## **Original research article**

# Biochemical correlates of diffuse non scarring alopecia in women

<sup>1</sup>Dr. Vandana D, <sup>2</sup>Dr. Suma D. Gudi, <sup>3</sup>Dr. C. Raghuveer, <sup>4</sup>Dr. Lakshmipathi Y. Pattar

<sup>1</sup>Specialist-Dermatologist, General Hospital, Harapanahalli Vijayanagar, Karnataka, India <sup>2</sup>Assistant Professor, Department of D V L, (Dermatology, Venereology and Leprosy), Vijaynagar Institute of Medical Sciences, Bellary, Karnataka, India

<sup>3,4</sup>Associate Professor, Department of D V L, (Dermatology, Venereology and Leprosy), Vijaynagar Institute of Medical Sciences, Ballari, Karnataka, India

#### **Corresponding Author:**

Dr. Lakshmipathi Y. Pattar

#### Abstract

Alopecia is defined as "absence or loss of hair". The word 'alopecia' was first coined from the Greek word for fox which refers to the constant shedding of hairs seen in the lifetime of this animal. Hair follicle loss may be due to follicular agenesis, dysgenesis, or destruction while loss of hair shafts can result from shedding, plucking, miniaturization, or breakage. A thorough general physical and systemic examination was carried out to look for any abnormalities. Scalp examination included type of hair loss, hair thinning, temporal recession and hair pull test was done in all cases. Diagnosis was made clinically based on the history and pattern of hair loss. The overall mean haemoglobin was  $11.0 \pm 2g/dl$ . Mean haemoglobin in ATE, CTE, FPHL was also 11g/dl. The overall mean S. Ferritin value is  $21.7 \pm 23.3 g/dl$ . Mean serum ferritin levels were lowest in patients with CTE  $(15.9 \pm 16.0)$  as compared to those with TE  $(23.3\pm 26.7)$  and highest in FPHL  $(27.8\pm 25.6)$  in our study. The overall mean S. TSH value is 3.7 µIU/ml. Mean S. TSH value in ATE, CTE, FPHL is 6.0 µIU/ml, 2.2 µIU/ml, 2.5 µIU/ml respectively. In our study,  $317.0 \pm 162.5$  pg/ml is the overall mean for serum vitamin B12. Mean value is lowest in  $300.9\pm111$  pg/ml in CTE, followed by  $308.8\pm97.1$  pg/ml in ATE, and highest in FPHL being  $355\pm277$ pg/ml. The overall mean for S Vitamin D3 is  $17.9 \pm 12.4$  ng/ml. Mean value is lowest in FPHL being  $15.5 \pm 5.9$  ng/ml, followed by  $18.3 \pm 13.3$  ng/ml in CTE, and highest in ATE being  $19 \pm 14.3$  ng/ml. Keywords: Biochemical correlates, diffuse non scarring alopecia, women

#### Introduction

Hair is a cutaneous appendage typical to mammalian skin and is considered as one of the most defining aspects of appearance of human beings <sup>[7]</sup>. Besides skin, hair is considered to contribute equally in the perception of oneself and projection of an individual's self-confidence and personality. Hair is essential in identity of many women and are more likely to have lowered quality of life and restrict social contacts as compared to men as a result of hair loss <sup>[1]</sup>. DHL is common and multifactorial problem in women which is quite challenging to treat. Psychiatric disorders are more prevalent in these patients suggesting that there may be a higher risk of developing serious depressive episodes, anxiety disorders, social phobia, or paranoid disorders <sup>[2]</sup>.

Alopecia is defined as "absence or loss of hair". The word 'alopecia' was first coined from the Greek word for fox which refers to the constant shedding of hairs seen in the lifetime of this animal.

Hair follicle loss may be due to follicular agenesis, dysgenesis, or destruction while loss of hair shafts can result from shedding, plucking, miniaturization, or breakage <sup>[3]</sup>.

The distribution of alopecia can be diffuse, patterned, or circumscribed/focal, with telogen effluvium and androgenetic alopecia being the most common forms of alopecia. Alopecias are broadly subdivided into scarring forms (cicatricial) and non-scarring forms (non-cicatricial)<sup>[4]</sup>.

