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Original Article

Pathology Section

Histopathological and Biochemical Evaluation of Diffuse Hair Loss in Women: A Cross-sectional Study

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ABSTRACT

Introduction: Diffuse hair loss in women has multiple aetiologies, which often present a diagnostic challenge due to the significant aetiological overlap between different types of alopecia. While clinical examination provides initial clues, histopathology remains a vital tool for definitive diagnosis. Furthermore, nutritional and endocrine factors, such as iron stores and thyroid function, are frequently implicated as systemic triggers or aggravators of hair loss.

Aim: To evaluate the histopathological spectrum of scalp biopsies in female patients presenting with diffuse hair loss.

Materials and Methods: This prospective cross-sectional analytical study was conducted at the Department of Pathology at Bangalore Medical College and Research Institute, Bengaluru, Karnataka, India over two years between January 2016 and December 2017. involving 50 female patients aged 15-45 years with diffuse hair loss. Scalp biopsies (4-mm punch) were examined in both horizontal and vertical sections to assess follicular density and morphology. Serum ferritin and thyroid profiles were estimated. Due to the non normal distribution of biochemical data, results were expressed as medians with Interquartile Ranges (IQR). Statistical analysis was performed using Kruskal-Wallis and Spearman's correlation tests {Statistical Package of Social Sciences (SPSS) version 28.0}.

Results: Diffuse Alopecia Areata (DAA) was the most frequent diagnosis in 28 (56%) cases, followed by Female Pattern Hair Loss (FPHL) 19 (38%) cases and Chronic Telogen Effluvium (CTE) in 3 (6%) cases. Follicular miniaturisation and perifollicular fibrosis were noted in 16 (84.2%) and 15 (78.9%) of FPHL cases, respectively, with a mean Terminal-to-vellus ratio (T:V) of 3:1. Additionally, 23 (82.1%) DAA cases showed peribulbar lymphocytic infiltration. CTE cases exhibited increased telogen hairs but maintained a normal terminal-to-vellus hair ratio (7.1:1). Suboptimal serum ferritin levels (<40 ng/mL) were identified in 18 (36%) cases, while 21 (42%) patients exhibited elevated TSH levels (>4.2 mIU/L). The FPHL group showed the lowest median ferritin levels (46.0 ng/mL; IQR: 10.0-58.4) and higher median TSH levels (5.05 mIU/L) compared to other groups. Although the correlation between the degree of severity of hair fall and TSH was not statistically significant, a clear numerical trend was observed, with median TSH levels rising from 3.8 mIU/L in Grade-I to 6.2 mIU/L in Grade-III cases.

Conclusion: Diffuse Alopecia Areata (DAA) is the most common cause of diffuse hair loss in females, highlighting the necessity of scalp biopsy for accurate diagnosis. The high frequency of suboptimal ferritin and elevated TSH, along with the numerical trend between TSH and hair loss severity, indicates that these biochemical factors likely act as significant clinical aggravators. Therefore, a comprehensive diagnostic approach incorporating histopathology and routine screening for iron and thyroid status is essential for the effective management of diffuse hair loss in women.

Keywords: Diffuse alopecia areata, Female pattern hair loss, Scalp biopsy, Serum ferritin

INTRODUCTION

Diffuse hair loss is one of the common complaints among women that significantly affect the quality of life because of its psychological and cosmetic implications [1]. The condition may be caused by various reasons, including hormonal imbalances, nutritional deficiencies and autoimmune disorders [2]. FPHL, DAA and CTE, are the most common causes of diffuse hair loss in women [3]. Accurate diagnosis is crucial for early diagnosis and effective treatment; however, differentiating between these conditions based only on clinical presentation leads to diagnostic uncertainty [4].

Many studies have highlighted the role of iron deficiency and thyroid dysfunction in the pathogenesis of diffuse hair loss [5]. Iron deficiency, before the anaemia sets in, has been associated with hair loss, particularly in women [6]. Similarly, thyroid dysfunction, especially elevated TSH levels, has been linked to hair cycle disruption and increased hair shedding [7]. Histopathological evaluation of scalp biopsies, along with biochemical markers such as serum ferritin and thyroid profile, can provide valuable insights into the underlying causes of diffuse hair loss [8]. The role of systemic factors in modulating the hair cycle is well-documented but often overlooked in routine screening. Accurate and early diagnosis may help identify underlying systemic or autoimmune contributors to diffuse hair loss and assist clinicians in selecting appropriate management strategies.

