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RESEARCH ARTICLE

Lactoferrin as a Marker of Chronic Telogen Effluvium in Premenopausal Women

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Abstract

Aim: Evaluation of serum Lactoferrin, Hb and ferritin levels in females with chronic telogen effluvium (CTE) or acute telogen effluvium (ATE), in order to validate their role in these common hair loss diseases. Methods: Fifty three females (aged range 18 - 45 years) with hair loss, in the form of TE (ATE=27 as G2, group aged range18-35), (CTE=26 as G3 group aged range 18-45), and 26 age-matched females with no hair loss (as G1 group), were included in the study. Diagnosis was based upon clinical examination as well as dermoscopy. Serum Lactoferrin and ferritin levels were determined for each participant. Results: Serum lactoferrin levels showed highly significant differences (p= 0.00) in the comparison of chronic TE (G3) and Acute TE (G2) (7.68±2.13 ng/ml vs. 4.65±2.18 ng/ml), Also between (G1) and (G3) (5.21±1.03 ng/ml vs. 7.68±2.13 ng/ml), but there is no significant difference between (G1) and (G2) (5.21±1.03 ng/ml vs.4.65±2.18 ng/ml).Plasma ferritin level showed highly significant decrease (p=0.001) in comparison (G1) group and (G2) group(50.72±11.17 µg/dl vs. 36.49 ±15.04µg/dl) respectively ,Also showed highly significant decrease (p=0.00) between (G1) group and (G3)(50.72±11.17 µg/dl vs.31.98± 15.98 µg/dl) but there is no significant difference(P=0.47) between (G2) and (G3) (36.49 ±15.04 µg/dl vs. 31.98± 15.98 µg/dl).Hemoglobin level showed highly significant decrease (p=0.028) in (G1) and (G2) (12.89±0.70 mg/dl vs. 12.15±1.19 mg/dl) respectively, Also showed highly significant decrease (p=0.00) between (G1) and (G3) (12.89±0.70 mg/dl vs. 11.60±1.09 mg/dl), but there is no significant difference(P=0.12) between (G2) and (G3) (12.15±1.19 mg/dl vs. 11.60±1.09 mg/dl). Conclusion: High serum Lactoferrin is associated with hair loss in females with CTE, while low levels of ferritin are associated in ATE and CTE. Screening to establish ferritin levels in cases of hair loss and supplementing with them when they are deficient may be beneficial in the treatment of disease.

Keywords: *Lactoferrin, Telogen effluvium, Ferritin, Transferrin.*

Introduction

Telogen effluvium (TE) was first termed by Kligman to define an increased shedding of normal club hairs the follicle tends to act in a similar manner undergoing a premature termination of anagen and precipitating telogen [1]. Every hair follicle follows three cyclical phases: anagen (growth), katagen (regression leading to apoptosis), and telogen (resting) [2]. Normally, every hair follicle follows an independent cycle. While some hairs grow, others rest or are shed.

Thus, the hair density remains unchanged, and the same amount of hair is preserved [3]. Telogen effluvium can be classified into two groups, according to the duration of disease, as acute and chronic. The duration of disease is shorter than 6 months in acute TE, while it takes more than 6 months in chronic state.

In acute TE, hair loss typically begins 2-3 months later than the triggering event, although the triggering factor is unknown in 33% of the cases. However, chronic TE is frequent in healthy females in the fourth to fifth decades [2]. The majority of TE is subclinical; however, its actual incidence or prevalence still remains unclear.

It has no predilection for particular racial or ethnic groups. Although it affects both sexes, women are more likely to present for the evaluation of acute TE than men [1]. A higher number of women also suffer from chronic TE than men. Chronic TE, which is less common than the acute variant, mostly affects women between the ages of 30 and 60 years [4].

One of the most common causes of hair loss in premenopausal women is nutritional deficiency of iron. Screening for serum ferritin and hemoglobin should be performed to clarify the possible cause of hair loss [5]. Severe protein, caloric restriction, vitamin D deficiency, zinc deficiency and chronic starvation can also induce diffuse telogen hair loss [5].

