

Evaluation of the relationship of digital phototrichogram findings of patients with diffuse hair loss with blood TSH, ferritin and vitamin B12 levels

 Leyla Bilik,¹  Ibrahim Kokcam,²  Mustafa Esen³

¹Department of Dermatology and Venereal Diseases, Mardin State Hospital, Mardin, Turkiye

²Department of Dermatology and Venereal Diseases, Adiyaman University Training and Research Hospital, Adiyaman, Turkiye

³Department of Dermatology and Venereal Diseases, Firat University Faculty of Medicine, Elazig, Turkiye

ABSTRACT

OBJECTIVE: Telogen effluvium (TE) is a type of alopecia that is frequently seen in women. Among factors resulting in hair loss, many reasons such as endocrine diseases, nutrition disorders, stress, anemia, low ferritin levels, vitamin B12 deficiency, and thyroid diseases are found. A digital phototrichogram is one of the non-invasive methods of diagnosis in the evaluation of alopecia. In this study, it was aimed to compare biochemical parameters of female patients with diffuse hair loss with phototrichogram findings.

METHODS: 108 female patients with diffuse hair loss were included in the study. Patients were divided into two groups: acute and chronic TE, and a hair pull test was applied. Total blood count, blood biochemistry, iron, iron binding capacity, ferritin, thyroid stimulating hormone (TSH), sT3, sT4, folic acid, and vitamin B12 levels were examined. The telogen/anagen ratios and hair densities of patients were determined with a phototrichogram.

RESULTS: The serum biochemical parameters (aspartate aminotransferase, alanine aminotransferase, urea, creatinine), TSH, sT3, sT4, and folic acid levels of patients were within normal limits. Telogen ratio, anagen ratio, hair density, number of shed hairs, family history, blood ferritin, TSH, and vitamin B12 levels were found to be similar between groups having acute and chronic TE. In our study, while mean anagen and telogen ratios with trichoscan were similar to literature data, no statistically significant correlation was determined between patients' ages and examined trichoscan findings ($p>0.05$). The hair pull test positivity of patients with chronic TE was higher compared to patients with acute TE ($p<0.05$). In patients with positive hair pull tests, the telogen ratio and hair density were found to be higher. In the group with <40 ng/mL ferritin level, the mean telogen ratio was detected to be significantly higher than the mean anagen ratio ($p<0.05$). No significant correlation was determined between vitamin B12 and TSH levels in patients and phototrichogram findings ($p>0.05$).

CONCLUSION: The findings of this study showed that ferritin has an important role in diffuse hair loss, and the phototrichogram method is an auxiliary method for the physician in the diagnosis of TE.

Keywords: Digital phototrichogram; ferritin; telogen effluvium.

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Telogen effluvium (TE), due to a defect in the hair cycle, is characterized by widespread loss of telogen hair and is seen more frequently in women [1, 2]. TE can be seen as acute or chronic TE based on various reasons,

such as underlying endocrine, nutritional, psychological, or physical stress [2, 3]. Frequent reasons for chronic diffuse telogen hair loss are thyroid diseases, iron deficiency anemia, acrodermatitis enteropathica, and malnutrition [4, 5].



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Correspondence: Mustafa ESEN, MD. Firat Universitesi Tip Fakultesi, Deri ve Zuhrevi Hastalıklar Anabilim Dalı, Elazig, Turkiye.

Tel: +90 424 233 35 55 e-mail: dottoreessen@hotmail.com

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Various methods have been used in the evaluation of alopecia; however, a golden standard does not exist. Different methods are used for the assessment of the type and course of alopecia and also for monitoring the response to treatment. These can be divided into three groups: invasive, semi-invasive, and non-invasive methods. Among the non-invasive methods, scoring systems, global photographing, collection of hairs shed daily, hair weight and hair number, trichoscopy, and computer-assisted methods like phototrichograms and digital phototrichograms are found. Semi-invasive methods are the hair pull test, the hair plucking test (trichogram), the unit area trichogram, and the measurement of linear growth of hair. Assessment of matrix cell kinetics and scalp biopsies are invasive processes [6, 7]. A digital phototrichogram is a non-invasive, pain-free, easily repeatable method independent of the operator that can be used to measure parameters related to hair development within a short period of time, like 20 min, and yield computer-recorded results [8–10].

