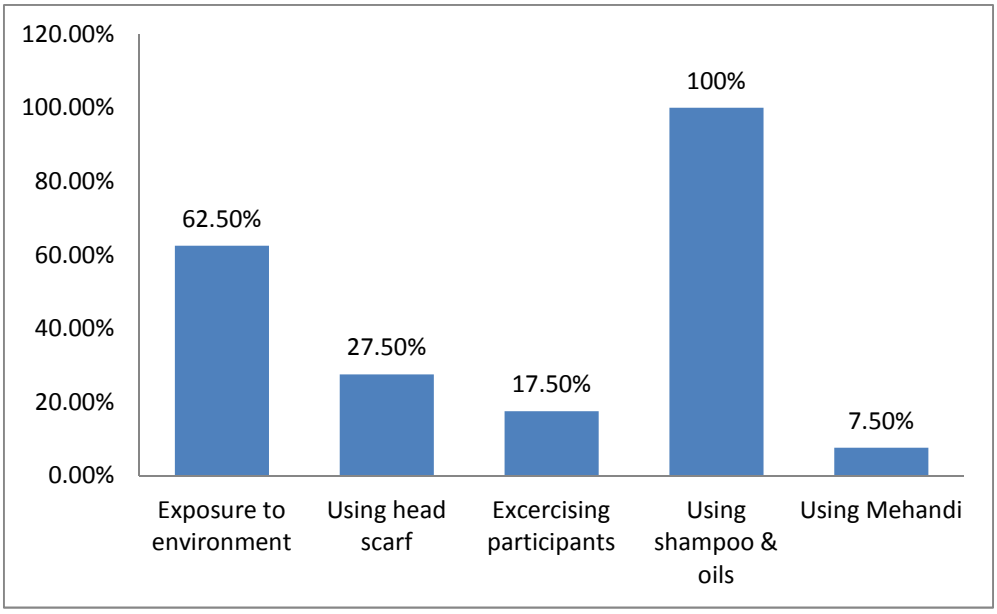
Parent of 2 (5%) participants had history of diabetes, 3 (7.5%) had hypertension, similar complaints of hair loss was present in the mother of 3 (7.5%) subjects.

25 (62.5%) participants gave the history of excess exposure to high external temperature and dusty environment. 11 (27.5%) subjects were using headscarf/helmet. 7 (17.5%) participants were exercising regularly like swimming, jogging and yoga. All the participants were using coconut oil, ayurvedic preparations and shampoo of different brands for hair application and washing. 3(7.5%) were in the habit of application of mehendi for coloring. They used to wash hair 1-3 times per week. 8(20%) participants had greying of hair.

Table No 19 : **Distribution of hair maintaining/application/washing behavior.**

Status	No of participants	%
Exposure to high changes in external environment and dust	25	62.5%
Subjects using head scarf/helmet	11	27.5%
Subjects exercising regularly	7	17.5%
Subjects using shampoo, coconut oil or ayurvedic preparations for hair	40	100%
Subjects using mehandi for coloring	3	7.5%

Graph 18 : Distribution of hair maintaining/application/washing behavior.



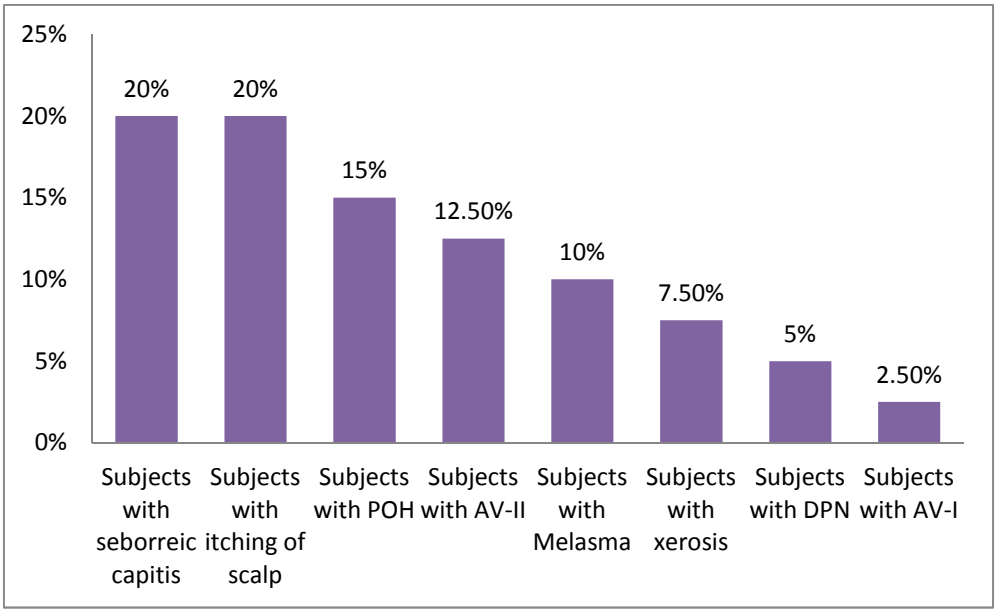
DISTRIBUTION OF ASSOCIATED CONDITIONS OF SCALP/HAIR:

8 (20%) subjects had seborrheic capitis. 8 (20%) participants had itching of scalp. 21 (52.5%) participants had suffered from other associated conditions *viz*; 6 (15%) had Peri-Orbital Hyperpigmentation (POH), 5 (12.5%) had Acne Vulgaris grade II (AV-II), 4 (10%) had melasma, 3 (7.5%) had xerosis, 2 (5%) had Dermatosis PapulosaNigra (DPN) and one (2.5%) had Acne Vulgaris grade I (AV-I).

Table No 20 : Distribution of associated conditions of scalp/hair.

Associated conditions	No of participants	%
Subjects with seborrheic capitis	8	20%
Subjects with itching of scalp	8	20%
Subjects with POH	6	15%
Subjects with AV-II	5	12.5%
Subjects with Melasma	4	10%
Subjects with xerosis	3	7.5%
Subjects with DPN	2	5%
Subjects with AV-I	1	2.5%

Graph 19 : Distribution of associated conditions of scalp/hair.



DISCUSSION

Hair loss is a common disorder in females, with an estimated life-time prevalence of 1.7%; however, this figure is not a reliable estimate, as very few epidemiological studies have been published in this regard, owing partly to under-reporting.[11] Hair loss has little or no physical harmful effects, but may lead to psychological consequences. Female pattern hair loss and chronic telogen effluvium, account for the majority of the cases of diffuse hair loss.[4] Conflicting observational data have failed to determine whether an association exists between hair loss and iron deficiency in the patients.[143] The relationship between body iron status and different types of hair loss has been investigated in a number of studies, however, with relatively discrepant findings.[139] Therefore this study has utilized an analytical cross-sectional methodology, to evaluate whether chronic telogen effluvium and female pattern hair loss in patients, are associated with decreased tissue iron stores. These findings have implications regarding understanding the triggers for hair loss, clinical trial design and therapeutics.

Diffuse hair loss affecting the entire scalp, for which no obvious cause can be found, indicates chronic telogen effluvium, while gradual diffuse hair loss with thinning of central scalp/widening of central parting line/frontotemporal recession indicates female pattern hair loss.

SAMPLE SIZE:

All the studies are performed on limited number of subjects drawn from the concerned population known as sample population. The data obtained is analyzed and conclusions are drawn which are extrapolated to the population under study. Hence

accurate sample size is basis of any study, to obtain unbiased results. The table below shows sample size of two types of hair loss in different studies.

Table No 21 : Comparison of sample size of various studies.

Studies	**Sample size in different types of hair loss**	
	CTE	**FPHL**
Present study	35 (87.5%)	5 (12.5%)
Shashikant Malkud[144]	28 (15.55%)	21 (11.66%)
Rasheed H, *et al*[138]	42(52.5%)	38 (47.5%)
Kirti Deo, *et al*[145]	84 (62.2%)	32 (23.7%)
Elise A Olsen, *et al*[146]	58 (25.43%)	170 (74.56%)

In this study, the total sample size was 40, out of which 35 (87.5%) subjects were having CTE and 5 (12.5%) subjects were diagnosed as FPHL. It shows that the number of subjects having CTE were more as compared to the FPHL, except in the study conducted by Elise A Olsen, where the subjects having FPHL were more in number comparatively, which may be due to inclusion of post-menopausal women in their study. Hence the most common type of alopecia was CTE as per our study and also in majority of other studies. Despite its exact prevalence being difficult to estimate due to its subclinical nature, CTE is generally considered to be the most common type of alopecia in females followed by FPHL. Although larger is the sample size, higher is the significance of study, due to time constraint only adequate number of subjects as per sample size calculation were included in the present study, strictly adhering to proper inclusion and exclusion criteria.

AGE DISTRIBUTION

The comparison of age distribution in present study with other studies is presented below.

Table No 22 : Comparison of age distribution in various studies.