Lactoferrin (Lf) belongs to the transferrin (Tf) family and is a non-heme iron binding glycoprotein with molecular weight of 78 kDa that contains around 690 amino acid residues. It is found in bovine milk as well as in humans [6]. In humans, it is one of the major proteins of all exocrine secretions including saliva, tears, semen, vaginal fluids, gastrointestinal fluids, nasal mucosa and bronchial mucosa [6]. The iron binding affinity of Lf is known to be the maximum amongst transferrin family. Lf can remain bound to iron in varying pH range [6].

Lactoferrin is considered to be an important host defense molecule and has a diverse range of physiological functions such as antimicrobial/antiviral activities, immune modulatory activity, and antioxidant activity [7]. The major role of Lf in humans is the transportation of iron in blood plasma [8].Lactoferrin, in its natural form, is partially saturated with iron and hence can be fully saturated with iron from the external environment [9]. Ferritin is a highly symmetrical and stable iron-containing protein that was defined as the major iron storage protein since it possesses a large cavity that can accumulate great amounts of iron [10].

One of the ferritin major properties is its capacity to attract iron ions and to induce their mineralization by using its ferroxidase activity together with the chemical properties specific of the cavity environment. Ferritins carry a ferroxidase activity consuming the same reagents of the toxic Fenton reaction; thus, they have antioxidant functions and control the intracellular availability of ferrous iron [11].The purpose of this study is to determine the role of lactoferrin in premenopausal Iraqi women suffering from hair loss in order to explore the role of Lf in this disease.

Materials and Methods

Study Design and Population

This study was carried out at Dermatologists Consultant Dr. Wesam Alsaraf clinic under the supervision of Dr. Wesam Ahmed Jawad Al-Saraf during the period from September 2018 to June 2019. Individuals enrolled in the present study were divided into healthy and women with hair loss.

The study included 79 Iraqi women (18 to 45 years old), 26 of them were healthy women (control, G1 group) and 49 women with hair loss. Women with hair loss were divided into two subgroup, acute (G2) and chronic (G3) hair loss women. Full clinical investigation was carried out by consultant physicians in the hospitals. A questionnaire list was filled for each subject. The diagnosis of hair loss was done on the basis of the American College of Obstetricians and Gynecologists criteria [3].

Exclusion Criteria for Cases and Control

Some clinical conditions were excluded in our study, women who have menstrual cycle disturbance or taking any supplement, women suffering from thyroid disease.

Blood Samples Collection

Ten ml of blood was obtained by venipuncture from premenopausal women (aged range 18-45 years) after fasting at least 10-12 hours. The blood sample was divided into two portions; 2 and 8 ml. The first portion was dispensed in tube containing ethylene diamine tetra acetic acid (EDTA) to collect plasma which used for the estimation of Ferritin. While the second portion was dispensed in a gel tube and left to clot at room temperature. The gel tube was centrifuged at (3000 r.p.m) for 10 minutes to collect serum which is used for estimation of lactoferrin, ferritin, iron, T.I.B.C. The serum was divided into portions (300µl) in Eppendorff tubes, and stored at (-20ºC) until use.

Laboratory Tests

Lactoferrin measured by using enzyme linked immunosorbent assay (ELISA) using the commercially available ELISA kit (Mybiosource, U.S.A). All procedures were carried out according to the manufacturer's instructions. Ferritin (Bio system, India), iron (HUMAN, Germany), TIBC (HUMAN, Germany).

Statistical Analysis

Data was statistically analyzed by SPSS software version 22. The variables were reported as means ± standard deviation. The groups were compared by using one way ANOVA and post hoc Tukey test, with a *P* value of <0.05 indicating the statistically significant difference.