In this study, it was aimed to assess the relationship between anagen ratio, telogen ratio, and hair density, which are calculated with a digital phototrichogram, and ferritin, thyroid stimulating hormone (TSH), and vitamin B12 levels in patients with diffuse hair loss.

MATERIALS AND METHODS

This study was performed in accordance with the principles of the Helsinki Declaration. Local ethical committee approval was obtained for this study. (No. 18.11.2014, 19/7) 108 non-pregnant and non-lactating patients over the age of 18 who applied to our dermatology polyclinic between May 2015 and November 2015 with diffuse hair loss complaints and without any other scalp dermatological diseases or a chronic disease and/or current drug usage were enrolled in the study. Consents from the patients related to their participation in the study were taken.

Information about all patients, such as age, sex, period of hair loss, number of hairs shed daily, and family history of hair loss, was obtained. Patients with a hair loss period of <6 months were considered to have acute TE, and patients with a hair loss period of more than 6 months were considered to have chronic TE. Patients were additionally divided into 2 groups of <100 and >100 according to their number of shedded hairs. A hair pull test was applied to all patients. For this process, approximately 50–60 hair strands were held tightly between the thumb and index finger and pulled slowly; if the number of epilated

Highlight key points

- Serum biochemical parameters (AST, ALT, urea, creatinine), TSH, sT3, sT4 and folic acid levels of patients were within normal limits.
- Telogen ratio, anagen ratio, hair density, number of shed hairs, family history, blood ferritin, TSH and vitamin B12 levels were found to be similar between groups having acute and chronic TE.
- No statistically significant correlation was determined between patients ages and examined trichoscan findings.
- In the group with <40 ng/mL ferritin level, mean telogen ratio was detected to be significantly higher than the mean anagen ratio.
- Ferritin has an important role in diffuse hair loss, and phototrichogram method is an auxiliary method for the physician in the diagnosis of TE.

hairs was >6, then the hair pull test was considered to be positive. To evaluate total blood count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinin, serum iron, serum iron binding capacity (IBC), ferritin, TSH, sT3, sT4, folic acid, and vitamin B12 levels, 5 mL of blood was drawn from all patients on an empty stomach and sent to the laboratory. Total blood counts of patients were analyzed using the Siemens Advia 2120i (Germany) device with the flow cytometry method; AST, ALT, urea, creatinin, serum iron, and serum IBC were determined with the spectrophotometric method (Siemens Advia 2400 and 1800, Germany); ferritin, TSH, sT3, sT4, folic acid, and vitamin B12 were determined with the immunoassay method (Siemens Advia Centaur XP, Germany). 7–276.8 ng/mL was accepted as the reference value for ferritin. Patients were divided into two groups: <40 ng/mL, and >40 ng/mL according to their serum ferritin levels. All the obtained data were recorded in the patient follow-up form.

Application of the Digital Phototrichogram Method

A plate with a hole of 1 cm² was placed on the right temporoparietal region of the scalp of all patients. The hair was first taken out of this hole and shortened, then trimmed off with a standard shaving machine to a length of 0.5 mm. Patients were recalled two days later. Hair within the trimmed-off region was dyed by mixing a temporary lash dye (Gschwenter Haarkosmetik, Austria, Refecto Cil[®], water, stearyl alcohol, diglyceryl-polyacyl-adipate-2, PEG-40 hydrogenated castor oil, sodium stearyl sulfate, and sodyum laureth sulfate) with the solution found within the set. It was waited for 12 min, and

