INTRODUCTION

Hair is essentially formed by a protein called “keratin.” There is more cysteine amino acid in keratin compared to other proteins. There are many peptide chains, disulfide ligaments (-S-S), H ligaments, and salt bridges among fibrils in keratin. Since disulfide ligaments (-S-S) among all mentioned are strong covalent ligaments, keratin has a stable structure. It is insoluble in water and durable against proteolysis, and this provides physical resistance.

Hair has a dynamic structure and it has a cycle consisting of three stages as follows: anagen, catagen, and telogen. Telogen effluvium (TE) which was first stated by Kligman is defined as extreme hair loss due to pathological hair cycle of telogen hair. Real incidence is not known. Hair loss that takes less than 6 months is called as acute TE, whereas those that take more than 6 months are assessed/identified as chronic TE. While the exact reasons are unknown, endocrinopathies, systemic diseases, drugs/chemicals, fever, emotional stress, weight loss, malnutrition, malabsorption, iron and Vitamin D deficiency, inflammatory scalp diseases, pregnancy, and labor lead to TE. In 33% of cases, no reasons have been found.

Reactive oxygen types (ROTs) are neutralized by antioxidant system in the organism and this keeps a balance. Oxidative stress (OS) has been shown to play an important role in the etiopathogenesis of so many diseases, the effects of OS on several skin diseases are researched and analyzed. Thiols are antioxidant components that include sulfur group, and the balance of thiol-disulfide has an important role in the formation and prevention of OS. This balance is destroyed in many diseases and its effect on TE is not clearly understood yet.

Objectives: In this study, we aimed to search the thiol–disulfide balance that could reveal OS in patients with TE.

Materials and Methods: Fifty-two patients with TE and control group of 46 persons were included in the study. Native thiol, disulfide, and total thiol levels were evaluated by a new, automatic spectrophotometric method. Disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol rates were calculated.

Results: There was no statistical difference between TE patients and control group in terms of native thiol, disulfide, and total thiol levels. Thiol–disulfide balance was fixed and not affected in TE patients.

Key words: Oxidative stress, telogen effluvium, thiol–disulfide balance
stress (OS) originates from the imbalance between oxidant system and antioxidant system, which was revealed as a result of the above-mentioned imbalance damage lipids, proteins, and DNA units in the organism and cause cell necrosis, cell death, and tissue damage. Especially, proteins are sensitive against oxidation. Among those proteins, thiols including sulfhydryl group are the most and quickest affected ones. Plasma thiols are strong and resistant antioxidants that remove free radicals physiologically. Serum levels of protein thiols are among the indicators of antioxidant status in the body.

It was asserted that ROT could have an important role in pathogenesis of several skin diseases and several information were reported about these assertions. However, there are articles published mostly about alopecia areata and hair whitening among hair diseases. OS mechanism in TE could not be revealed clearly and completely. There are several studies about TE etiopathogenesis in the literature, but those are mostly oriented for provocative factors. As far as we know, there is no publishing about TE and OS. Furthermore, thiol–disulfide homeostasis which is one of OS indicators has not been researched yet.

MATERIALS AND METHODS

This concentric prospective case study was done between January and August 2015. We included female patients over 18 years of age, who applied to our outpatient clinic with the hair loss complaint and who have no thinning on central scalp area and no scar or atrophic findings and who were diagnosed with TE by means of history, physical examination, and hair pulling test.

Age of patients, systemic diseases, nutritional status, medicines that they used in the last 1 year, birth history, menstrual irregularity, stress history, diet, operation history, cosmetic applications of hair, and their family history were all questioned. Those patients, who had any systemic or neoplastic diseases and who used medicines for any reasons and who smoke, were not included in this study.

Fifty-two women patients who were diagnosed with TE clinically by means of hair pulling test and 46 healthy women were all included in this study. Control groups were chosen among persons who did not smoke, did not have any other skin diseases, and who were healthy and came to our outpatient clinic for general control. Before the study, approval was taken from the ethical committee of our hospital. The study was realized in accordance with good clinical practice and the Declaration of Helsinki.