Results

In our study,79 premenopausal Iraqi women(aged range 18-45) were divided into three groups: control group(n=26,G1), acute telogen effluvium (ATE) group (n=27,G2), and chronic telogen effluvium (CTE) group (n=26,G3). The results of our study showed in Table (1) revealed that lactoferrin showed highly significant increase (p= 0.00) in the comparison of G3 group and G2 group (7.68±2.13 ng/ml vs. 4.65±2.18 ng/ml), Also between G3 and G1 (7.68±2.13 ng/ml vs. 5.21±1.03 ng/ml), while there is no significant difference between G2 and G1 (4.65±2.18 ng/ml vs. 5.21±1.03 ng/ml). Plasma ferritin level showed highly significant decrease (p=0.001) in comparison G1 group and G2 group (50.72±11.17 µg/dl vs.36.49 ±15.04 µg/dl) .The results also showed highly significant decrease (p=0.00) between G1 group and G3group (50.72±11.17 µg/dl vs.31.98± 15.98 µg/dl) but there is no significant difference (p=0.474) between G2 and G3 (36.49 ±15.04 µg/dl vs. 31.98± 15.98 µg/dl).

Table 1: The characteristics of participants of lactoferrin, ferritin, Hb, Fe, TIBC, and transferrin among different groups (n=79)

Variables	G1 (mean± SD) control N=26	G2 (mean± SD) Acute TE N=27	G3 (mean± SD) Chronic TE N=26	*P* -Value	
Lactoferrin (ng/ml)	5.21±1.03	4.65±2.18	7.68±2.13	G1*G2	0.519
				G1*G3	0.000**
				G2*G3	0.000**
Ferritin (µg/dl)	50.72±11.17	36.49 ±15.04	31.98±15.98	G1*G2	0.001**
				G1*G3	0.000**
				G2*G3	0.474
Hb (g/dl)	12.89±0.70	12.15±1.19	11.60±1.09	G1*G2	0.028**
				G1*G3	0.000**
				G2*G3	0.122
Fe (µg/dl)	84.28±52.79	53.11± 36.64	40.09±23.65	G1*G2	0.026**
				G1*G3	0.008**
				G2*G3	0.888
TIBC (µg/dl)	376.19±72.00	361.74±188.57	405.37±90.28	G1*G2	0.912
				G1*G3	0.693
				G2* G3	0.437
Transferrin (µg/dl)	263.33±50.40	253.21±132.00	283.75±63.19	G1*G2	0.912
				G1*G3	0.693
				G2*G3	0.437

G1:- Control group, G2:- Acute Telogen effluvium (ATE), G3:- Chronic Telogen effluvium (CTE).*P < 0.05; **P < 0.001; no significant: P > 0.05

Hemoglobin level showed highly significant decrease (p=0.028) in G2group (12.15±1.19 mg/dl) and G3 group (11.60±1.09 mg/dl) as compared with G1group (12.89±0.70 mg/dl), Also showed highly significant decrease (p=0.00) between (G1) group and (G3) (12.89±0.70 mg/dl vs. 11.60±1.09 mg/dl), but there is no significant difference(*P*=0.122) between (G2) and (G3) (12.15±1.19 mg/dl vs. 11.60±1.09 mg/dl). Serum iron (Fe) showed highly significant decrease (p=0.000) in chronic TE (G3) and acute TE (G2) group (40.10±23.65µg/dl vs.53.11± 36.65µg/dl) as compared with control group (G1) (84.29±52.79 µg/dl). Also Fe showed highly significant decrease (p=0.00) in chronic TE (G3) (40.10±23.65µg/dl) as compared to acute TE (G2)group (53.11±36.65 µg/dl), while TIBC levels showed no significant differences between groups (G1, G2, G3) (*p*=0.459), Also transferrin level showed no significant differences between groups(G1, G2, G3) (*p*=0.459). The correlations of serum lactoferrin with other biochemical parameters in acute (G2) and chronic telogen effluvium (G3) were summarized in Table (2).

The results showed that lactoferrin was correlated positively with ferritin in G2 group only (r=0.388, p=0.045), while there were no correlation relationship between lactoferrin and other parameters in G2 and G3 respectively.