Diffuse shedding of hair was originally termed 'defluvium capillorum'. Sabouraud as early as 1932, confined the term to a sudden diffuse loss of hair following a severe emotional shock, all though others applied it to all forms of alopecia. Diffuse hair shedding can result from disruption in any one phase of the hair cycle, anagen (active hair growth), catagen (involution), or telogen (resting)<sup>[5]</sup>.

Due to subclinical nature of TE, the true incidence or prevalence is widely unknown. Women who seek treatment are usually over-represented, probably due to unawareness or underreporting in males. ATE can occur in either sex, but due to hormonal changes in the postpartum period of women, they have a greater tendency to experience this condition. Women tend to find the hair shedding more troublesome than men and seek medical attention for the condition. The effect of age is unclear, however elderly women are reported to be more susceptible to acute telogen effluvium (ATE) following high fever,

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 07, 2023

surgical trauma, severe haemorrhage, or immense psychological stress. In children, TE has been reported to be responsible for only a minority of cases with hair loss (2.7%). CTE seems to affect only women for uncertain reasons, CTE affects middle aged women of 30-60 Years<sup>[6]</sup>.

### Methodology

## Inclusion criteria

• All consenting females 18 to 45 years of age with non- scarring diffuse hair loss and diffuse thinning of hair.

#### **Exclusion criteria**

- Not willing to participate.
- Participants on iron therapy, medications for systemic disorders.
- Trichotillomania.
- Alopecia areata.
- Anagen Effluvium.

#### Study design

Cross sectional study.

#### Method

#### **History taking**

A detailed history and clinical examination findings were noted after taking a written informed consent from the informant. Questionnaires regarding age, duration, onset and progression of hair loss, family history, systemic illness and associated symptoms like scalp itching, pain, seborrhea were recorded in a pre- structured case proforma. History of trigger factors like stress, febrile illness, chronic or acute blood loss, hair care products usage, recent childbirth and abortions, recent hospitalizations and surgeries were noted. Previous similar history in the past with menstrual and obstetric history were recorded. Hamilton anxiety and depression scoring was done.

#### **Clinical examination**

A thorough general physical and systemic examination was carried out to look for any abnormalities. Scalp examination included type of hair loss, hair thinning, temporal recession and hair pull test was done in all cases. Diagnosis was made clinically based on the history and pattern of hair loss. Patients were categorised into three groups of Acute Telogen Effluvium, Chronic Telogen Effluvium and Female pattern hair loss. Patients of FPHL were sub grouped based on pattern as Ludwig type, Olsen type and Hamilton type. Patients with Ludwigs type of FPHL were graded with Sinclaire scale (Type I to V).

#### Investigations

The following laboratory investigations were done and recorded.

- Haemoglobin.
- Serum Ferritin.
- Serum Vitamin B12.
- Serum Vitamin D3.
- Thyroid function test-TSH, T3, T4.
- LFT.
- RFT.

#### Special investigation if necessary

- Serum androgen profile/Ultrasound abdomen was done in participants with Impaired fertility, associated menstrual disturbances, hirsutism, recrudescence of acne.
- Scalp Biopsy was done wherever clinical diagnosis was in doubt.

The data was entered into a case record form specially designed for the study.

#### **Statistical Analysis**

The study was a hospital based descriptive cross-sectional study. All characteristics were summarized descriptively. Categorical variables were expressed in percentage. For continuous variables, mean $\pm$ 2SD were used. Multiple group comparisons for the continuous variables were done by using Analysis of Variance (ANOVA). In case the p-value based on Multiple group comparison was found to be significant, post hoc test was applied to test the significance between two groups. Categorical variables were compared by using Chi- square test. The categorical variables having expected cell frequency less than 5 were compared using Fisher's exact test. P- value <0.05 was considered to be significant for all the tests.