Consent was obtained from the patient and control group. Complete blood count, full biochemistry tests, iron, ferritin levels, Vitamin B12 levels, and thyroid functions tests were assessed as laboratory tests.

Blood samples, which were kept in deep freezer at −80°C, were centrifuged at 1500 speed for 10 min, and thiol/disulfide homeostasis tests were measured using the automatic spectrophotometric method.

Sodium borohydride and disulfide ligaments were regressed, and free functional thiol groups were revealed.

Unused reduced sodium borohydride was reacted with formaldehyde to prevent reduction of 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB). Total thiol groups that consist of reduced and native thiol groups were identified after reaction with DTNB. The quantity of dynamic disulfide was found by dividing the difference between total thiol and native thiol into two. Disulfide/native thiol (index 1), disulfide/total thiol (index 2), and native thiol/total thiol (index 3) rates were calculated.

Total protein and albumin were measured using colorimetric methods as g/dl in Roche-Hitachi Cobas c501 automatic analyzer.

The compliance of continuous variables in the study such as age, total protein, total thiol ferritin, and Vitamin B12 to normal distribution was analyzed with Shapiro–Wilk test. T-test or Mann–Whitney U-test was applied in independent groups depending on whether the distribution of variables and groups was in balance or not. Thiol values were examined; Pearson r coefficient and rho coefficient were calculated based on the distribution of variables. Statistical significance value was accepted as P < 0.05.

IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. IBM Corp. Armonk, NY, USA) program was used for statistical analyses.

RESULTS

It was determined that the ages of 52 TE patients and 46 healthy control groups included in this study vary between 18 and 68 and 18 and 66, respectively. Median age value was calculated as 28.0 (interquartile range [IQR] = 17.0) and 32.0 (IQR = 22.0) and it was determined that the two groups are of similar age (Z = 1.443 and P = 0.149).

Laboratory values of patients are shown in Table 1. It was determined that the total protein values of patients and...
control group are similar \((t = -0.786\) and \(P = 0.434\)). There are no significant differences between patients and control group statistically in terms of index 1, index 2, albumin, native thiol, total thiol, and disulfide \((P > 0.05)\). Index 3 median was calculated as 0.93 (IQR = 0.04) for patients and as 0.91 (IQR = 0.04) for control group which indicated that index 3 values of patients are higher than the control group \((Z = 2.120\) and \(P = 0.034)\). Scatter tables of native thiol, disulfide, and total thiol values are given in Table 2.

It was understood that there is a negative direction moderate relation between age and native thiol and total thiol and positive direction weak relation between age, index 1, and index 2, and there is no significant relation between age and disulfide statistically [Table 3]. Positive direction weak relation was observed between total protein, native thiol, and total thiol. It was determined that there is positive direction moderate relation between albumin, native thiol, and total thiol. It was determined that there is no significant relation thyroid-stimulating hormone (TSH), thyroglobulin antibody, and thyroid peroxidase antibody (AntiTPO), and thiol values statistically \((P > 0.05)\).

**DISCUSSION**

Thiol and disulfides are in balance normally and they have a role in cellular redox homeostasis. As a result of OS, structures and functions of enzymes that are bound to thiols are damaged and this causes a change in the thiol/sulfide ratio in the cell media. This ratio shows that a decrease in plasma thiol concentration shall cause an increase in free radical production.\(^{[22,23]}\)

There are few many studies about dynamic thiol/disulfide homeostasis in skin diseases OS researches.\(^{[24-27]}\) In some of them, it is shown that thiol/disulfide balance is sliding to oxidative side. However, this balance is not affected in others.