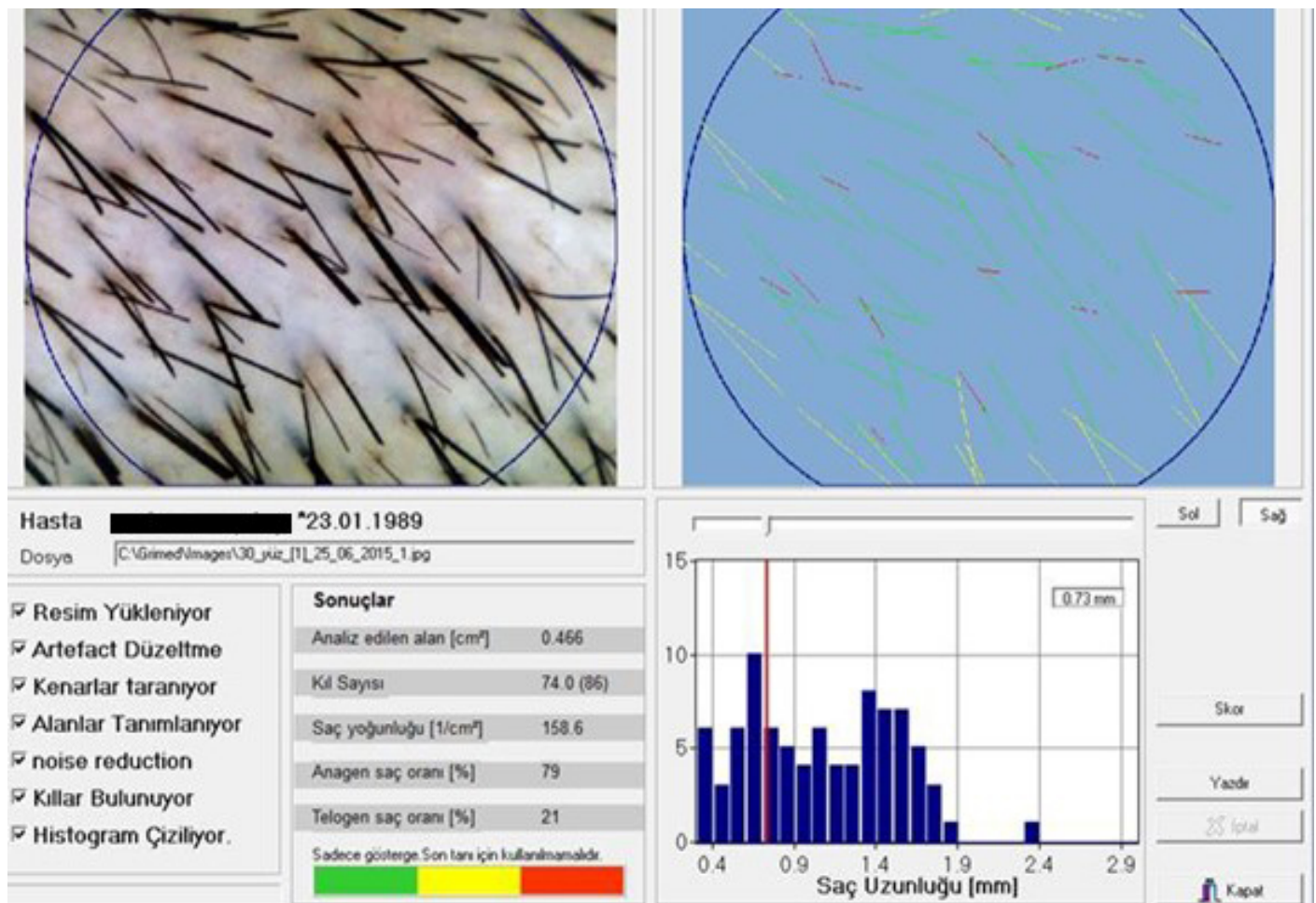


FIGURE 1. Telogen (red), anagen (green), end-of-cycle (yellow) hair in digital phototrichogram analysis.

the trimmed hair was let to absorb the dye. After this period, the trimmed region was cleaned with the alcoholic solution provided in the set (Schülke and Mayr GmbH, Germany, Kodan[®], 45.0 g 2-propanol, 10.0 g 1-propanol, and 0.20 g 2-biphenylol). Digital images were obtained from this area while still humid with the Grimed, China (Griscope) digital epiluminescence system. The obtained images were recorded in the Grimed version of the digital phototrichogram (trichoscan) software program. The 0.466 cm² region of the cut area of 1 cm² was analyzed. During the analysis, non-growing hair (telogen, red), growing hair (anagen, green), hair at the end of the cycle (yellow), and the amount of hair falling to the area of 1 cm² (hair density) were calculated (Fig. 1).

Statistical Analysis

SPSS (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL) 22 package programs were used for the analyses in the study. Mean and standard deviation val-

ues were used in the descriptive statistics of the data. In the correlation analysis, relationships among all the parameters were examined with the Pearson and Spearman correlation coefficients. In the intergroup comparisons, the Student t-test was used for parametric values, and the Mann–Whitney U test was applied for nonparametric values. Significance was assessed at levels of $p < 0.05$.

RESULTS

The age range of 108 women with diffuse alopecia included in the study was 18–60; the age average was determined to be 27.90 ± 8.51 . The clinical characteristics of the patients are given in Table 1. 81 patients (75%) had more than 100 shedded hairs daily. In the group having more than 100 shedded hairs daily, telogen ratio and hair density were found to be higher and anagen ratio was found to be lower compared to the group having <100 shedded hairs daily ($p < 0.05$).