The present study aimed to evaluate the histopathological spectrum of scalp biopsies in females with diffuse hair loss and to correlate these findings with serum ferritin levels and thyroid profiles to improve diagnostic evaluation of diffuse hair loss.

MATERIALS AND METHODS

This prospective cross-sectional analytical study was conducted at the Department of Pathology, Bangalore Medical College and Research Institute, Bengaluru, Karnataka India over two years duration between January 2016 and December 2017 after obtaining the ethical clearance (No- BMC/PGs/159/2015-16). Informed consent was obtained from all participants prior to inclusion in the study.

Sample size calculation: The sample size was calculated using an estimated iron deficiency prevalence of 40% based on previously published studies evaluating diffuse hair loss in women [9].

$$n=\frac{Z^2\times p\times q}{d^2}$$

Where n=required sample size,

Z=value for desired confidence level (1.96 for 95% CI)

P=estimated prevalence (0.40)

q=1-p (0.60)

d=margin of error (0.14)

$$n=\frac{(1.96)^2\times0.4\times0.6}{(0.14)^2}$$

$$n=\frac{3.84\times0.24}{0.0196}=\frac{0.9216}{0.0196}=47.04\approx47$$

Assuming a prevalence of 40%, a 95% confidence interval and a margin of error of 14%, a minimum sample size of 47 patients was required. A total of 50 patients were enrolled.

Inclusion and Exclusion criteria: Total 50 female patients aged 15-45 years with diffuse hair loss were included. The pregnant and lactating women, individuals exposed to radiotherapy or chemotherapy and those with scarring alopecia or malignant scalp lesions were excluded from the study.

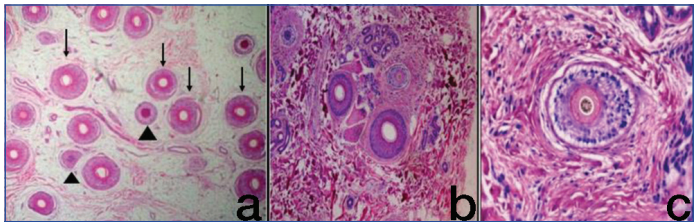
Detailed clinical histories were recorded and scalp biopsies were performed. Two 4 mm punch biopsy specimens were collected from each patient, processed and stained with Haematoxylin and Eosin (H&E). Horizontal and vertical sections were examined under a light microscope. Vertical sections were used for assessment of follicular morphology and perifollicular changes and horizontal sections for evaluation of follicular density, anagen to telogen ratio and miniaturisation. Cases were grouped into, based on histopathological findings, DAA, FPHL and CTE. Serum ferritin and thyroid profiles (TSH, T3, T4) were estimated using radioimmunoassay.

STATISTICAL ANALYSIS

Continuous variables were assessed for normality. As serum ferritin and TSH values showed non normal distribution, they were expressed as median with Interquartile Range (IQR) and range. Categorical variables were expressed as frequencies and percentages. For comparison between the three histopathological groups (FPHL, DAA and CTE), Kruskal-Wallis test was used for continuous variables and Chi-square test was applied for categorical variables. Correlation between disease severity (Ludwig grading) [10] and biochemical parameters was assessed using Spearman's rank correlation. A p-value of <0.05 was considered statistically significant. Data was analysed using Statistical Package of Social Sciences (SPSS) version 28.0.

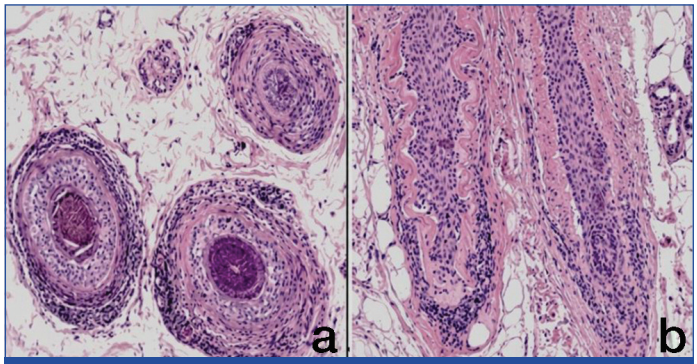
RESULTS

The age of patients ranged from 15-45 years with mean age of 28.6±7.1 years and majority of patients were in age group 26-35 years. DAA was the most common histopathological diagnosis in 28 cases (56%), followed by FPHL in 19 (38%) cases and CTE in three cases (6%). The mean age of FPHL, DAA and CTE was 29±7.1, 32±8.1 and 24±3.5 years, respectively. Follicular miniaturisation was noted in 84% of FPHL cases and 78% of FPHL showed perifollicular fibrosis with mean T:V ratio (3:1) [Table/Fig-1a-c], while 82% of DAA cases showed peribulbar lymphocytic infiltration [Table/Fig-2a,b] CTE cases exhibited increased telogen hairs but maintained a normal terminal-to-vellus hair ratio (7.1:1) (Normal terminal to vellus ratio- >7:1) [Table/Fig-3] [10].