In this study, we try to determine the existence of OS in cases with TE by researching thiol/disulfide balance. While comparing native thiol, total thiol and disulfide values and disulfide/native thiol, disulfide/total thiol and native thiol/total thiol rates of patients with the control group, we determined that native thiol, total thiol, and disulfide values do not change in TE. There is a balance between SH and S-S ligaments in the structure of hair proteins. If the disulfide ligaments are damaged, hair shall be thinned, but it shall not splinter as long as there are salt bridges. The contrary is also true. If salt bridges are damaged, as long as disulfide ligaments are there, hair shall not be splintered. However, at pH 12, disulfide ligaments shall be broken and hair shall be dissolved.\(^{[1]}\)

In our study, thiol/disulfide balance was not damaged in patients. However, although there is no sufficient difference between patient and control group, native thiol has not been changed but disulfide decreased due to keratin loss. Since disulfide decreased, total thiol decreased and the ratio of native thiol/total thiol also decreased. It was determined that index 3 value of patients \((nt/ht)\) is higher than control group. This shows the relation of keratin (S-S-disulfide) and OS.

Recently, several hypotheses were asserted for TE etiopathogenesis. Segregation of hair gradually increases due to exogenic and endogenic reasons such as smoking, irritation, ultraviolet rays, microbial inflammation, contamination, and psychoemotional reasons.\(^{[27-29]}\) It

### Table 1: Some clinical features and laboratory values of patients

| Clinical Features | Patient, n (%) | Laboratory values | Median (IQR) |
|-------------------|----------------|------------------|--------------|
| **Period of disease** | | | |
| Acute | 28 (53.8) | Ferritin (ng/ml) | 2.0 (1.77) |
| Chronic | 24 (46.2) | Vitamin B12 (pg/ml) | 37.0 (19.8) |
| **Localization** | | | |
| Frontal | 6 (11.5) | FT3 (pg/ml) | 2.9 (0.58) |
| Occipital | 2 (3.9) | Minimum-maximum | 0.82 (37.2) |
| Parietal | 4 (7.7) | FT4 (ng/dl) | 1.26 (0.20) |
| Temporal | 2 (3.9) | Minimum-maximum | 0.44 (1.78) |
| Vertex | 6 (11.5) | TSH (µmol/L) | 1.78 (1.79) |
| Diffuse | 32 (61.5) | Minimum-maximum | 0.35 (49.2) |
| Stress | | Anti-TPO (IU/ml) | 16 (56.5) |
| Yes | 50 (96.2) | Minimum-maximum | 8.0 (7.36) |
| No | 2 (3.8) | Anti-TG (IU/ml) | 10.77 (9.36) |
| | | Minimum-maximum | 5.00 (203.40) |

IQR – Interquartile range; AntiTPO – Thyroid peroxidase antibody

### Table 2: Distribution of laboratory findings of patient and control group

| Patient group | Control group | Test statistics | \(P\) |
|---------------|---------------|----------------|------|
| Total protein (g/dl) | 7.34±0.33 | 7.43±0.41 | \(t = -1.185\) | 0.239 |
| Albumin (g/dl) | 4.72 (0.31) | 4.69 (0.48) | \(Z = 0.555\) | 0.579 |
| Native thiol (µmol/L) | 455.29±53.39 | 454.53±53.39 | \(t = 0.071\) | 0.943 |
| Disulfide (µmol/L) | 17.82±6.75 | 20.16±7.83 | \(t = -1.591\) | 0.115 |
| Total thiol (µmol/L) | 495.40±85.78 | 501.15±70.25 | \(Z = 0.634\) | 0.526 |
| Index 1 | 0.04 (0.02) | 0.05 (0.02) | \(Z = 1.935\) | 0.053 |
| Index 2 | 0.04 (0.02) | 0.04 (0.02) | \(Z = 2.120\) | 0.034 |
| Index 3 | 0.93 (0.04) | 0.91 (0.04) | \(Z = 2.120\) | 0.034 |

Disulfide/native thiol (index 1), disulfide/total thiol (index 2) and native thiol/total thiol (index 3).