Studies	Age range (in years)	Mean age (in years)
Present study	15 to 45	25.2±8.94
Shashikant Malkud[144]	12 to 54	25.9±7.99
Rasheed H, *et al*[138]	18 to 45	29.8±9.3
Kirti Deo, *et al*[145]	15 to 60	30.96±8.49

The mean age and age range of subjects in all studies appeared to be similar, though in the studies conducted by Shashikant Malkud and Kirti Deo, *et al*, upper age limits were 54 and 60 years respectively, as they have included post-menopausal women also. But in our study we have not included post-menopausal women, to avoid the influence of changed hormonal pattern on results of the study, because hormonal status can affect hair loss. In our study maximum number of subjects (n=28, 70%) were in the younger age group (15 to 25 years), as in case of CTE. Hence prevalence of CTE was highest in the younger age group. In this study, all the five subjects (12.5%) having FPHL were in the age group of 36 to 45 years, indicating this type of hair loss was prevalent in aged women.

DURATION OF HAIR LOSS

The mean duration of hair loss and its range in various studies is shown in the table below.

Table No 23 : Comparison of duration of hair loss in various studies.

Studies	Mean duration of hair loss (in months)	Range in months
Present study	20.28±16.84	6 to 60
Kirti Deo, *et al*[145]	15.64±16.003	1 to 84
Dr Pushpa Sarkar[147]	7.1±1.42	---
Shashikant Malkud[144]	---	7 days to 6 months

Mean duration of hair loss of our study (20.28 months) was comparable to the study conducted by Kirti Deo, *et al* (15.64 months). Range of duration of hair loss in the study conducted by Shashikant Malkud (7 days to 6 months), appeared to be short as compared to our study and mean duration of hair loss was not enumerated in his study. The mean duration of hair loss and its range of present study did not correspond to other studies because of different sample and inclusion, exclusion criteria. In this study lower limit of duration of hair loss was taken as six months because the patients take at least 3-5 months to seek consultation for their hair loss problem. In our study the number of subjects having hair loss duration for less than a year were higher in number (n=23, 57.5%). In this study the mean duration of hair loss was less in case of CTE (18.37 months), as compared to FPHL (33.60 months).

HEMOGLOBIN LEVELS

Hemoglobin concentration can be used to screen for iron deficiency, whereas serum ferritin concentration can be used to confirm iron deficiency.[12] Hence both parameters were estimated in this study. Hemoglobin concentration is frequently used to screen for iron deficiency because of their low cost and wide standard availability. However, hemoglobin concentration is only decreased in full-blown iron deficiency anemia (IDA), not only in iron deficiency. The mean hemoglobin levels and subjects having Hb level less than 12gm/dL, in various studies is shown in the table below

Table No 24 : Comparison of mean hemoglobin levels and subjects having Hb level less than 12gm/dL in various studies.

Studies	Mean hemoglobin levels (in gm/dL)	Range of Hb levels (in gm/dL)	Subjects with Hb less than 12gm/dL
Present study	11.5±1.78	6.9 to 13.9	18 (45%)
Kirti Deo, *et al*[145]	10.97±1.32	7.20 to 13.40	99 (73.4%)
Elise A Olsen, *et al*[146]	13.64	13.53 to 13.75	---
Shashikant Malkud[144]	---	---	50 (60.24%)

In this study mean hemoglobin level (11.5 gm/dL) and range of Hb levels (6.9 to 13.9 gm/dL) of subjects was almost similar to the study conducted by Kirti Deo, *et al* (10.97 gm/dL and 7.20 to 13.40 gm/dL). But in the study conducted by Elise A Olsen, *et al*, the mean Hb level was above minimal range (13.64gm/dL), since the sample included in their study was different.

According to World Health Organization (WHO), anemia is defined as hemoglobin level <12gm/dl. In our study 18 out of 40 participants (45%) had hemoglobin level less than 12 gms/dL, which was below the normal range. In the study conducted by Shashikant Malkud, low haemoglobin (<12gm/dl) was observed in 50/83 (60.24%) subjects and in 99 (73.4%) subjects in the study conducted by Kirti Deo, *et al*. Hence these data supports the fact that hair loss may be associated with low Hb and iron deficiency.

Table No 25 : Comparison of mean hemoglobin levels less than 12 gms/dL in different types of hair loss of various studies.

Studies	CTE	FPHL
Present study	18 (51.42%)	0%
Kirti Deo, *et al*[145]	63 (75%)	20 (62.5%)
Shashikant Malkud[144]	10 (50%)	6 (30%)

The above table shows the number of subjects along with percentage having Hb levels less than the normal levels (Normal range:12-16gm/dL) in different types of hair loss (CTE and FPHL).

In the present study the percentage of subjects having low Hb levels was 51.42%, in CTE, which was comparable to the study conducted by Shashikant Malkud in which it was 50%. In the study conducted by Kirti Deo, *et al*, the percentage of subjects having low Hb levels in CTE was higher (75%). These studies indicate that hair loss in CTE may be associated with iron deficiency.

On the contrary, in case of FPHL, the percentage of subjects having Hb levels less than the normal were minimal (0% in the present study and 30% in the study conducted by Shashikant Malkud), except in the study conducted by Kirti Deo, *et al*. where it was higher (62.5%), which may be due to different sample size and higher age group (15-60 years) included in their study.

SERUM FERRITIN LEVELS

Generally, serum ferritin is directly related to intracellular ferritin and thus total body iron stores. Investigators consider serum ferritin to be the most powerful screening tool for iron deficiency. One large review concluded that serum ferritin had a greater predictive value than other tests of iron status, such as transferrin saturation and erythrocyte zinc protoporphyrin.[135] The next table shows means serum ferritin levels of all subjects and in CTE, FPHL of various studies

Table No 26 : Comparison of mean serum ferritin levels of various studies.

Studies	Mean serum ferritin levels of all subjects	Mean serum ferritin levels in FPHL	Mean serum ferritin levels in CTE
Present study	18.6±21.08 µg/L (ranges from 0 to 85.6 µg/L)	36.64 µg/L (ranges from 14.1 to 85.6 µg/L)	16 µg/L (ranges from 0 to 58.9 µg/L)
Bregy A. Trüeb R.M.[142]	53.14 µg/L (ranges from 2 to 304 µg/L)	54.95 µg/L (ranges from 3 to 304 µg/L)	40.09 µg/L (ranges from 2 to 209 µg/L)
Shashikant Malkud.[144]	---	37.11 µg/L	39.39 µg/L
Elise A Olsen, *et al*.[146]	---	61.01 µg/L	51.81 µg/L
Rasheed H, *et al.*[138]	---	23.9±38.5 µg/L (ranges from 2.4 to 225.8 µg/L)	14.7±22.1 µg/L (ranges from 2.2 to 131.3 µg/L)
Kirti Deo, *et al.*[145]	54.73±41.5 µg/L (ranges from 5.10 to 200.70 µg/L)	---	---

Mean serum ferritin levels of this study were low (18.6µg/L), as compared to the studies conducted by Bregy A. Trüeb R.M and Kirti Deo, *et al*, where higher levels were evident (53.14 µg/L and 54.73 µg/L respectively).

Mean serum ferritin levels of our study in FPHL (36.64 µg/L) was comparable to the study conducted by Shashikant Malkud (37.11 µg/L), while it was still lower in the study conducted by Rasheed H *et al* (23.9 µg/L).Mean serum ferritin levels in

FPHL was higher in the studies conducted by Elise A Olsen, *et al* (61.01 µg/L)and Bregy A. Trüeb R.M (54.95 µg/L), as compared to our study (36.64 µg/L).

Mean serum ferritin levels of our study in case of CTE (16 µg/L) was comparable to the study conducted by Rasheed H *et al* (14.7µg/L), indicating association between iron deficiency and hair loss. Mean serum ferritin levels in the studies conducted by Shashikant Malkud (39.39 µg/L), Bregy A. Trüeb R.M (40.09 µg/L) and Elise A Olsen, *et al* (51.81 µg/L), in case of CTE were found to be higher, as compared to this study (16 µg/L). The lower values observed in the present study as compared to other studies is because of non-inclusion of post-menopausal women, inclusion of minimum duration of hair loss as six months and proper selection criteria of subjects, which eliminates confounding variables. In the study subjects of other studies, the range of serum ferritin was very wide (2-304µg/L), as compared to the present study (0-85.6µg/L), which was less. Further the mean serum ferritin levels were less in CTE as compared to FPHL, in all the above studies except in the study conducted by Shashikant Malkud, where it was almost similar. Also the range of serum ferritin in study subjects was very wide in FPHL as compared to CTE in all above studies. In the present study, 17 (42.5%) of subjects had serum ferritin level less than the lower range (6µg/L) and 2 participants (5%) had serum ferritin levels as zero µg/L.

Table No 27 : Comparison of different levels of serum ferritin with other study.