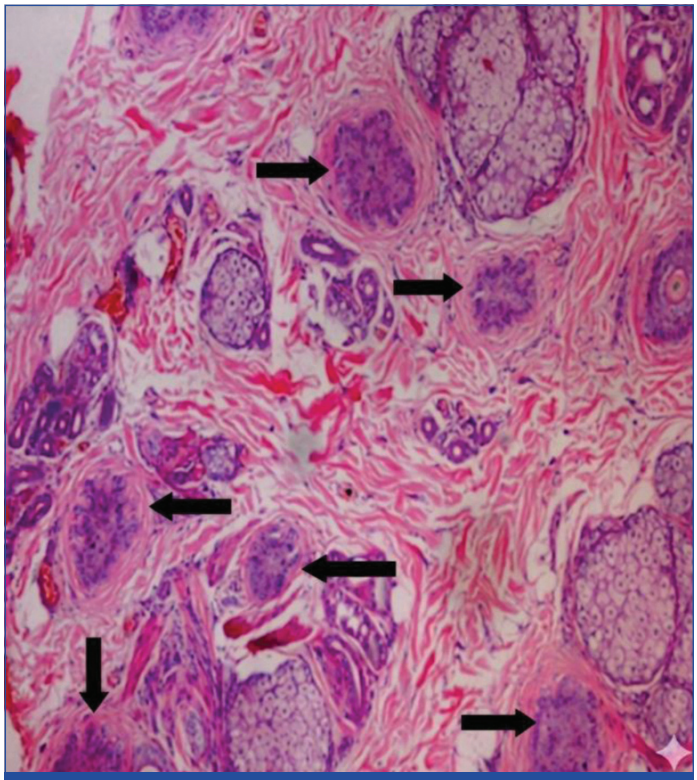


[Table/Fig-1]: Histopathological examination of FPHL: a) Transverse section showing variability in diameter of the follicles. Arrows showing terminal follicle and arrow heads showing vellus hair (H&E 4x); b) Miniaturised follicles (H&E 4x); c) Miniaturised follicle with perifollicular fibrosis (H&E 40x) (original image).

Ludwig's scale was used to assess hair loss severity. Grade-II hair loss (moderate thinning) was the most common (58%), followed by Grade-I (30%) and Grade-III (12%) [Table/Fig-4].



[Table/Fig-2a,b]: Histopathological examination of DAA: a) Transverse section showing perifollicular fibrosis and inflammation (H&E, ×40); b) Vertical section showing peribulbar lymphocytic infiltrates (H&E, ×40) (original image).



[Table/Fig-3]: Histopathological examination of CTE. Transverse section showing (arrow) increase in telogen follicular units (H&E, ×10) (original image).

Ludwig type	DAA n (%)	FPHL n (%)	CTE n (%)
I	10 (20%)	05 (10%)	0
II	13 (26%)	13 (26%)	03 (6%)
III	05 (10%)	01 (2%)	0

[Table/Fig-4]: Pattern of hair loss according to the degree of hair loss (Ludwig type).

Low serum ferritin level (<40 ng/mL) was observed in 36% of patients [Table/Fig-5]. The overall serum ferritin levels showed a non normal distribution with a median value of 51.0 ng/mL (IQR: 20.15-64.15; range: 3.2-90 ng/mL).

Serum ferritin levels (ng/mL)	DAA (%)	CTE (%)	FPHL (%)
<12	4 (8%)	0	6 (12%)
12-20	2 (4%)	0	1 (2%)
21-40	4 (8%)	0	1 (2%)
41-70	13 (26%)	3 (6%)	9 (18%)
≥71	5 (10%)	0	2 (4%)
Total	28 (56%)	3 (6%)	19 (38%)

[Table/Fig-5]: Distribution according to serum ferritin levels.