TABLE 1. Clinical characteristics of patients (n=108)

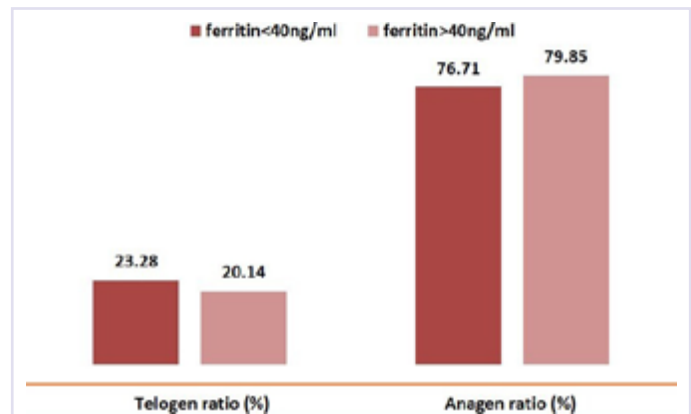
Patients	%
Family history of hair loss	
Present	69.4
None	30.6
The amount of hair shed per day	
<100	25
>100	75
Hair loss period	
Acute	15.7
Chronic	84.3
Hair pull test	
Positive	54.6
Negative	45.4

TABLE 2. Number of hair shedding in relation to ferritin levels and anagen-telogen ratios by ferritin category

Parameters	Relation to ferritin	
	Ferritin level (Mean±SD)	
Number of hair shedding		
<100	24.27±16.32	
>100	25.97±20.14	
Ferritin category		
	Ferritin	Ferritin
	<40 ng/mL	>40 ng/mL
Telogen ratio (Mean±SD)	23.28±7.47	20.14±4.38
Anagen ratio (Mean±SD)	76.71±7.47	79.85±4.38

SD: Standard deviation.

Ferritin levels were determined as <40 ng/mL in 87 patients (80.6%) and as >40 ng/mL in 21 patients (19.4%). Ferritin levels were determined to be lower than 7 ng/mL in 12 patients (11.1%). The telogen ratio mean of patients with blood ferritin levels <40 ng/mL was (23.28%±7.47%), and the anagen ratio mean was (76.71%±7.47%). The anagen ratio mean (79.85%±4.38%) of 21 patients with ferritin levels >40 ng/mL was determined to be higher than the telogen ratio mean (20.14%±4.38%) (Fig. 2). In the group with <40 ng/mL ferritin level, the mean telogen ratio was detected to be significantly higher than the mean anagen ratio ($p<0.05$). The mean ferritin level was found to be 24.27±16.32 in those who shed <100 hairs per day,

**FIGURE 2.** Anagen-telogen ratios in relation to ferritin levels.

while the mean ferritin level was 25.97±20.14 in those who shed more than 100 hairs per day. No significant difference was found in terms of ferritin levels according to the number of hairs shed daily ($p>0.05$) (Table 2). No significant difference was found between the groups in respect to hair density ($p>0.05$).

Serum biochemical parameters (AST, ALT, urea, creatinine), TSH, sT3, sT4, and folic acid levels of patients were within normal limits (Table 3). While vitamin B12 levels were below the normal level in 4 patients (3.7%), this level was determined to be within normal limits in 104 patients. A significant correlation could not be determined in the blood vitamin B12 and TSH levels, telogen ratio, anagen ratio, or hair density of patients ($p>0.05$).

A significant correlation was determined between telogen ratio and number of shedded hairs, hair density, anagen ratio, and serum folic acid levels ($p<0.05$). The digital phototrichogram findings of patients are presented in Table 4. A statistically significant correlation could not be detected between age and ferritin, vitamin B12, TSH, telogen ratio, anagen ratio, and hair density ($p>0.05$). 42 patients (38.8%) had substandard serum iron levels.

DISCUSSION

In this study, the relationship between anagen ratio, telogen ratio, and hair density calculated with a digital phototrichogram and ferritin, TSH, and vitamin B12 levels was evaluated in patients with diffuse hair loss. Although chronic TE was reported to be less frequent than acute TE, most of the patients in our study suffered from chronic TE. The reason for this situation may be that patients in the acute phase apply to tertiary health institutions at a later stage.