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Results

	Overall	(n=100)	ATE (1	1=39)	CTE (	n=37)	FPHL	(n=24)	D l
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	r value
Age	25.5	7.3	25.2	7.2	23.6	6.0	28.9	8.4	0.0192
Haemoglobin	11.0	2.0	11.0	2.3	11.0	1.9	11.0	1.6	0.987
S.Ferritin (g/dl)	21.7	23.3	23.3	26.7	15.9	16.0	27.8	25.6	0.126
TSH (µIU/ml)	3.7	14.9	6.0	23.7	2.2	1.8	2.5	2.0	0.49
S. Vit B12 (pg/ml)	317.0	162.5	308.8	97.1	300.9	111.0	355.0	277.0	0.416
S. Vit D3 (ng/ml)	17.9	12.4	19.0	14.3	18.3	13.3	15.5	5.9	0.546

Table 1: Mean Values

The overall mean age was 25.5 ± 7.3 years. Mean age in ATE, CTE, FPHL was 25.2, 23.6, 28.9 years respectively. The overall mean haemoglobin was  $11.0 \pm 2g/dl$ . Mean haemoglobin in ATE, CTE, FPHL was also 11g/dl. The overall mean S. Ferritin value is  $21.7 \pm 23.3$  g/dl. Mean serum ferritin levels were lowest in patients with CTE (15.9± 16.0) as compared to those with TE (23.3± 26.7) and highest in FPHL (27.8± 25.6) in our study. The overall mean S. TSH value is 3.7 µIU/ml. Mean S. TSH value in ATE, CTE, FPHL is 6.0  $\mu IU/ml,$  2.2  $\mu IU/ml,$  2.5  $\mu IU/ml$  respectively. In our study, 317.0  $\pm$  162.5 pg/ml is the overall mean for serum vitamin B12. Mean value is lowest in 300.9 ±111 pg/ml in CTE, followed by  $308.8 \pm 97.1$  pg/ml in ATE, and highest in FPHL being  $355 \pm 277$  pg/ml. The overall mean for S Vitamin D3 is  $17.9 \pm 12.4$  ng/ml. Mean value is lowest in FPHL being  $15.5 \pm 5.9$  ng/ml, followed by  $18.3 \pm 13.3$  ng/ml in CTE, and highest in ATE being  $19 \pm 14.3$  ng/ml.

Table 2: Haemoglobin

Hb (g%)	Overall	ATE (n=39)	CTE (n=37)	FPHL (n=24)	P value		
1.<8	7	4 (10.3%)	3 (8.1%)				
2.8-10.9	42	14 (35.9 %)	14 (37.8%)	14 (58.3 %)	0 4005		
3. 11-11.9	19	7 (17.9%)	9 (24.3%)	3 (12.5%)	0.4903		
4.>12	32	14 (35.9%)	11 (29.7 %)	7 (29.2%)			
p value (TE vs FPHL) 0.2209							

Haemoglobin less than 12 mg/dl was present in 68% women and 32 % patients had haemoglobin more than or equal to 12mg/dl. Severe anaemia (Hb <8gm/dl) was noted in 4% cases of acute telogen effluvium, and 3% cases of chronic telogen effluvium. Anemia was noted in all cases of ATE (64.1%), CTE (70.27%), FPHL (70.83%) in our study.

Table	3:	S.	Ferritin
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S. Fe	erritin (g/dl)	Overall	ATE (n=39)	CTE(n=37)	FPHL (n=24)	P value			
1.	<15	58	24 (61.5%)	25 (67.6%)	9 (37.5 %)				
2.	15-40	23	5 (12.8%)	8 (21.6%)	10 (41.7 %)	0.04014			
3.	41-70	14	6 (15.4%)	4 (10.8%)	4 (16.7%)	0.04914			
4.	>70	5	4 (10.3%)		1 (4.2%)				
n val	value (TE vs EPHL) 0.05629								

As shown in the Table, S. Ferritin levels <15g/dl was present in 58% of total patients which included 61.5% of ATE and 67.6% of CTE. Only 37.5% of total FPHL patients had S. Ferritin levels <15g/dl. Low serum ferritin (<70g/dl) was noted in 95% women including 89.7% ATE, 95.8% FPHL and all CTE patients.