On comparison between different histopathological groups: CTE: median ferritin level 62.0 ng/mL (IQR: 57.0-63.5; range: 55-65 ng/mL), DAA: median ferritin level 49.0 ng/mL (IQR: 32.0-64.65; range: 12-90 ng/mL), FPHL: median ferritin level 46.0 ng/mL (IQR: 10.0-58.4; range: 3.2-85 ng/mL). Relatively lower ferritin levels were observed in patients with FPHL; however, the difference between groups was not statistically significant (Kruskal-Wallis test, p=0.28). Correlation of biochemical parameters with severity of hair loss (Ludwig grading) using Spearman's correlation did not show a statistically significant association between serum ferritin levels and severity of hair loss (Spearman's correlation, p=0.41, r=0.12) [Table/Fig-6].

Degree of hair loss (Ludwig Grade)	Serum ferritin (ng/mL) Median (IQR; Range)	TSH (mIU/L) Median (IQR; Range)
Grade-I	48.0 (22.0-60.0; 5.0-85.0)	3.8 (3.2-6.5; 0.6-18.0)
Grade-II	45.0 (18.0-58.0; 3.2-80.0)	4.5 (3.8-8.2; 1.5-20.0)
Grade-III	52.0 (30.0-65.0; 10.0-90.0)	6.2 (4.5-9.8; 2.5-22.0)
p-value (Spearman's correlation)	0.41	0.36
r-value	0.12	0.04

[Table/Fig-6]: Comparison of serum ferritin and TSH with degree of hair loss (Ludwig Grading).

Hypothyroidism (TSH >4.2 mIU/L) was detected in 42% (21) of cases. Among these, sub-clinical hypothyroidism constituted the majority, accounting for 40% (20) of cases, while overt hypothyroidism was identified in 2%(1) of cases. TSH values were non normally distributed with a median value of 4.0 mIU/L (IQR: 3.6-8.2; range: 0.6-22 mIU/L). Serum T3 levels showed a median of 2.0 nmol/L (IQR: 1.1-2.6; range: 1.0-3.8 nmol/L) (normal range- 1.1-2.8 nmol/l), while serum T4 levels had a median of 77 nmol/L (IQR: 70-92; range: 58-112 nmol/L) (normal range- 64-155 nmol/L).

On sub-group analysis:

- **FPHL:** TSH median 5.05 mIU/L (IQR: 4.0-8.73; range: 2.8-12.2 mIU/L), T3 median 2.08 nmol/L (IQR: 1.35-2.6; range: 1.0-3.8 nmol/L), T4 median 82 nmol/L (IQR: 72-91; range: 60-102 nmol/L).
- **DAA:** TSH median 3.9 mIU/L (IQR: 3.35-9.41; range: 0.6-22 mIU/L), T3 median 1.8 nmol/L (IQR: 1.08-2.6; range: 1.0-3.8 nmol/L), T4 median 73 nmol/L (IQR: 69.5-93; range: 58-112 nmol/L).
- **CTE:** TSH median 2.8 mIU/L (IQR: 2.3-4.8; range: 1.8-6.8 mIU/L), T3 median 2.0 nmol/L (IQR: 1.95-2.45; range: 1.9-2.9 nmol/L), T4 median 92 nmol/L (IQR: 80-96; range: 68-100 nmol/L).

Higher TSH levels were observed in patients with FPHL; however, the difference between groups was not statistically significant (Kruskal-Wallis test, p=0.34). The association between Thyroid-stimulating Hormone (TSH) levels and severity of hair loss (Ludwig grading) was analysed. Median TSH levels appeared higher in Grade-III cases (6.2 mIU/L) compared to Grade-I (3.8 mIU/L), though this was not statistically significant in the present study (Spearman's correlation, p=0.36, r=0.04)

DISCUSSION

In the present study, elevated TSH levels were observed in a substantial proportion of cases, with sub-clinical hypothyroidism constituting the majority. This high rate of thyroid involvement mirrors recent findings by Nikhila R Nair et al. (2025), who advocated for routine thyroid screening in all women presenting with diffuse thinning [11]. Similarly, the study by Malkud S highlighted the importance of thyroid screening in women with diffuse hair loss, as thyroid dysfunction was found to be a common underlying cause [4]. Lee S et al., reported a higher prevalence of thyroid abnormalities and thyroid autoantibodies in patients with alopecia areata, supporting a possible association between thyroid dysfunction and autoimmune-mediated hair loss [7]. The elevated TSH levels in DAA patients indicate a potential link between thyroid dysfunction and autoimmune hair loss [7]. These findings underscore the need for a comprehensive diagnostic approach, including serum ferritin and thyroid profile.