Table 2: Pearson correlation of lactoferrin in acute telogen effluvium (G2) and in chronic telogen effluvium (G3)

Parameters	Lactoferrin (ng/ml)			
	G2		G3	
	r	p	r	p
Ferritin (µg/dl)	0.388	0.045*	0.061	0.765
Hb (g/dl)	0.123	0.540	-0.160	0.436
Fe (µg/dl)	-0.029	0.887	-0.178	0.383
TIBC (µg/dl)	-0.225	0.258	0.293	0.146
Transferrin (µg/dl)	-0.225	0.258	0.293	0.146

R, Pearson coefficient
*Statistically significant at $p \leqslant 0.05$

Discussion

Telogen Effluvium (TE) is the most common form of hair loss encountered in clinical practice [12]. It is characterized by loss of hair in its telogen phase. Chronic Telogen Effluvium(CTE) is a diffuse, generalized form of hair loss of unknown cause that is common in middle aged women [13].Lactoferrin (Lf) was found in most exocrine secretions and in the secondary granules of neutrophils. Antimicrobial and anti-inflammatory activity reports on lactoferrin identified its significance in host defense against infection and extreme inflammation [6].

Lactoferrin (Lf) and transferrin (Tf) ensure that the proper 10^{-18} M free iron concentration in human fluids is maintained, thus avoiding iron precipitation, ROS induction and microbial colonization[6]. To the best of our knowledge, the current study is the first to investigate increase in circulating lactoferrin (Lf) associated with hair loss.

The results obtained from our study showed compensatory twofold significant increase of Lf levels in the G3(CTE) group as compared to G2(ATE) and their G1(control)group. We did not find any literature survey on the relationship of Lf with hair loss in women, but there has been many researches of Lf with other diseases, especially inflammatory diseases such as inflammatory bowel diseases [14], type 2 diabetic mellitus [15] and other diseases. Therefore, more studies on Lf and its role in hair loss should be done with large group numbers of women suffering from hair loss with different age. In a previous study, researchers found that bovine Lf (bLf) promoted cell proliferation in dermal papilla cells and hair growth in mice, so they believed that Lf exerts an effect on cell growth and differentiation [16].

In other study researchers suggest that bLf can act as a novel treatment agent for alopecia. Oral administration of Lf also increases the number of red blood cells and serum ferritin concentrations also decreased IL-6 concentration [17]. Our results revealed that iron, ferritin and hemoglobin have an association with hair loss in women as they gave effective indicators depend on their low levels in women who suffered from acute or chronic telogen effluvium. Serum iron is a measure of circulating iron that is bound to transferrin, which is a major serum iron transport protein.

In the present study, there was statistically highly significant decrease in serum iron levels in TE women compared with control women. This finding was in agreement with Hamad et al [18].But in contrast to Moeinvaziri et al [19]. Further analysis of serum iron was performed in our study, according to the clinical type of TE, and we found a statistically significant decrease in serum iron levels in ATE (53.11± 36.64 µg/dl) and CTE (40.09±23.65 µg/dl) patients in comparison with the control group (84.28±52.79 µg/dl).Several studies have evaluated the relationship between iron deficiency and hair loss.

Almost all of these studies have focused exclusively on women, and some suggest that iron deficiency even in the absence of iron deficiency anemia may cause hair loss [20]. Nevertheless, it is common practice for dermatologists to prescribe iron supplementation in women under the assumption that low iron stores may cause hair loss. The deleterious effects of iron deficiency are partly due to impaired oxygen delivery to the rapidly proliferating hair follicle matrix cells.

Another mechanism for the possible effect of iron on hair growth stem cells is its requirement as a cofactor for ribonucleotide reductase enzyme, which is involved in DNA synthesis. Iron depletion could prevent proper function of this enzyme, resulting in inhibition of proliferation [19]. Ferritin is a protein that plays a key role in iron metabolism and allows storage of iron. Its serum level is used to evaluate iron reserves, and thus help in early detection of iron deficiency. In the present study, we found statistically significant difference in serum ferritin levels between TE and healthy women. As serum ferritin levels accurately reflect body iron stores, our study clearly demonstrated an association between low iron stores and telogen hair loss. The results of our study were in agreement with Moeinvaziri et al [19].