Studies	Serum ferritin levels			
	≤ 12 µg/L	**13-20 µg/L**	**21-70 µg/L**	**≥ 71 µg/L**
Present study	23 (57.5%)	6 (15%)	10 (25%)	1 (2.5%)
Shashikant Malkud[144]	7 (20.58%)	5 (14.70%)	14 (41.17%)	8 (23.52%)

In this study subjects were categorized based on their serum ferritin levels into 4 groups:[144] ≤12µg/L (iron deficiency), 13-20 µg/L (iron depletion), 21-70 µg/L (serum ferritin level lower than required for normal hair cycle) and ≥71 µg/L (normal ferritin level).

In this study, 57.5% subjects had serum ferritin levels ≤ 12 µg/L, as compared to only 20.58% subjects in the other study, indicating majority of our subjects were having iron deficiency. The percentage of subjects having serum ferritin levels from 13-20 µg/L in this study (15%) was comparable to other study (14.70%), which indicates iron depletion in the subjects. The percentage of subjects having serum ferritin levels from 21-70 µg/L was more in other study (41.17%) as compared to our study (25%), indicating serum ferritin level was lower than required for normal hair cycle in these subjects. Only one subject (2.5%) had serum ferritin level ≥ 71 µg/L, in this study, as compared to other study, in which there were 8 (23.52%) subjects having serum ferritin level ≥ 71 µg/L. This indicates only one subject in our study had normal ferritin level and all other subjects were having low storage iron as per the definition.

Cut-off levels of serum ferritin

The relationship between iron deficiency and hair loss has been examined in several studies, some of which suggest that iron deficiency (ID) even in the absence of IDA may be associated with certain kinds of hair loss. Many of these studies have different definitions of iron deficiency. The normal range of serum ferritin taken in this study, as per the specification of lab literature was 6-160 µg/L. As there is wide range in level of serum ferritin, there has been controversy over the cut-off level of serum ferritin, below which it can be defined as iron deficiency, triggering hair loss. Hence it has become necessary to use a cut off level of serum ferritin as adopted in other studies. Only iron deficiency causes very low serum ferritin concentrations. Using serum ferritin level as a marker for iron storage deficiency, the definition of iron deficiency (but not specifically iron deficiency anemia) in various studies has ranged from a serum ferritin level of ≤15µg/l to ≤70µg/l. Variety of serum ferritin levels are used in literature to define ID, Elise A Olsen, *et al* used 3 different definitions of ID: ≤ 15 µg/L, ≤ 40 µg/L and ≤ 70 µg/L, to evaluate prevalence of ID in their patients.[146]

Although many laboratories use serum ferritin concentrations of 10 to 15 µg/L as the lower limits of normal based on reference sample groups, this only gives a sensitivity of 59% and a specificity of 99% for diagnosing iron deficiency. In women of childbearing age, using a cutoff of 10 to 15 µg/L yields a sensitivity of 75% and specificity of 98%. A cutoff of 30 µg/L yields a sensitivity of 92% and a specificity of 98%, while a cutoff of 41 µg/L yields a sensitivity of 98% and a specificity of 98%. Serum ferritin levels above 70 µg/L are considered as normal. Hence same criteria are also used in this study.[134]

Table No 28 : Comparison of percentage of subjects at different cut-off levels of serum ferritin in various studies.

Studies	FPHL				CTE			
	≤ 15 µg/L	**≤ 20 µg/L**	**≤ 40 µg/L**	**≤ 70 µg/L**	**≤ 15 µg/L**	**≤ 20 µg/L**	**≤ 40 µg/L**	**≤ 70 µg/L**
Present study	20% (1/5)	60% (3/5)	60% (3/5)	80% (4/5)	68.57% (24/35)	74.28% (26/35)	85.71% (30/35)	100% (35/35)
Elise A Olsen, *et al*[146]	12.5%	---	58.8%	75.4% (215/285)	12.1%	---	63.8%	75% (72/96)
Sinclair R[16]	---	---	---	---	---	6% (12)	---	---
Rushton *et al*[148]	---	---	65% (200)	---	---	---	---	95% (200)
Shashikant Malkud[144]	---	---	---	82.35% (14/17)	---	---	---	88.23% (15/17)
Zhang *et al*[149]	---	---	---	35%	---	---	---	---

FPHL: In the present study 20% of subjects had serum ferritin levels ≤ 15 µg/L, while in the study conducted by Elise A Olsen, *et al* 12.5% subjects had serum ferritin levels ≤ 15 µg/L.

Our study results were comparable to other studies when cut off level of ≤ 40 µg/L used, in which 60% of our subjects fall under this group, while in the studies conducted by Elise A Olsen, *et al* and Rushton *et al*, 58.8% and 65% of subjects fall under this group respectively.

Even our study results were comparable to other studies when cut off level of ≤ 70 µg/L used, in which 80% of our subjects fall under this group. In the studies conducted by Elise A Olsen, *et al* and Shashikant Malkud, 75.4% and 82.35% of subjects fall under this group respectively. A study by Zhang *et al* reports lower value of 35%, when cutoff of ≤ 70 µg/L was used.

CTE: In the present study 68.57% of subjects had serum ferritin levels ≤ 15 µg/L, while in the study conducted by Elise A Olsen, *et al* only 12.1% subjects had serum ferritin levels ≤ 15 µg/L. This shows majority of our subjects with CTE had iron deficiency when cut off levels of serum ferritin was used as ≤ 15 µg/L.

When serum ferritin cut off level of ≤ 40 µg/L was used, 85.71% subjects in the present study and 63.8% of subjects in the study conducted by Elise A Olsen, *et al* were falling in this group. This also indicates majority of our subjects with CTE had iron deficiency when cut off levels of serum ferritin was used as ≤ 40 µg/L.

Our study results are definitely comparable to other studies when cut off level of ≤ 70 µg/L used, in which 100% of our subjects fall under this group. In the studies conducted by Elise A Olsen, *et al*, Rushton *et al* and Shashikant Malkud, 75%, 95% and 88.23% of subjects fall under this group respectively.

This shows majority of our study subjects had ID, when cut off level of serum ferritin was used as ≤ 70 µg/L (FPHL=80% and CTE=100% of subjects). Even when cut off level of serum ferritin was used as ≤ 40 µg/L, 60% of subjects in FPHL and 85.71% of subjects in CTE, showed ID. When cut off level of serum ferritin was used as ≤ 20 µg/L, 60% of subjects in FPHL and 74.28% of subjects in CTE, showed ID. To conclude, our study subjects had low serum ferritin levels at different definitions

of ID and shows CTE, FPHL are associated with decreased iron stores. As ferritin levels accurately reflect body iron stores, our study clearly demonstrated the association between low iron stores and hair loss.

PSYCHOLOGICAL STRESS

Psychological stress is one of the commonest etiological factor and also an exacerbating factor for hair loss. The presence of psychological stress in various studies is shown in the table below.

Table No 29 : Comparison of presence of psychological stress in various studies

Studies	Presence of stress in number and percentage of subjects
Present study	19 (47.5%)
Shashikant Malkud[144]	48 (36.92%)
Kirti Deo, *et al*[145]	86 (63.7%)
Rustom *et al*[150]	21 (42%)

In the present study, 47.5% of subjects gave the history of psychological stress, which is comparable to the study conducted by Rustom *et al* (42%). The occurrence of psychological stress in the subjects was higher in the study conducted by Kirti Deo, *et al* (63.7%) and lower in the study conducted by Shashikant Malkud (36.92%), as compared to our study. In case of subjects with presence of stress, the mean Hb in those subjects was 10.76 gms/dL, which was significantly less than the normal lower range of Hb ('t'=2.8420, 'p'=0.0111*) and mean serum ferritin in those subjects was 11.34 µg/L, which was not significantly less than the normal lower range

of serum ferritin ('t'=1.1860, 'p'=0.2510). This shows that the psychological stress could be a triggering factor for hair loss in our subjects with iron deficiency.

PRESENCE OF PALLOR (ANEMIA)

The prevalence of iron deficiency in adolescent girls and women of childbearing age (16-49 years of age) is 12% to 16%, whereas the prevalence of IDA is 2% to 4%. The finding of clinical pallor (anemia) in various studies is shown in the table below.

Table No 30 : Comparison of presence of pallor (anemia) in various studies.

Studies	Presence of pallor in number and percentage of subjects
Present study	16/40 (40%)
Shashikant Malkud[144]	21/83 (25.30%)
Kirti Deo, *et al*[145]	63/84 (75%)

The presence of clinical pallor was in 40% of subjects in the present study, while prevalence of anemia was higher in the study conducted by Kirti Deo, *et al* (75%) and lower in the study conducted by Shashikant Malkud (25.30%), as compared to our study.

In case of subjects with presence of clinical pallor, the mean Hb in those subjects was 9.92 gms/dL, which was significantly less than the normal lower range of Hb ('t'=5.0030, 'p'=0.0001*) and mean serum ferritin in those subjects was 3.89 µg/L, which was significantly less than the normal lower range of serum ferritin ('t'=3.0358, 'p'=0.0090*), in the present study. Thus presence of clinical pallor in

40% of subjects in the present study supports the laboratory findings of low hemoglobin and low serum ferritin levels.