Table 4:	Thyroid	Disorders
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Thyroid disorders:	Overall (n=100)	ATE (n=39)	CTE (n=37)	FPHL (n=24)	P value
1. Euthyroid	85	33 (84.6%)	31 (83.8 %)	21 (87.5 %)	
2. Subclinical hypothyroid	4	2 (5.1%)	1 (2.7%)	1 (4.2%)	0.0966
<ol><li>Overt hypothyroid</li></ol>	8	3 (7.7%)	3 (8.1%)	2 (8.3%)	0.9800
4. Hyperthyroid	3	1 (2.6%)	2 (5.4%)		

As depicted in the Table, euthyroid status was noted in 85% women. 8% women had overt hypothyroidism and 4% women had subclinical hypothyroidism. Hyperthyroidism was present in 3% women.

Table 5: S Vitamin B12

S V	it B12 (pg/ml)	Overall	ATE (n=39)	CTE (n=37)	FPHL (n=24)	p value
1.	<200	16	5 (12.8%)	8 (21.6 %)	3 (12.5%)	0.2926

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 07, 2023

2.	200-300	40	12 (30.8%)	17 (45.9 %)	11 (45.8 %)	
3.	>300	44	22 (56.4%)	12 (32.4 %)	10 (41.7 %)	
p value (TE vs FPHL) 0.8597						

As shown in the Table, low serum vitamin B12 level (<200 pg/mL) was noted in 16% women, and 40% women had depleted serum vitamin B12 (200-300 pg/ml). Low serum vitamin B12 (<300 pg/mL) was noted in 56% of total patients which included 43.6% ATE, followed by 67.5% CTE, 58.3% of FPHL cases (p value 0.2926).

Table 6:	S	Vitamin D3	
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S Vi	it D (ng/ml)	Overall	ATE (n=39)	CTE (n=37)	FPHL (n=24)	P value			
1.	<20	70	27 (69.2%)	25 (67.6 %)	18 (75%)				
2.	20-29	25	10 (25.6%)	9 (24.3%)	6 (25%)	0.799			
3.	>30	5	2 (5.1%)	3 (8.1 %)					
n val	value (TE ve EDUI ) 0.6531								

p value (TE vs FPHL) 0.6531

As depicted in the Table, low serum vitamin D3 level (<20 pg/mL) was noted in 70% women, and 25% women had insufficient serum vitamin D3 (20-29 pg/ml). Low serum vitamin D3 (<30 pg/mL) was noted in ATE (94.8% cases of ATE cases), followed by CTE (91.9% of CTE cases), all FPHL cases (p value-0.799).

#### Discussion

In our study, 68% women had haemoglobin less than 12 g/dl which included 19% cases with mild anaemia, 42% with moderate anaemia and 7% with severe anaemia, where as 32% patients had haemoglobin more than or equal to 12 g/dl.

Severe anaemia (Hb <8g/dl) was noted in 4% cases of acute telogen effluvium, and 3% cases of chronic telogen effluvium but was absent in FPHL. Anaemia (Hb <12g/dl) was noted in all cases of ATE (64.1%), CTE (70.27%), FPHL (70.83%) in our study. On comparing the Telogen Effluvium (ATE and CTE) with FPHL group, the p value was found to be 0.2209 which was not statistically significant.

In a study of 100 females conducted by Patel KB *et al.*, severe anaemia ( $\langle 8g/dl \rangle$ ) was found in 9% patients, moderate anaemia (8-10g/dl) in 10% patients while mild anaemia (10-12g/dl) was found in 68% patients, rest having normal haemoglobin level and was statistically not significant (p value-0.8).7 According to study by Kirti Deo *et al.* 73.4% cases had Hb  $\langle 12g/dl \rangle$ , 50.63% had mild anaemia of Hb 10-11.9g/dl, 23% had moderate anaemia of Hb 7-9.9g/dl and none had Hb levels  $\langle 7g/dl \rangle$ . 8 Malkud S noted anaemia in 60.24% patients, and 30% of FPHL patients; but no significant relationship was observed between CTE, haemoglobin level and serum ferritin level<sup>[1]</sup>.