Elethawi and Jabbar [21], Rasheed et al [20].And Fatani et al [12].Where they showed that serum ferritin levels in TE patients were significantly lower compared with the control group, and concluded that there was a significant association between low serum ferritin and TE. In contrast to our results, Chisti et al [22].And Ibrahem et al [23].Concluded that there was no closely linked relationship between iron metabolism and TE. Further analysis was carried out on serum ferritin and we found no significant differences between ATE and CTE groups (P >0.05).

Women with ATE (37.1%) and CTE (53.9%) had serum ferritin levels below 30 ng/ml. accordingly; we could suggest that decreased serum ferritin levels might be considered as a potential risk factor for developing CTE. Our results were in agreement with Moeinvaziri et al [19].Who used serum ferritin levels of 30 ng/ml as the cut-off point, and Sarkar et al [24].Who used serum ferritin levels of 20 ng/ml as the cut-off point. Both of them concluded that women with lower serum ferritin levels than the proposed cut-off point had a significant risk of developing telogen hair loss. In addition, Fatani et al [12]. Found that serum ferritin levels in 61.25% of TE patients were less than 30 ng/ml, which proved to be statistically significant. Furthermore, Chisti et al [22]. Found a statistically significant number of CTE patients with serum ferritin levels less than 20 ng/ml when compared with those of controls. Total iron-binding capacity is the sum of plasma iron and unsaturated iron-binding capacity, which is calculated from the level of transferrin in blood. In this study, serum TIBC and transferrin showed no statistically significant difference between TE patients (neither ATE nor CTE) and healthy controls.

This finding was in contrast to Moeinvaziri et al [19].Who found that serum TIBC was statistically significantly higher in TE patients than in controls. It is important to note that Hb may be within normal limits in women with iron deficiency and hair loss. This is because decreased iron stores in body will lead to hair shedding before the development of microcytic anemia [25]. In this study, further analysis was performed using Hb concentrations of 12 g/dl as the cut-off point, and we found a statistically significant number of TE patients with Hb concentrations less than 12 g/dl, with the lowest Hb levels seen in CTE women (11.60 g/dl),than in ATE(12.15 g/d). These results are in agreement with Moeinvaziri et al [19].

Who stated that patients with iron-deficiency anemia had telogen hair loss, while Deloche C. [26].Stated that women with ATE seem to have lower hemoglobin value compared to women with CTE. However, these differences were not statistically significant. Hard et al, showed that iron supplementation irrespective of anemia improved hair loss supports the role of anemia as a causative factor [22].

Conclusion

Lactoferrin levels were significantly elevated in women with chronic telogen effluvium in comparison with acute telogen effluvium and healthy subjects, so it can be used as a useful diagnostic tool for diagnosis of chronic telogen effluvium. Lactoferrin had positive correlation with ferritin in acute telogen effluvium; we could suggest that serum ferritin levels might be considered as a potential risk factor for developing CTE.

References

1. Ozlu, E, AS Karadag (2017) Telogen effluvium. Hair and Scalp Disorders. InTech, 125-39.
2. Grover C, A Khurana (2013) Telogen effluvium. Indian Journal of Dermatology, Venereology, and Leprology, 79(5): 591.