HAIR PULL TEST

In this study hair pull test was done properly, as per correct methodology and following necessary precautions. When 10% of hairs pulled away from scalp, it constitutes positive hair pull test and implies active hair shedding.[46] In this study hair pull test was positive in all 35 participants of CTE and negative in all 5 participants of FPHL, indicating that active hair shedding was prevalent in all subjects with CTE. The percentage of subjects having positive hair pull test in various studies is shown in the table below.

Table No 31 : Comparison of positive hair pull test in various studies.

Studies	**Presence of positive hair pull test in No and percentage of subjects**
Present study	35 (87.5%)
Shashikant Malkud[144]	54 (41.53%)
Kirti Deo, *et al*[145]	46 (54.8%)
M.I.Fatani *et al*[151]	168 (61%)

In the present study, hair pull test was positive in 87.5% of subjects, which was higher than that of other studies. Positive hair pull test was observed in 61% of subjects in the study conducted by M.I.Fatani *et al*, in 54.8% of subjects in the study conducted by Kirti Deo, *et al* and in 41.53% of subjects in the study conducted by

Shashikant Malkud. The difference may be because of selection of different sample population and different types of hair loss.

ETIOLOGICAL FACTORS

Though the exact cause of hair loss is not fully understood, but it is usually related to many factors like family history (hereditary), medical conditions and medications, trigger events like surgery etc. Comparison of these factors in various studies is shown in the tables.

Table No 32 : Comparison of past history of subjects in various studies

Studies	H/O similar complaints in the family	H/O previous surgeries, caesarian sections	H/O diabetes in self or family	H/O hypertension in self or family
Present	3 (7.5%)	4 (10%)	2 (5%)	3 (7.5%)
Kirti Deo, *et al*[145]	6 (4.4%)	14 (10.3%)	21 (15.6%)	11 (8.1%)
Shashikant Malkud[144]	---	3 (2.30%)	---	---
M.I.Fatani, *et al*[151]	(10.4%)	---	(5.7%)	(2.5%)

The most common cause of hair loss is hereditary especially in case of FPHL. Heredity also affects the age at which the patient begin to lose hair, the rate of hair loss and the extent of baldness. History of hair loss in the family was observed in 7.5% of subjects in the present study as compared to 4.4% in the study conducted by Kirti Deo, *et al* and 10.4% in the study conducted by M.I.Fatani *et al*.

Surgical stress and anesthesia can trigger hair loss, though temporary, can continue to loose hairs if they have strong genetic predisposition for hair loss. History of previous surgeries was present in 10% of subjects in the present study which was similar to the study conducted by Kirti Deo, *et al* in which it was 10.3%, but was lower in the study conducted by Shashikant Malkud (2.30%).

Though none of the participants in this study had active diabetes, 5% of subjects gave history of diabetes in their family, while 5.7% of subjects were having diabetes in the study conducted by M.I.Fatani *et al.* and higher percentage of subjects (15.6%) had diabetes in the study conducted by KirtiDeo, *et al*. The difference seen in different studies is because of exclusion criteria, wherein this study has excluded the subjects with active diseases like diabetes, hypertension, thyroid and hormonal disorders and other systemic diseases, since they all affect test results.

Similarly, though none of the participants in this study had hypertension, 7.5% of subjects gave the history of hypertension in their family, while 8.1% in the study conducted by Kirti Deo, *et al.* and 2.5% in the study conducted by M.I.Fatani *et al* were having hypertension in their study subjects. Again the observed difference is because of strict exclusion criteria in the present study, in which patients with active diabetes and hypertension were excluded.

Associated conditions

It has been documented that the type of diet, bathing practices, application of different types of hair-oils, shampoos, chemicals like dyes and scalp disorders has effect on hair fall. It has been postulated that the iron requirements for vegetarians are approximately 1.8 times higher than that for non- vegetarians because of the

bioavailability of ingested iron. Many individuals reduce their shampooing frequency due to fear of losing more hair, but this increases the amount seen in subsequent shampooing. Many of the scalp affections lead to scaly patches and hair loss. The next table shows such practices and associated disorders in various studies.

Table No 33 : Comparison of associated conditions of various studies

Studies	Vegetarians	Application of chemicals, shampoo, mehandi	Suffering from seborrheic capitis/ dandruff
Present	18 (45%)	40 (100%)	8 (20%)
Kirti Deo, *et al.*[145]	53 (65%)	72 (53.3%)	63 (46.7%)
Shashikant Malkud. [144]	---	8 (6.15%)	---
M.I.Fatani *et al*[151]	---	---	(17%)

In the present study 45% of subjects were vegetarians as compared to 65% in the study conducted by Kirti Deo, *et al.*

In this study all the subjects (100%) were using shampoo, coconut oil, dyes and mehendi of different brands, as compared to 53.3% in the study conducted by Kirti Deo, *et al* and 6.15% of subjects in study conducted by Shashikant Malkud.

Percentage of subjects having seborrheic capitis/ dandruff in the present study was 20%, which is similar to the study conducted by M.I.Fatani *et al* (17%), while it was higher in the study conducted by Kirti Deo, *et al* (46.7%).

The large differences noticed in the parameters of above studies are due to the characteristics of samples, which were derived from different ethnical, cultural, socioeconomic backgrounds.

Some of parameters could not be compared due to lack of data in other studies. However in our studies 32.5% subjects were married and 11/13 (84.6%) were having children (ranging from 1 to 4). The diet habits of 47.5% of subjects were irregular. 62.5% of subjects gave the history of exposure to high external environmental changes and only 27.5% subjects were using head scarf for protection. Only 17.5% of subjects were regularly exercising. They used to oil and wash the hair 1 to 3 times per week. 60% of subjects were combing the hair daily and 40% of subjects were combing the hair weekly. 20% of subjects had greying of hair.

The mean BMI of all subjects in this study was 21.61 (CTE=21.28, FPHL= 23.92), indicating BMI of all the subjects were in the normal range.

In this study, age of the participants was compared with Hb levels, which showed positive correlation (‘r’=0.314, ‘p’= 0.048*) and also age of the participants was compared with serum ferritin levels, which also showed positive correlation. (‘r’=0.313, ‘p’= 0.049*).

In this study correlation between duration of hair loss and Hb was studied statistically (r =- 0.020 and p= 0.903), which showed negative correlation and correlation between duration of hair loss and ferritin was studied statistically (r=- 0.202 and p= 0.211), which also showed negative correlation. Hence as duration of hair loss increases, the Hb and ferritin levels decreases further, which is an important documentation.

Correlation between Hb and ferritin was studied statistically (r=0.396 and p= 0.012*), which showed positive correlation and is the most important finding of this study. This supports the fact that Hb and serum ferritin decrease/increase concurrently. There was no data available with other studies, to compare above said relations.

Hence to conclude, present study shows that association exists between hair loss and iron deficiency in the participants of this study.

CONCLUSION

The relationship between body iron status and different types of hair loss has been investigated in a number of studies, however, with relatively discrepant findings.[139] Therefore this study was conducted to evaluate whether different types of hair loss were associated with decreased tissue iron stores. This study has found that, chronic telogen effluvium and female pattern hair loss in patients, were associated with decreased tissue iron stores.

All subjects of study

Adequate number of subjects as per sample size calculation were included in the present study and the total sample size was 40. In this study post-menopausal women were not included, to avoid the influence of changed hormonal pattern on results. Though the age range was 15-45 years, mean age was 25.18 years, indicating prevalence of younger population in the study. Mean duration of hair loss was 20.3 months which ranged from 6 to 60 months. Duration of hair loss was less than 12 months in higher number of (57.5%) subjects. This shows that younger populations were more concerned about hair loss and they approached the doctor early for evaluation, diagnosis and management of hair loss. The average BMI of all subjects was 21.61, indicating the study subjects were of average built and not obese, which rules out obesity as cause for hair loss in these study subjects.

In this study hemoglobin concentration was used to screen for iron deficiency and serum ferritin concentration used to confirm iron deficiency. Mean hemoglobin level was 11.5 gms/dL, which ranged from 6.9 to 13.9 gms/dL and 45% of study participants had hemoglobin levels less than 12 gms/dL. Iron being most important

constituent of Hb, these Hb levels showed that iron deficiency could be the cause of hair loss in these study subjects. There was negative correlation between duration of hair loss and Hb, indicating that as the duration of hair loss increased, the Hb levels decreased further.

Investigators consider serum ferritin to be the most powerful tool for diagnosis of iron deficiency. Subjects of this study had iron deficiency, which was revealed by their mean serum ferritin level which was low (18.6 µg/L), and also the range was narrow (0 to 85.6 µg/L). Serum ferritin of 42.5%subjects was less than the lower limit of serum ferritin level (6 µg/L)and 5%had serum ferritin levels as zero µg/L, which further shows the presence of iron deficiency. 57.5% of participants had serum ferritin levels less than 12 µg/L indicating iron deficiency in majority of them.15% of participants had serum ferritin levels ranging from 13-20 µg/L, indicating iron depletion and 25% of the participants had serum ferritin levels ranging from 21-70 µg/L, indicating serum ferritin levels were lower than required for normal hair cycle. Hence iron deficiency as a cause for hair loss was evident. As there is a wide range in serum ferritin levels, different cut-off levels of serum ferritin, below which it can be defined as iron deficiency, triggering hair loss was used, with which also many subjects showed ID. When cut off level of serum ferritin was taken as 41µg/L, there was statistically significant difference between the mean serum ferritin levels of all subjects as compared to cut off level of serum ferritin value. There was positive correlation between Hb and ferritin levels, which supports the fact that Hb and serum ferritin decrease/increase concurrently.