TABLE 3. Laboratory characteristics of patients

Parameters	Normal (n)	Low (n)	High (n)	Mean±SD
Hemoglobin (g/dL)	105	3	0	13.04±1.13
Hematocrit (%)	105	3	0	40.38±3.07
Iron (ug/dL)	66	42	0	76.12±43.03
IBC (ug/dL)	105	0	3	351.68±43.11
Ferritin (ng/mL)	96	12	0	25.55±19.20
Vitamin B12 (pg/mL)	104	4	0	313.57±116.79
Folic acid (ng/mL)	108	0	0	9.41±2.91
AST (U/L)	108	0	0	20.19±6,14
ALT (U/L)	108	0	0	17.25±8.13
Urea (mg/dL)	108	0	0	26.25±6.74
Creatinine (mg/dL)	108	0	0	0.55±0.10
TSH (mIU/L)	106	0	2	1.96±1.73
T3 (pg/mL)	108	0	0	3.15±0.37
T4 (ng/dL)	108	0	0	1.15±0.12

SD: Standard deviation; IBC: Iron binding capacity; TSH: Thyroid stimulating hormone; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; TSH: Thyroid stimulating hormone;.

The hair pull test is one of the methods used in the assessment of hair loss. The goal of this technique is to roughly assess the amount of hair loss and determine whether active and excessive hair loss is present or not [11]. Yuruker et al. [12], evaluated the phototrichogram findings of 55 female patients with long-term hair loss, found the hair pull test positive in 10 patients and negative in 31 patients, and emphasized that clinical tests such as the hair pull test and trichogram could be superior to the phototrichogram in the evaluation of patients with TE. A hair pull test was reported to be positive in TE and especially in acute TE; however, a negative hair pull test was not reported to exclude TE [13, 14]. In our study, a hair pull test was determined to be positive in 59 patients. In patients with positive hair pull tests, the telogen ratio and hair density were found to be higher.

One of the most frequently examined parameters of hair loss is hair density. Hair density is defined as the number of non-vellus hairs within 1 cm². According to the performed literature search, different results were found to be related to hair density in patients with alopecia. Rushton et al. [15] determined hair density as 181 hair/cm² with a phototrichogram and as 237 hair/cm² with a unit area trichogram in their study, in which phototrichogram and unit area trichogram were compared in a to-

TABLE 4. Digital phototrichogram characteristics of patients

Parameters	Lowest	Highest	Mean±SD
Telogen ratio (%)	9	44	22.67±7.07
Anagen ratio (%)	56	91	77.32±7.07
Density	110.40	303.30	209.61±42.64

SD: Standard deviation.

tal of 12 cases. Tajima et al. [16] determined hair density as 205.5±50.5/cm² in their study that they carried out with a phototrichogram in 159 female patients between the ages of 14–68 with diffuse hair loss. In our study, hair density with Trichoscan was found to be similar to literature data. Another important parameter examined in diffuse alopecia is the telogen and anagen ratio. In the human scalp, on average, 85–89% of hair follicles are at the anagen phase, 13% of them are at the telogen phase, and <1% are at the catagen phase [17]. Saraogi and Dhurat [18] found a weak correlation between clinical severity and telogen ratios in trichoscan in a study that they performed on 77 patients with hair loss and 22 control group members. In addition, when compared with the control group, the telogen ratio was paradoxically reported to be higher in the control group compared to the patient group. Lopez et al. [19] reported that the average telogen ratio was 37.4%, the average anagen ratio was 62.4%, and the average hair density was 239/cm² in their study that they performed with 180 female patients with diffuse hair loss. They reported that a significant correlation was present between the ages of the patients and hair density, i.e., there was a decrease in hair density with advanced age. They emphasized that a significant correlation did not exist between age and other trichoscan parameters. In our study, while mean anagen and telogen ratios with trichoscan were similar to literature data, no statistically significant correlation was determined between patients' ages and examined trichoscan findings (p>0.05).