3. Shrivastava SB (2009) Diffuse hair loss in an adult female: approach to diagnosis and management. Indian Journal of Dermatology, Venereology, and Leprology, 75(1): 20.
4. Trüeb RM (2010) Systematic approach to hair loss in women. JDDG: Journal der Deutschen Dermatologischen Gesellschaft, 8(4): 284-297.
5. França K, et al (2013) Comprehensive overview and treatment update on hair loss. Journal of Cosmetics, Dermatological Sciences and Applications, 3(3): 720-726.
6. Kanwar JR, et al (2015) Multifunctional iron bound lactoferrin and nanomedicinal approaches to enhance its bioactive functions. Molecules, 20(6): 9703-9731.
7. Burrow H, R K Kanwar, JR Kanwar (2011) Antioxidant enzyme activities of iron-saturated bovine lactoferrin (Fe-bLf) in human gut epithelial cells under oxidative stress. Medicinal Chemistry, 7(3): 224-230.
8. Iafisco M, et al (2011) Adsorption and spectroscopic characterization of lactoferrin on hydroxyapatite nanocrystals. Dalton Transactions, 40(4): 820-827.
9. Kanwar JR, et al (2008) 'Iron-saturated'lactoferrin is a potent natural adjuvant for augmenting cancer chemotherapy. Immunology and cell biology, 86(3): 277-288.
10. Arosio P, L Elia, M Poli (2017) Ferritin, cellular iron storage and regulation. IUBMB life, 69(6): 414-422.
11. Putburee R, et al (2018) Mitochondrial ferritin expression in erythroid cells from patients with alpha-thalassaemia. Hematology, 23(10): 844-848.
12. Fatani M, et al (2015) Prevalence and factors associated with telogen effluvium in adult females at Makkah region, Saudi Arabia: a retrospective study. Journal of Dermatology & Dermatologic Surgery, 19(1): 27-30.
13. Malkud S (2015) A hospital-based study to determine causes of diffuse hair loss in women. Journal of clinical and diagnostic research: JCDR, 9(8): WC01.
14. Mosli MH, et al (2015) C-reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. The American journal of gastroenterology, 110(6): 802.
15. Mohamed WA, MF Schaalan (2018) Antidiabetic efficacy of lactoferrin in type 2 diabetic pediatrics; controlling impact on PPAR-γ, SIRT-1, and TLR4 downstream signaling pathway. Diabetology & metabolic syndrome, 10(1): 89.
16. Naot D, et al (2005) Lactoferrin-a novel bone growth factor. Clinical Medicine & Research, 3(2): 93-101.
17. Huang H-C, H Lin, M-C Huang (2019) Lactoferrin promotes hair growth in mice and increases dermal papilla cell proliferation through Erk/Akt and Wnt signaling pathways. Archives of dermatological research, 1-10.
18. Hamad WA, AF Said, A Hamid (2010) Role of some trace elements in the pathogenesis of telogen effluvium in Egyptian females. J. Egypt Women Dermatol Soc., 7(1): 44-8.
19. Moeinvaziri M, et al (2009) Iron status in diffuse telogen hair loss among women. Acta Dermatovenerologica Croatica, 17(4): 0-0.
20. Rasheed H, et al (2013) Serum ferritin and vitamin d in female hair loss: do they play a role? Skin pharmacology and physiology, 26(2): 101-107.
21. Elethawi AMD (2012) Comparison Between Hemoglobin Serum Level and Serum Ferritin level in Detecting Low Iron Store in Adult Menstruating Females with Chronic Telogen Effluvium. Iraqi Academic Scientific Journal, 11(1): 87-91.
22. Chisti MA, et al (2017) Serum ferritin levels in non-scarring alopecia of women: a case-control study. Journal of Pakistan Association of Dermatology, 22(1): 4-11.
23. Ibrahem M, et al (2012) Estimation of serum ferritin level in female patients with telogen effluvium. Hair Ther. Transplant, 2(104): 2167-0951.1000104.
24. Sarkar P, et al (2013) A case control study to determine the correlation between serum ferritin levels and chronic telogen effluvium in a Tertiary Hospital, Mandya.
25. Al-asady HMK, NH Al-dulaimy (2019) Assessment of Some Hematological Parameter in People with Hair Loss. Indian Journal of Public Health Research & Development, 10(8): 2411-2414.
26. Karadağ AS, et al (2011) The role of anemia and vitamin D levels in acute and chronic telogen effluvium. Turkish Journal of Medical Sciences, 41(5): 827-833.