40% of the subjects appeared pale on clinical examination, in whom, the mean Hb was 9.92gms/dL, and mean serum ferritin was 3.89 µg/L, which were significantly

less than normal lower range. This shows that presence of clinical pallor can be a rough guide for low hemoglobin and low serum ferritin levels.

Psychological stress is one of the commonest etiological factor and also an exacerbating factor for hair loss and 47.5%of subjects in this study gave the history of having stress in their life. The mean Hb in the subjects with presence of stress was 10.76 gms/dL and the mean serum ferritin was 11.34 µg/L, which were significantly less. This shows that the psychological stress could be a triggering factor for hair loss in these subjects with iron deficiency.

Hair loss in iron deficient subject can be triggered by various factors. History of hair loss in the family was present in 7.5% of the subjects, history of previous surgery was present in 10% of subjects. Many of the subjects were vegetarians (45%) and with irregular diet habits, which could be the cause for the iron deficiency. Usage of shampoo, dyes for the hair and presence of dandruff may be precipitating factors for hair loss in these subjects. None of the participants of this study were having active diabetes, hypertension and thyroid disorders.

Subjects with chronic telogen effluvium (CTE)

As per this study, CTE was the most common type of alopecia in females, which is also true with majority of other studies. Hair pull test was positive in all subjects having CTE, which implies active hair shedding and helps to assess the severity and location of hair loss. Maximum number of subjects with CTE (70%) was in the younger age group (15 to 25 years), indicating prevalence of CTE was highest in the young. The mean duration of hair loss (18.37 months) and BMI (21.28) were less in case of CTE as compared to FPHL.

Iron deficiency as a cause for hair loss in subjects with CTE was evident by their lower mean Hb levels (11.33 gms/dL) and mean serum ferritin levels (16 µg/L). There was statistically significant difference between the mean Hb levels of subjects with CTE as compared to normal minimum value of Hb. Majority of the study subjects with CTE showed ID, when different cut off levels of serum ferritin were used viz; ≤ 15 µg/L(68.57%), ≤ 20 µg/L(74.28%), ≤ 40 µg/L(85.71%) and ≤ 70 µg/L (100%). There was statistically significant difference between the mean serum ferritin levels of subjects with CTE as compared to cut off level of serum ferritin value as 41µg/L. Further 45.7%of subjects were found to have pallor during clinical examination and 48.5%subjects reported to be having stress during history taking, which shows that the psychological stress could be a triggering factor for hair loss in these subjects of CTE with iron deficiency. Thus all above supports the fact that iron deficiency was the cause for hair loss in subjects having CTE.

Subjects with female pattern hair loss (FPHL)

In this study, number of subjects with FPHL was less, because of non-inclusion of post-menopausal women and hair pull test was negative in them. Severity of hair loss was assessed in subjects with FPHL by Ludwig scale, in which majority of subjects (80%) had grade II hair loss. The subjects having FPHL were in the age group of 36 to 45 years, indicating this type of hair loss was prevalent in the aged women. Mean duration of hair loss was also more in FPHL (33.60 months) as compared to CTE. As the subjects with FPHL were in the older age group, the average BMI of these subjects (23.92), though normal, was higher as compared with CTE.

As compared to CTE, the mean Hb levels in FPHL was in the normal range (12.94 gms/dL). Even the mean serum ferritin level of FPHL was higher (36.64 µg/L), as compared to CTE. But significant number of subjects with FPHL also showed ID, when different cut off levels of serum ferritin were used as ≤ 15 µg/L(20%), ≤ 20 µg/L(60%), ≤ 40 µg/L(60%) and ≤ 70 µg/L (80%).40% of subjects with FPHL gave the history of stress in life.

Hence to conclude, present study shows that association exists between hair loss and iron deficiency in the participants of this study. Further scientific studies are required with larger sample size to postulate quantitative association between different etiological factors and hair loss in iron deficient female.

SUMMARY

This was a cross-section study conducted from January 2015 to December 2015 at KLES Dr Prabhakar Kore Hospital & MRC, Belagavi, on the female patients attending dermatology clinic, having chronic diffuse hair loss. All the female patients aged between 15 to 45 years, having chronic telogen effluvium and female pattern hair loss, were recruited as per the inclusion and exclusion criteria. Aim of the study was to evaluate whether Chronic Telogen Effluvium (CTE) and Female Pattern Hair Loss (FPHL) in patients, was associated with decreased tissue iron stores, as measured by serum ferritin and hemoglobin levels. The patients included in the study, should undergo blood tests like serum ferritin (by ADVIA centaur ferritin assay) and hemoglobin levels (by spectrophotometry), which requires drawing of blood. Hence, this intervention was stated in the informed and written consent and also the study was carried out after institutional ethical clearance. The study protocol was briefed to the patients and only those subjects who came forward voluntarily to participate in the study were included for the study. None of these participants were taking treatment for hair loss.

The inclusion criteria were; all consenting female subjects of age group 15-45 years, with chronic diffuse hair loss, subjects having diffuse thinning of hair and subjects with patterned hair loss.

The exclusion criteria were; subjects who did not give consent, subjects who were on iron therapy, undergone GI/scalp surgeries, suffering from trichotillomania, hormonal abnormalities and subjects who were on regular medications for other systemic disorders.

The primary objective of study was to measure serum ferritin and hemoglobin levels in patients with hair loss of the age group between 15 to 45 years, and secondary objective was to analyze correlation between serum ferritin and haemoglobin levels with hair loss by statistical methods.

The sample size was 40 subjects with hair loss. A detailed history was taken along with photographs of the condition. Systemic and dermatological examination was carried out. Diagnosis of CTE and FPHL was made by clinical examination and by performing hair pull test and they were graded by using appropriate grading scales.

All the results were tabulated; analysis of result was made by using mean ± standard deviation and Pearson correlation coefficient, one sample‘t’ test was used wherever applicable. The results were expressed as percentages and significance.

In this study, the total sample size was 40 and number of subjects having CTE was more (35) as compared to the FPHL (5). All the subjects had normal BMI (21.61). Hair pull test was positive in all subjects having CTE and negative in subjects with FPHL. Severity of hair loss was assessed in the subjects with FPHL by Ludwig Scale (Type II grade: 80%, Type III grade: 20% subjects).

Mean age of all participants was 25.18 years (range: 15-45 years), CTE was prevalent in the younger age group (15 - 25 years) and FPHL was prevalent in older women (36 - 45 years). Mean duration of hair loss was 20.28 months (range 6-60 months), which was less in CTE (18.37 months), as compared to FPHL (33.60 months).

Hemoglobin concentration was used to screen for iron deficiency. Mean hemoglobin levels of all participants was 11.5gms/dL (range: 6.9-13.9 gms/dL), 45%

of participants had hemoglobin level less than 12 gms/dL (Normal range: 12-16 gms/dL). Hb levels were less in subjects having CTE (11.33 gms/dL) as compared to the subjects with FPHL (12.94 gms/dL). In CTE the percentage of subjects having Hb levels less than 12 gms/dL, was 51.42%.There was no correlation between age with duration of hair loss and duration of hair loss with Hb.

Serum ferritin concentration has a greater predictive value than other tests of iron status and was used to confirm iron deficiency in this study. Mean serum ferritin levels of all subjects was 18.6µg/L (range: 0-85.6 µg/L). 57.5% of the subjects had serum ferritin levels less than 12 µg/L indicating iron deficiency, 15% of participants had serum ferritin levels ranging from 13-20 µg/L indicating iron depletion. 25% of participants had serum ferritin levels ranging from 21-70 µg/L indicating serum ferritin levels were lower than required for normal hair cycle. Only one participant (2.5%) had serum ferritin level more than 71 µg/L indicating serum ferritin levels were within the normal range in that subject. 42.5% participants had serum ferritin levels less than 6 µg/L, which is definitely below the normal lower range as per specification of test. Two participants (5%) had serum ferritin levels as zero µg/L. Serum ferritin levels were low in subjects with CTE (16 µg/L), as compared to FPHL (36.64 µg/L) and the difference was statistically significant ('p'=0.0389*). As range of serum ferritin is very wide, cut off levels of serum ferritin (≤ 15 µg/L, ≤ 40 µg/L and ≤ 70 µg/L) were adopted in this study. Majority of subjects of this study had ID, when cut off level of serum ferritin was used as ≤ 70 µg/L (FPHL=80% and CTE=100% of subjects). When cut off level of serum ferritin was used as ≤ 40 µg/L, 60% of subjects in FPHL and 85.71% of subjects in CTE, showed ID. Even when cut off level of serum ferritin was used as ≤ 20 µg/L, 60% of subjects in FPHL and 74.28% of subjects in CTE, showed ID. Hence, subjects of this study had low serum

ferritin levels at different definitions of ID and CTE, FPHL were associated with decreased iron stores. As ferritin levels accurately reflect body iron stores, this study clearly demonstrated the association between low iron stores and hair loss. There was positive correlation between age with Hb and serum ferritin levels. There was positive correlation between Hb and ferritin levels and negative correlation between duration of hair loss and serum ferritin levels.