Rustom and Pasricha in their study noted 50% of patients had haemoglobin less than 12 g/dl. Severe anaemia (Hb <8g/dl) was noted in 6.7% cases of acute telogen effluvium and 14.3% cases of chronic telogen effluvium. According to study by Jayashankar CA *et al.* in 100 females with diffuse hair loss, anaemia was observed in 66% of the patients reflecting a strong correlation between telogen effluvium and anaemia which was similar to our study <sup>[10]</sup>. Moeinvaziri *et al.*, noted Hb <12g/dl in 26.6% patients of telogen effluvium, and anaemia was more commonly associated with telogen effluvium <sup>[11]</sup>.

The overall mean haemoglobin in our study was 11 g/dl. Mean haemoglobin in ATE, CTE, FPHL was 11g/dl. This was in contrast to study conducted by Agarwal S *et al.*, in which the mean haemoglobin levels in patients with CTE (10.36  $\pm$  1.72) were significantly lower as compared to TE (11.34  $\pm$  1.54) and FPHL (12.36  $\pm$  1.30). They observed that 32% of the patients to be mildly anaemic (11-11.9 g%), 18% moderately anaemic (8-10.9 g%) and the rest 2% to be severe anaemic (< 8 g%) <sup>[12]</sup>.

The lower haemoglobin value in our study could be due to increased prevalence of anaemia in females who were the study population.

In our study, 58% of total patients had overall S. Ferritin levels <15g/dl which included 61.5% of ATE and 67.6% of CTE. Only 37.5% of total FPHL patients had S. Ferritin levels <15g/dl.

Low serum ferritin (<40g/dl) was noted in 81% women including 74.3% ATE, 89.2% CTE and 79.2% FPHL patients. Low serum ferritin (<70g/dl) was noted in 95% women including 89.7% ATE, 95.8% FPHL and all CTE patients. The overall mean S. Ferritin value was  $21.7 \pm 23.3$  g/dl and was lowest in patients with CTE (15.9 $\pm$  16.0) as compared to those with TE (23.3 $\pm$  26.7) and highest in FPHL (27.8 $\pm$  25.6) in our study. On comparing Telogen Effluvium group (included ATE and CTE) with FPHL group, the p value was found to be 0.0562 which was statistically significant.

Our study showed similar findings as the mean serum ferritin levels were lowest in CTE patients (21.21  $\pm$  15.86) as compared to those with TE (29.44  $\pm$  21.64) and FPHL (30.38  $\pm$  18.78) in a study conducted by Agarwal S *et al.*, <sup>[10]</sup>. In the study conducted by Malkud S *et al.*, S. ferritin level <70g/dl was seen 93.24% patients with mean serum ferritin level for FPHL and CTE was 36.11 g/dl and 39.39 g/dl respectively. 82.35% of FPHL patients and 88.23% of CTE patients showed serum ferritin level <70g/dl <sup>[11]</sup>. In study conducted by Raichur *et al.*, 18.6 g/dl was the mean S. Ferritin value of all participants.

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 07, 2023

Mean serum ferritin levels were lowest in patients with CTE 16 g/dl and highest in FPHL with 36.64 g/dl [13].

In a retrospective study by Olsen *et al.*, when ferritin less than or equal to 15 g/dl was used as the cut-off limit, iron deficiency was found to occur in 12.4% and 12.1% of premenopausal women with FPHL and CTE respectively. When ferritin less than or equal to 40 g/dl was used as the cut-off, iron deficiency was observed in 58.8% and 63.8% of premenopausal women with FPHL and CTE respectively. When ferritin less than or equal to 70 g/dl was used as the cut-off, iron deficiency was observed in 87.1% and 86.2% of premenopausal women with FPHL and CTE respectively. When ferritin less than or equal to 70 g/dl was used as the cut-off, iron deficiency was observed in 87.1% and 86.2% of premenopausal women with FPHL and CTE respectively. Mean serum ferritin level of the patients for FPHL was 61.01 g/dl and 51.81 g/dl with CTE in the study by Olsen *et al.*, In a cross-sectional study conducted by Gowda D *et al.*, in 100 patients with hair loss, low ferritin levels were observed in 41.67% FPHL, 11.76% MPHL, 40.74% TE (p value 0.012) and a relatively higher proportion of participants with TE had iron deficiency (P = 0.069). In a study conducted by Tamer F *et al.*, the mean serum ferritin level was 14.72 ±10.70 ng/ml in patients and 25.30 ±14.41 ng/ml in healthy individuals. Also, serum ferritin level was decreased in 20.4% patients while 79.6% patients had normal serum ferritin levels, also 9.1% healthy individuals had a decreased serum ferritin level, 90.9% healthy individuals had normal serum ferritin levels (p value-0.09) <sup>[14]</sup>.