In literature studies, low ferritin levels, vitamin B12 deficiency, and thyroid diseases were reported to be among the most frequent factors contributing to telogen hair loss [2, 13]. In this study, telogen ratio, anagen ratio, hair density, number of shedded hairs, family history, blood ferritin, TSH, and vitamin B12 levels were found to be similar between groups having acute and chronic TE. In studies evaluating the ferritin levels of patients with diffuse hair loss, different views were reported related to

ferritin levels. Moeinvaziri et al. [17] found that mean ferritin levels were found to be lower in the hair loss group compared to the control group in their study, in which 30 female hair loss patients and 30 control patients without hair loss between the ages of 15–45 were included. They suggested that serum ferritin levels of 30 ng/mL or lower were strongly related to telogen hair loss. In their study, in which 5110 pre-menopause patients between the ages of 35–60 were evaluated, Deloche et al. [20] reported that 59% of patients with severe hair loss had serum ferritin levels lower than 40 ng/mL. When patients having ferritin levels under 40 ng/mL were compared with patients having ferritin levels above 70 ng/mL, patients having ferritin levels lower than 40 ng/mL had significantly more hair loss. In our study, ferritin levels were found to be low, in accordance with literature data. In patients with ferritin levels <40 ng/mL, the telogen ratio was found to be higher and the anagen ratio was found to be lower; in other words, more severe TE was observed ($p < 0.05$).

Folic acid deficiency is another factor that is blamed for diffuse hair loss. Ozturk et al. [21] determined folate deficiency in 6 (2.6%) and excessive amounts of folate in 11 (4.8%) patients out of 248 patients in a retrospective study that they had performed to evaluate serum ferritin, vitamin B12, and folate levels of women with telogen alopecia who were in the pre-menopause period. In our study, we found that folic acid levels were within normal limits in all 108 patients. However, when patients with and without family history of hair loss were compared, folic acid levels were found to be significantly higher in patients without family history. In their study, in which they carried out to evaluate the frequency of serum ferritin, vitamin B12, folic acid, and thyroid function test defects, Avci et al. [22] determined that 51 (9.06%) out of 563 female patients who had applied with the complaint of telogene effluvium had folate deficiency.

One of the blood parameters investigated in the etiology of diffuse alopecia is vitamin B12 deficiency. Though routinely and frequently sought for, the number of studies demonstrating the relationship between chronic TE and vitamin B12 deficiency is quite low. In a study in which 563 female patients with TE were evaluated, 17 of the cases (3.02%) were determined to have vitamin B12 deficiency, and it was suggested that TE and vitamin B12 deficiency could not be associated with each other directly [22]. In the study by Ozturk et al. [21], they pointed out that 52 out of 245 patients (21.2%) had vitamin B12 deficiency in the biochemical examinations. In our study, 4 cases (3.7%) were found to have subnormal

vitamin B12 levels. A statistically significant correlation was not found between serum vitamin B12 level and telogen ratio, anagen ratio, or hair density ($p > 0.05$).

One of the frequently studied parameters in diffuse hair loss is thyroid function testing. In thyroid hormone deficiency, inhibition of cell division in the epidermis and skin annexes and a decrease in the anagen/telogen ratio are held responsible for hair loss. The relationship between hyperthyroidism and hair loss is not fully understood [2, 23]. Different views are reported in literature studies related to thyroid disorders. In the study by Lo Sicco et al. [24], in which they retrospectively evaluated the thyroid abnormalities of 367 patients with alopecia, abnormalities in thyroid functions were determined in 8 patients. It was emphasized that blood tests were not sufficient for the determination of thyroid abnormalities in patients with alopecia, and manual thyroid examination and thyroid ultrasonography should also be performed.

In a study in which comorbid diseases were investigated in 100 women with widespread hair loss for more than 6 months, Guler Ozden et al. [25] determined that thyroid disorders and euthyroid goiter were present in 18 patients. In our study, TSH levels were found to be above normal levels in only 2 out of 108 patients; however, sT3 and sT4 levels were found to be within normal limits. The TSH, sT3, and sT4 levels of all other patients were found to be within normal limits. No statistically significant correlation was found between TSH, sT3, and sT4 values and the telogen ratio, anagen ratio, and hair density of the cases ($p > 0.05$).

Conclusion

As a result, we think that the diagnosis of TE with trichoscan can be made more easily, and the level of serum ferritin plays an important role in diffuse TE.

Ethics Committee Approval: The Firat University Clinical Research Ethics Committee granted approval for this study (date: 18.11.2014, number: 19/07).

Authorship Contributions: Concept – LB, IK; Design – LB, IK; Supervision – LB, IK; Fundings – LB, IK; Materials – LB, IK; Data collection and/or processing – LB, IK; Analysis and/or interpretation – LB, IK; Literature review – LB, IK, ME; Writing – LB, IK, ME; Critical review – LB, IK, ME.

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