The presence of clinical pallor in 40% of the study subjects was supported by the low hemoglobin (9.92 gms/dL) and low serum ferritin levels (3.89µg/L), in them.

In the subjects with presence of stress (47.5%), the mean Hb was significantly low (10.76 gms/dL) and the mean serum ferritin in those subjects with stress was 11.34 µg/L, which was significantly less, when cut off of serum ferritin level was taken as 41µg/L.

Different etiological factors of hair loss like , history of hair loss in the family was present in 7.5% of the subjects, 20% of the subjects were having seborrheic capitis/ dandruff, 62.5% of the subjects gave the history of exposure to high external environmental changes and only 27.5% subjects were using head scarf for protection.

The relationship between body iron status and different types of hair loss has been investigated in a number of studies, however, with relatively discrepant findings. This study confirms that chronic telogen effluvium and female pattern hair loss in patients, are definitely associated with decreased tissue iron stores.

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ANNEXURE-I

INFORMED CONSENT FORM

I.D.No :

Name of the participant (In block letters)	
Address	
E-mail	
Contact number	

Title of the study: "*A one year cross sectional study, to observe correlation of serum ferritin levels in female patients with chronic diffuse hair loss, attending dermatology clinic at KLES Dr Prabhakar Kore Hospital & MRC, Belagavi."*

Respected Sir/Madam,

We invite you to participate in our study as, you are eligible for the same. During the study you will be asked some questions in detail regarding your present complaints.

Need and purpose of the study:

Hair loss is a common problem in females, which may be associated with decreased body iron. Hence this study intends to find whether hair loss is associated with decreased body iron stores, which is measured by serum ferritin and hemoglobin levels. This will help us to find the cause for hair loss and its treatment. Hence we want to measure serum ferritin and hemoglobin levels in the female patients of the age group 15-45 years, with hair loss. You are being requested to participate in this research

because you have been diagnosed to have chronic diffuse hair loss (chronic telogen effluvium / female pattern hair loss).

Procedure and treatment:

Should you choose to participate, you will be asked to give a detailed history of your disease, undergo a physical examination, hair pull test and consent for investigations like estimation of serum ferritin and hemoglobin levels, which requires drawing of 2 ml of blood.

Risks and benefits:

While drawing of 2 ml blood for investigations like estimation of serum ferritin and hemoglobin levels, you may experience slight pain due to needle prick. The hair pull test (pulling of a few hairs) required for the diagnosis, may cause minimal pain. However all necessary steps and precautions will be taken to ensure your safety. The result of you taking part in this research would help health care providers towards a better understanding of this disease, and thus we will be able to provide improved patient care

Alternatives:

If you decide not to participate in this study, you will still be receiving the usual standard care for your disease.

Privacy and confidentiality:

Your privacy will be respected and all information collected about you during the course of this study will be kept confidential. Your identity will remain undisclosed.

Relations with the Institutional policy:

The J. N. Medical College will provide, within the limitations of the laws of the State of Karnataka, facilities and medical attention to patients who suffer injuries as a result of participating in this project.

Queries:

If you have any questions regarding the study, you may contact KLES Dr. Prabhakar Kore Hospital and MRC on Telephone No. +_____

Financial incentives:

You shall not be receiving any payment or any financial incentives for participating in this study.

Authorization to publish results:

The results of this study may be published for scientific purpose or presented to a scientific group. Your identity, however, will be maintained confidential at all times.

Voluntary participation:

Your participation in this study is voluntary. Your decision whether or not to participate will neither affect the care of your current disease, nor your future relations with the doctor or the hospital. In case you need further information regarding your rights as a study participant, you may please contact Dr. Ganga. S. Pilli, Chairman of the ethical committee, J. N. Medical College, Belagavi, on telephone no. 08312473777.

STATEMENT OF CONSENT

I.D. No:

I Ms/Mrs ---------- volunteer and consent to participate in this study. I have read the consent document or it has been read to me in my vernacular language. I accept to participate in the study. All the information regarding this study is provided to me and I have understood the same. I have been given the opportunity to ask questions and obtain appropriate answers.

Participant's name:

Signature or left thumb print of participant:

Witness name:

Signature of witness:

Signature of the investigator:

Date:

STATEMENT OF ASSENT

I.D.NO:

I Mr/Ms/Mrs ---------- parent/guardian/ward/ of---------- consent to enroll my daughter to participate in this study. I have read the consent document or it has been read to me in my vernacular language. I give my acceptance on behalf of my daughter for her participation in the study. All the information regarding this study is provided to me and I have understood the same. I have been given the opportunity to ask questions and obtain appropriate answers.

Participant's name:

Participant's parent/guardian name:

Signature or left thumb print of parent/guardian of participant:

Witness name:

Signature of witness:

Signature of the investigator:

Date:

ANNEXURE-II

PROFORMA

TITLE OF STUDY: "A one year cross sectional study, to observe correlation of serum ferritin levels, in female patients with chronic diffuse hair loss, attending dermatology clinic of KLES Dr Prabhakar Kore Hospital & MRC, Belagavi.

CASE No: **IP/OP No:**

NAME:

AGE:

SEX:

OCCUPATION:

ADDRESS:

CONTACT NO:

CHIEF COMPLAINTS:

HISTORY OF PRESENT ILLNESS:

TREATMENT HISTORY:

PAST HISTORY: Diabetes mellitus/ Thyroid disorders / Hypertension / SLE / RA/ bleeding disorders/severe illness/poisoning

Obstetrics history: Number of pregnancies

Menstrual history: Excessive bleeding

Gynecological history: Polycystic ovarian syndrome/others

Surgical history: Major surgeries/Injury/ piles

Skin: Skin/Scalp disorders

Stress: present/absent

H/O similar complaints in the past

H/O intake of any drugs

H/O hair/scalp infections or infestations

FAMILY HISTORY: Endocrine disorder Yes / No

Respiratory/CVS/CNS disorders: Yes/ No

Hematological/renal/malignancies: Yes / No

Hypertension: Yes / No

Patterned hair loss: Yes/ No

Similar complaints: Yes/ No

PERSONAL HISTORY: Exposure : Pollutants/excessive sunlight /chemicals

Diet : Vegetarian / Non-vegetarian

Adequate nutrients / Balanced diet

Dieting / weight loss

Drugs intake: Yes / No Specify : Duration

:

Radiotherapy: Yes / No Duration:

Chemotherapy: Yes / No Duration:

Smoking: Yes / No Duration:

Alcohol: Yes / No Duration:

Bowel habits: Bladder habits:

Use of Cosmetics/hairoils/shampoo/dyes/indigenous products/

Salon visits for hair treatment/ spa (with frequency):

Use of headscarfs/ helmets :

Itching over scalp:

Regular yoga practice/ exercise/ swimming:

Bathing habits (frequency per week):

Combing/ hair-oiling habits:

GENERAL EXAMINATION:

Height: Weight: BMI:

Temperature: Pulse: RR: BP:

Pallor: Icterus: Cyanosis:

Clubbing: Pedal oedema: Lymphadenopathy:

Deficiency signs of Iron/ Calcium/ vitamins/ proteins :

SYSTEMIC EXAMINATION:

CVS:

RS:

P/A:

CNS:

ENDOCRINES:

DERMATOLOGICAL EXAMINATION:

Skin:

Palms and Soles:

Oral Mucosa:

Genitalia:

Nails:

Examination of scalp:

Hair:

- Signs of infection/ infestation:
- Texture/ quality of hair :
- Canitis :
- Alopecia areata :
- Hair pull test :
- Grade of female patterned hair loss according to Ludwig's classification: | I | | II | | III |

INVESTIGATIONS:

Hemoglobin (in gm%):

Serum ferritin: (in ng/dl):