In our study, euthyroid status was noted in 85% women, 8% women had overt hypothyroidism, 4% women had subclinical hypothyroidism and 3% women had hyperthyroidism. The overall mean S. TSH value was 3.7  $\mu$ IU/ml. Mean S. TSH value in ATE, CTE, FPHL was 6.0  $\mu$ IU/ml, 2.2  $\mu$ IU/ml, 2.5  $\mu$ IU/ml respectively. (p value 0.98)

Hyperthyroidism was present in 3% women and hypothyroidism was detected in 10.86% cases in study by Srivastav *et al.*, Thyroid function test was undertaken in 17 out of 35 patients and evidence of subclinical hypothyroidism and hyperthyroidism was recorded in 1 patient each <sup>[4]</sup>.

In a study conducted by Poonia K, 11% cases were found to be suffering from newly diagnosed thyroid disorder which was hypothyroidism <sup>[15]</sup>. According to the study by Kirti Deo *et al.*, in 135 female patients, 17% patients were found to be suffering from thyroid disorders which included 9.63% with hypothyroidism and 7.4% with hyperthyroidism, however 6.7% were newly diagnosed thyroid disorder in their study which was statistically not significant <sup>[8]</sup>.

In a study conducted by Tamer F *et al.*, the mean serum TSH level was  $1.32 \pm 0.96 \mu$ IU/ml in patients and  $1.56 \pm 1.18 \mu$ IU/ml in healthy individuals (p = 0.10) (normal range of 0.4 to 4.2  $\mu$ IU/ml). The serum TSH level was decreased in 1.9% patient, within normal limits in 98.1% patients. The serum TSH level was decreased in 1.8% healthy individual and was increased in 1.8% healthy individual. 96.4% healthy individuals within the control group had normal serum TSH levels (p = 0.56 statistically NS) <sup>[16]</sup>. Our study differs from the other studies as we had excluded the known cases of thyroid disorders.

In our study,  $317.0 \pm 162.5$  pg/mL was the overall mean for serum vitamin B12. Mean value was lowest in 300.9 ±111 pg/mL in CTE, followed by 308.8 ± 97.1 in ATE, and highest in FPHL being 355 ± 277 pg/mL.

Low serum vitamin B12 level (<200 pg/mL) was noted in 16% women, and 40% women had depleted serum vitamin B12 (200-300 pg/ml). Low serum vitamin B12 (<300 pg/mL) was noted in 56% of total patients which included 43.6% ATE, followed by 67.5% CTE, 58.3% of FPHL cases (p value 0.2926). On comparing the Telogen Effluvium (ATE and CTE) with FPHL group, the p value was found to be 0.8597 which was statistically not significant.

In study conducted by Poonia K *et al.*, Serum vitamin B12 < 210 pg/mL was found in 76% of cases. Serum vitamin B12 < 211 pg/mL was observed in 63.6% of FPHL patients, 79% of CTE, 81.3% of ATE patients with p value of 0.38 which was statistically not significant <sup>[15]</sup>.

In a cross-sectional study conducted by Gowda D *et al.*, in 100 patients with hair loss, low Vitamin B12 levels were observed in 16.67% FPHL, 8.82% MPHL, 9.26% TE (p-0.713) with no significant difference in other micronutrients between TE and androgenetic alopecia similar to our study. They could demonstrate noticeable prevalence of amino acid and micronutrient deficiencies irrespective of the type of alopecia <sup>[14]</sup>.