Diffuse hair loss is one of the most common complaints in females attending dermatology clinics. There are various causes for this condition with aetiological overlap and hence histopathological and biochemical evaluation is essential for an accurate diagnosis. In the present study of 50 patients, DAA was the most frequent diagnosis (56%), followed by FPHL (38%). The predominance of DAA in the present cohort aligns with previous studies, which have reported a high prevalence of autoimmune-related hair loss in women [1]. In contrast, many global studies have reported FPHL is the common cause of non scarring alopecia [12,13]. The high prevalence of DAA in the present cohort highlights the importance of scalp biopsy in differentiating sub-clinical autoimmune presentations from other causes of hair loss [14].

The present findings in DAA cases showed peribulbar lymphocytic infiltration in 82% of cases. This "swarm of bees" appearance is a critical histopathological hallmark that differentiates DAA from CTE [15]. This leads the anagen hair to enter into catagen phase, finally leading to increase in catagen and telogen hairs. The FPHL patient usually complains of slowly progressive hair thinning that may or may not be associated with increased shedding. The affected areas usually involve the vertex and upper parietal scalp and sometimes also frontoparietal areas of the head, unlike in men the frontal hair line is typically preserved [16]. In the FPHL sub-group, Authors observed follicular miniaturisation in 84% of cases with a T:V ratio of 3:1. This aligns with established diagnostic criteria suggesting that a T:V ratio of less than 4:1, viewed in horizontal sections, is highly indicative of androgenetic hair loss [16]. Telogen follicles in excess of 15% are considered to be abnormal and suggest a diagnosis of telogen effluvium in the absence of significant inflammation and miniaturisation [17].

Iron serves as an essential cofactor for ribonucleotide reductase, the rate-limiting enzyme for DNA synthesis in the rapidly dividing hair follicle matrix [18]. While standard laboratory reference ranges often define deficiency at <15 ng/mL, hair follicles exhibit higher metabolic demands. The present study utilised a threshold of <40 ng/mL, identifying a deficiency in 36% of patients. Recent literature supports this higher cut-off; Almohanna HM et al., emphasised that iron stores must be significantly higher than the levels required to prevent anaemia to maintain the anagen (growth) phase [19]. Iron deficiency, even in the absence of anaemia, has been investigated in women with hair loss, including FPHL, although the association remains inconsistent across studies [9]. Similarly, a study conducted by Sunil M et al., showed that among 263 CTE patients, only 32% had anaemia (Hb <12) but 86% had S. ferritin <40 ng/mL with the mean S. ferritin being 23.93 ng/mL [20]. This suggests that "pre-latent" iron deficiency acts as a significant aggravator across all types of diffuse hair loss. Additionally, Thompson JM et al., highlighted the potential role of micronutrient deficiencies, including iron deficiency, in the pathogenesis of alopecia and emphasised the importance of nutritional assessment in patients presenting with hair loss [8]. Thyroid hormones directly modulate hair follicle oxygen consumption and protein synthesis [21].

In the present study, the association between TSH levels and the severity of hair loss assessed by the Ludwig grading system was not statistically significant (Spearman correlation, r = 0.04, p = 0.36). Although median TSH values were numerically higher in Grade III compared with Grade I hair loss, this descriptive difference should not be interpreted as evidence of a directional or graded association between TSH levels and hair loss severity. The observed findings

therefore suggest that, within present study population, TSH levels were not significantly correlated with the clinical severity of hair loss. Larger studies with adequate representation across different severity grades and longitudinal follow-up may be required to determine whether thyroid dysfunction has any clinically relevant relationship with progression of hair loss.

Limitation(s)

Although the calculated sample size requirement was achieved, the relatively small sub-group sizes, particularly in the CTE group, may have limited the detection of weaker statistical associations. This is a single-centre study, which may not be generalisable to different ethnic or geographical populations with varying dietary and genetic profiles. This is a cross-sectional design and the lack of longitudinal follow-up prevented the assessment of hair regrowth following iron supplementation or thyroid correction. The absence of age-matched healthy controls limited the comparison of biochemical markers against a baseline "normal" population. Other relevant markers, such as Vitamin D, Zinc and androgens, were not included in the study.

CONCLUSION(S)

The DAA was the predominant cause of diffuse hair loss in women in the present study. Suboptimal ferritin levels and elevated TSH were frequently observed, suggesting that nutritional and endocrine factors may co-exist with primary alopecia as important clinical aggravators. A comprehensive diagnostic approach incorporating scalp biopsy and biochemical evaluation may aid in accurate diagnosis and management.

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