DIAGNOSIS: TELOGEN EFFLUVIUM -

PATTERNED HAIR LOSS -

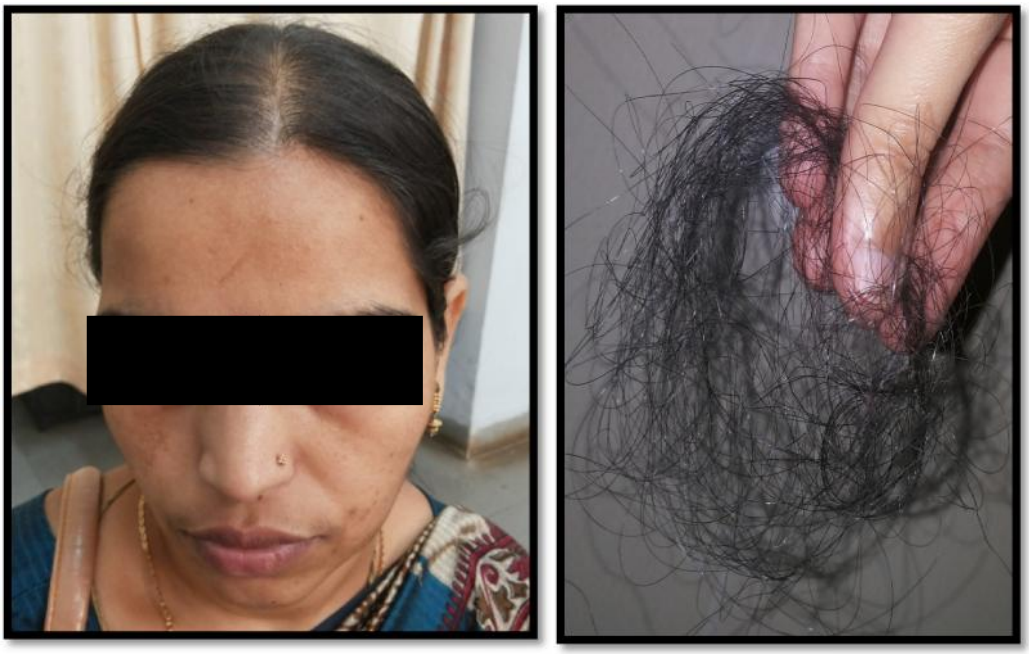
TYPE -

GRADE -

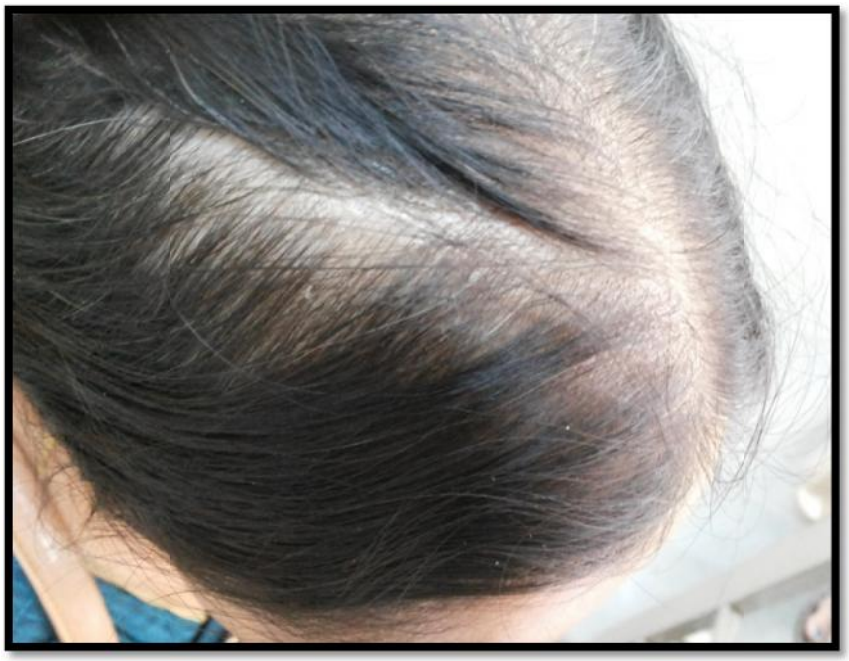
ANNEXURE - III PHOTOGRAPHS

PHOTOGRAPH 1 : CHRONIC TELOGEN EFFLUVIUM SHOWING DIFFUSE THINNING AND LOSS OF HAIR OVER SCALP.

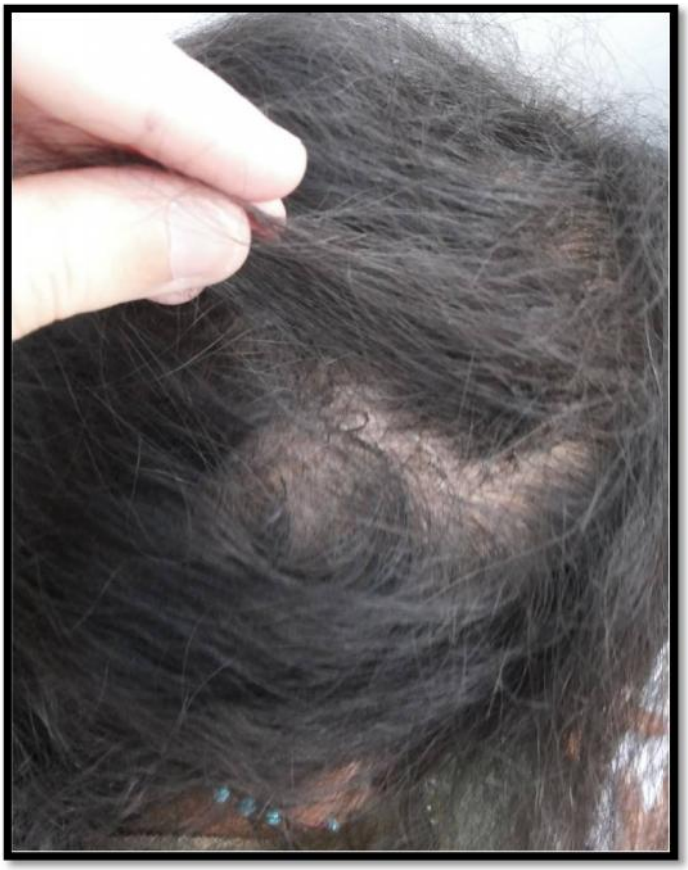
PHOTOGRAPH 2 : CHRONIC TELOGEN EFFLUVIUM SHOWING DIFFUSE THINNING OF HAIR OVER SCALP.



PHOTOGRAPH 3 : CHRONIC TELOGEN EFFLUVIUM PATIENT SHOWING DAILY LOSS OF HAIRS.



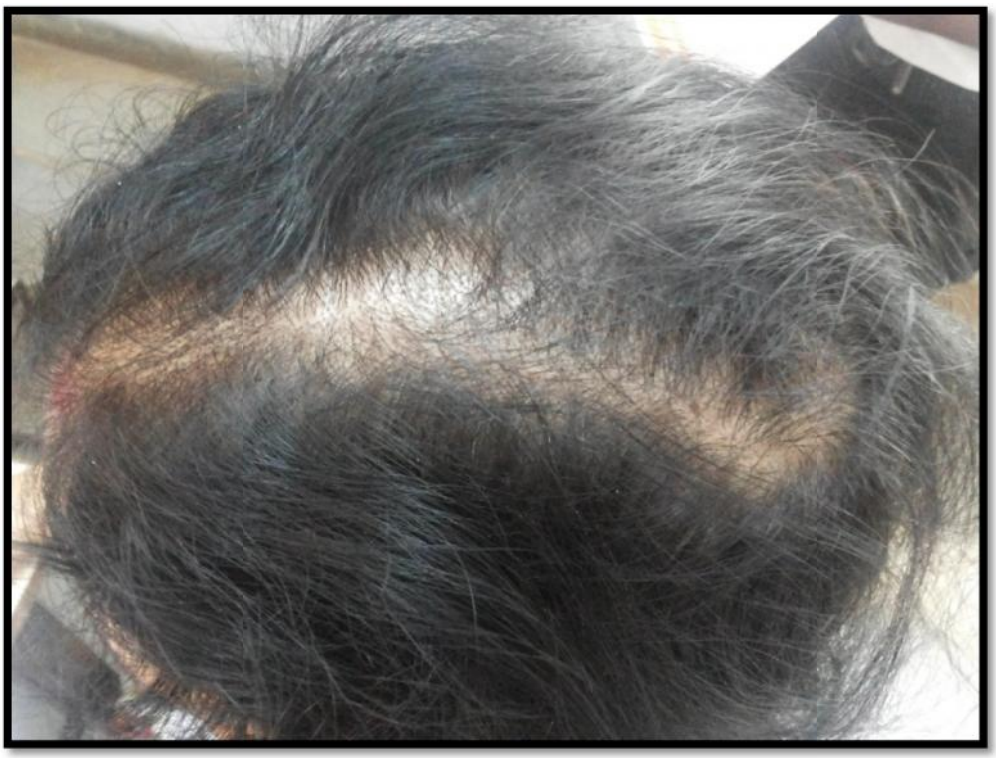
PHOTOGRAPH 4 : CHRONIC TELOGEN EFFLUVIUM SHOWING DIFFUSE THINNING AND LOSS OF HAIR OVER SCALP.