In a study conducted by Tamer F *et al.*, the mean serum vitamin B12 level was  $291 \pm 233$  pg/ ml in patients and  $330 \pm 247$  pg/ml in healthy individuals (p = 0.18) (normal range is 190 to 880 pg/ml). The serum vitamin B12 level was decreased in 13% patients while 47 (87%) patients had a normal serum vitamin B12 level. However, 7.3% healthy individuals had a decreased serum vitamin B12 level, while 92.7% healthy individuals had normal serum vitamin B12 levels (p = 0.33 statistically NS) <sup>[16]</sup>.

Variations in the percentage could be because of the dietary habits in the study population as vitamin B12 is largely found in nonvegetarian food such as meat, chicken, fish, and eggs. Thus, it can be inferred that nutritional deficiencies could be a cause of hair loss irrespective of pattern or type of alopecia.

In our study,  $17.9 \pm 12.4$  ng/ml was the overall mean for serum vitamin D. Mean value was lowest in FPHL being  $15.5 \pm 5.9$  ng/ml, followed by  $18.3 \pm 13.3$  ng/ml in CTE, and highest in ATE being  $19 \pm 14.3$  ng/ml.

Low serum vitamin D3 level (<20 ng/mL) was noted in 70% women, and 25% women had insufficient serum vitamin D3 (20-29 ng/ml). Low serum vitamin D3 (<30 ng/mL) was noted in ATE (94.8% cases

ISSN:0975 -3583,0976-2833 VOL14, ISSUE 07, 2023

of ATE cases), followed by CTE (91.9% of CTE cases), all FPHL cases (p value- 0.799).

On comparing the Telogen Effluvium group (included ATE and CTE) with FPHL group, the p value was found to be 0.6531 which was statistically not significant.

In a study conducted by Mohammad NM *et al.*, no significant relation between serum vitamin D and duration of TE was observed. Mean vitamin D was  $26.69 \pm 44.1$ ng/ml in ATE and  $12.77 \pm 29.8$ 6ng/ml in CTE group which was statistically not significant (p value- 0.32). In a study conducted by Poonia K *et al.*, serum vitamin D3 level was found to be below normal range <30 ng/dl in 81% females. Serum vitamin D3 level <30 ng/dl was seen in 90.9% of FPHL patients, 79% of CTE, 75% of ATE patients with p value of 0.38 which was statistically not significant. Low vitamin D3 level was statistically significant. Low vitamin D3 level was statistically significantly associated with DHL, but not with any particular pattern of hair loss.15 In a case–control study by Nayak K *et al.*, 81.8% cases had Vitamin D deficiency compared to 45.5% of controls and difference was statistically significant (p- 0.007). Half of the controls (50%) were in the Vitamin D insufficiency category and none of the cases had normal Vitamin D values, only 4.5% controls were in the normal category <sup>[17]</sup>. According to a study conducted by Banahashemi M *et al.*, mean serum Vitamin D3 level in patient and control group was 13.45 and 17.16 respectively and there was a significant difference between the two groups in Vitamin D3 serum levels (p value - 0.04) <sup>[18]</sup>.

In a study conducted by Tamer F *et al.*, the mean serum Vitamin D level was 14.2  $\pm$ 8.09 ng/ml in patients and 17.01  $\pm$ 8.59 ng/ml in healthy individuals (*p*-0.016). The serum Vitamin D level was decreased in 79.6% patients and 70.9% healthy individuals and was normal in 20.4% patients and 29.1% healthy individuals in the control group (*p* = 0.29 statistically NS)<sup>[16]</sup>.

#### Conclusion

Majority of the females in our study had low haemoglobin, serum ferritin, serum vitamin B12, serum vitamin D3 and abnormal thyroid levels showing an association with hair loss. Psychological stress was observed in most of the patients. Even in patients with normal haemoglobin levels, it is required to determine serum ferritin levels to confirm iron deficiency.

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