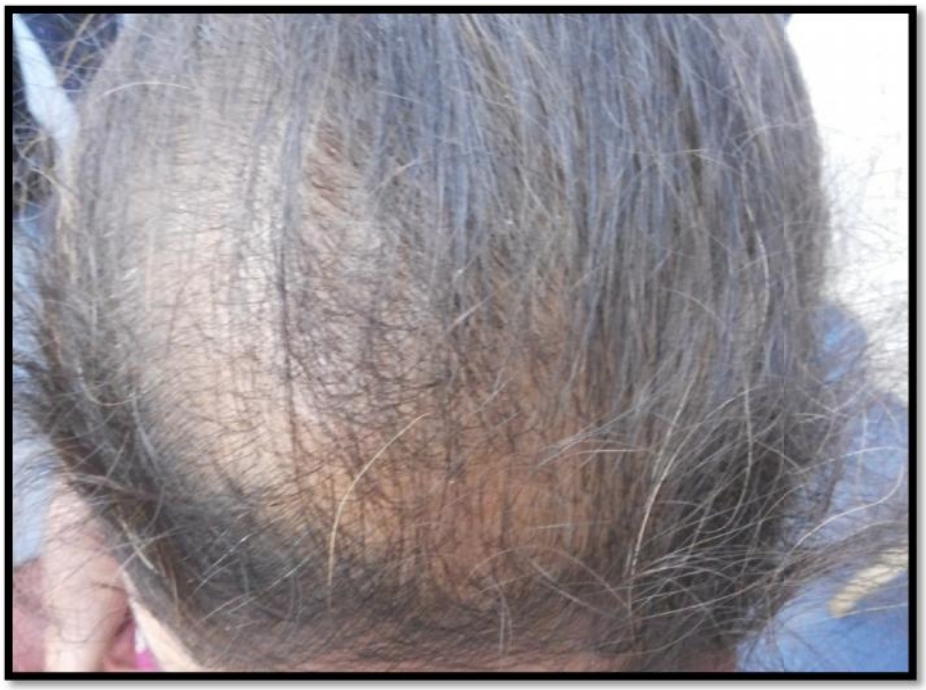
PHOTOGRAPH 5 : CHRONIC TELOGEN EFFLUVIUM SHOWING DRY AND LUSTRELESS HAIR.

PHOTOGRAPH 6 : DEMONSTRATING HAIR PULL TEST IN A PATIENT OF CHRONIC TELOGEN EFFLUVIUM.

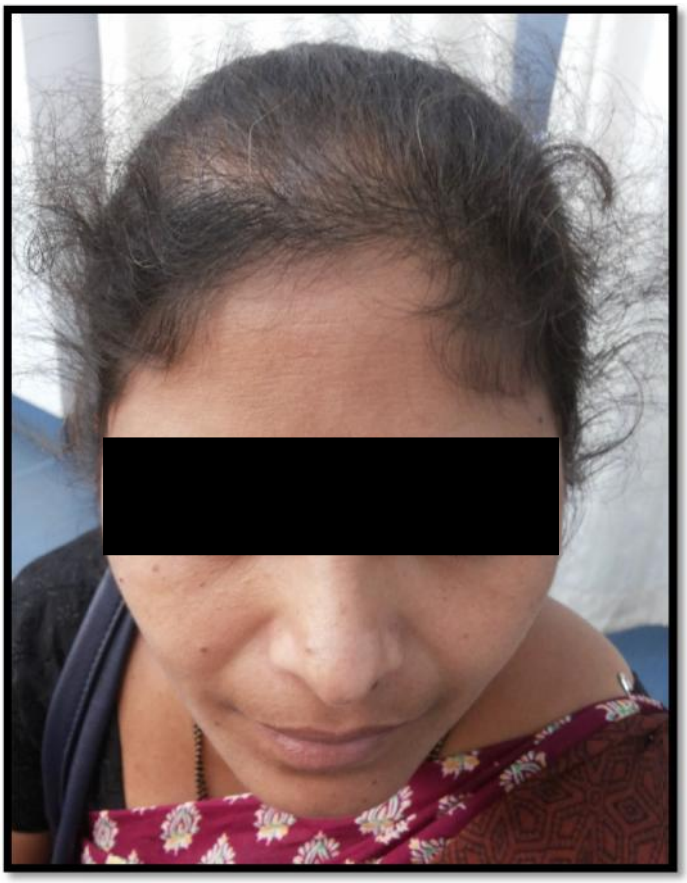
PHOTOGRAPH 7 : HAIR PULL TEST.



PHOTOGRAPH 8 : GRADE II FEMALE PATTERN HAIR LOSS WITH LOSS OF LUSTRE.

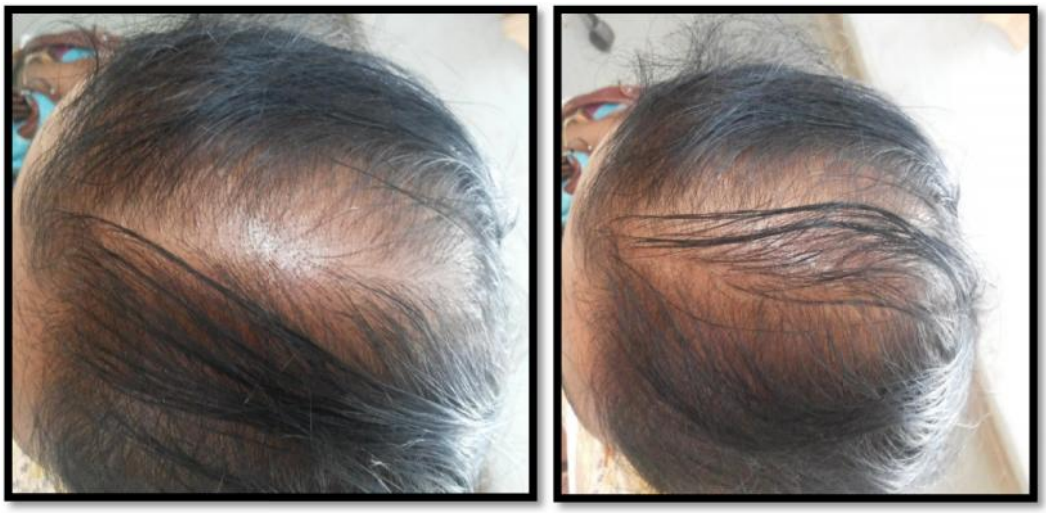


PHOTOGRAPH 9 : FEMALE PATTERN HAIR LOSS.



PHOTOGRAPH 10 : FEMALE PATTERN HAIR LOSS GRADE II, SHOWING LOSS OF HAIR OVER THE FRONTAL AND VERTEX REGION.

PHOTOGRAPH 11 : FEMALE PATTERN HAIR LOSS GRADE II.



PHOTOGRAPH 12 : FEMALE PATTERN HAIR LOSS GRADE III SHOWING VISIBLE EXTENSIVE THINNING AND LOSS OF HAIR WITH EASY VISIBILITY OF THE SCALP.

ANNEXURE-IV

KEY TO MASTER CHART

Marital status:	M=Married
	U=Unmarried
Occupation:	H=House wife
	C=Clerk
	S=Student
	T=Tailor
	N=Nurse
	B=Business
Stress:	P=Present
	A=Absent
Type of diet:	V=Vegetarian
	NV=Non-vegetarian
Dietary habits:	R=Regular
	I=Irregular
H/O Past surgeries:	LSCS=Lower Segment Cesarean Section
	YB=Years Back
	APPY=Appendectomy
	H/O= History Of
H/O DM in family:	DM= Diabetes Mellitus
	M=Mother
	F=Father

H/O HTN in family: HTN= Hypertension

M=Mother

F=Father

H/O Similar complaints in family:

M=Mother

F=Father

H/O Environmental exposure:

P=Present

A=Absent

Using of head scarf/helmet:

HS= head scarf

H= helmet

Regular Exercise: P= Present (exercising)

SKIP= Skipping

SWIM= Swimming

JOG=Jogging

YOGA=Yogic exercise

A=Absent (Non-exercising)

Application of shampoo:

AYURVD=Ayurvedic

CLNPLS=Clinic plus

SUNSLK=Sunsilk

Application of hair oil:

AYURVD=Ayurvedic

COCONT=Coconut

Using of Mehandi/ hair color:

M=Mehendi

Frequency of hair washing habit:

1=once/week

2=Twice/week

3=Thrice/week

Combing habits:

D=Dry

W=Wet

H/O greying of hair:

P=Present

A=Absent

Seborreic capitis/dandroff:

P=Present

A=Absent

Scalp itching:

P=Present

A=Absent

Associated conditions of scalp/hair:

O= Nil

AV I= Acne vulgaris grade I

AV II= Acne vulgaris grade II

DPN= Dermatosis Papulosa Nigra

POH= Peri-Orbital Hyperpigmentation

Pallor:

P=Present

A=Absent

Hair pull test:

P=Positive

N=Negative

Type of hair loss:

CTE=Chronic Telogen Effluvium

FPHL=Female Pattern Hair Loss

FPHL 2= Female Pattern Hair Loss grade I

FPHL 3= Female Pattern Hair Loss grade III