SD – Standard deviation; IQR – İnterquartile range
is recorded that ultraviolet rays affect hair growth and pigmentation by Trueb.[27] Cysteic acid residues increase in gray hair and cysteine decreases then fiber activity increases to decrease the oxidation, and thus, follicular stem cells are affected and cause acute TE. Arck et al. showed in their experimental study on stem cells that OS, which occurred due to endogenous reasons, affects the antioxidant activity in hair follicles and decreases the number of melanocytes and causes permanent damage by affecting those cells to become apoptosis.[17] Again it is shown that as a result of OS occurred in hair follicles during hair transplantation, hair is lost.[30] Chemotherapeutic medicines cause increase in OS in hair follicles.[31] Furthermore, in humans, scalp pigmented hair, melanin for selective binding of heavy metals from the body, chemicals, and toxins can be a rapid excretion.[32] The pigment depletion potential of each individual hair follicle genetically regulated. Pigment reduction results from a decrease in tyrosinase activity in hair bulbar melanocytes. The damage to the nuclear and mitochondrial DNA by reactive oxygen species causes mutation in bulbar melanocytes.[33]

Thiol/disulfide balance is not affected in our study, and the reason may be that our patients do not have a history of smoking, irritation, ultraviolet radiation, microbial inflammation, and contamination.

Food, hormones, and enzymes affect hair growth. In case of protein deficiency, hair shall be affected, becomes dull colored, thinner, and loses its elasticity without decreasing blood albumin, and could cause hair whitening. As a result of studies, it is shown that especially, insufficient protein taking causes hair loss.[34] Keratin that is included in the hair structure has a high percentage of sulfur. This is caused by sulfur cysteine and it is shown that cysteine is also effective in hair growth.[34,33]

Vitamin B complex has no well-known role in hair growth. Vitamin B6 shall ease the entrance to hair follicles by cysteine, and cysteine contributes the hair growth by means of detoxification of cysteine with glutathione.[34] In literature, there are also reports stated that amino acid which includes sulfur such as l-cysteine and keratin. B-complex vitamin and medical yeast complex shall be given, and tensile strength of hair strand shall increase and shall cause less hair loss.[34,35] In case of congenital and acquired biotin deficiency, hair fall could be observed.[36]

Iron is a key factor in antioxidant enzyme function. It is a strong catalyst which forms ROT in the organism. Free forms of iron could have a toxic effect on living cells. Ferritin binds iron and tries to remove that negative effect.[2,34] Decrease in ferritin could also show an antioxidant effect.[37] Iron effects nuclear proteins by means of lipid peroxidase and DNA damage. Thus, in iron deficiency, matrix cell proliferation shall decrease and causes TE.[2,3,34] It is determined that even standardization is made in ferritin levels for ideal hair growth, when patient’s serum ferritin levels shall be lower than 40 ng/ml, then telogen phase occurs. Without anemia, iron deficiency could also cause hair loss.[2,34,38] In so many previous studies, the lowest margin for ferritin is determined as 40–70 ng/dl.[39,40] The blood ferritin levels were varying between 2 and 174 ng/dl (mean 25) in our patients. Although our patients have no anemia, the blood ferritin levels were determined below 40 ng/dL in 65% of them. In this case, we can say that the lack of ferritin has very little effect on OS.

OS which occurs with the effect of thyroid hormones could also cause change in hair. It is a matter of decrease in anagen/telogen ratio in hypothyroidism of which relation with hair loss was proven. In hypothyroidism, there is inhibition of epidermis and cutaneous adnexal and this causes TE.[2] Replacement therapy could turn the case back. In this study,
the relation between thyroid tests and thioldisulfide values was also assessed in patients with TE. Blood serum free triiodothyronine and free thyroxine values of our cases are statistically significant as per control group, but there is no significant difference found between TSH and AntiTPO and antithyroglobulin values. With those results, it could be said that TE might have a correlation with thyroid disease. There are several studies that show the existence of OS in thyroid patients. In patients with TE who have also thyroid disease, OS could increase. Since iron deficiency commonly accompanied with thyroid disease due to negative effects of thyroid hormones on erythropoiesis, this could trigger TE.

In extreme psychological stress, it is shown that corticotropin-releasing hormone inhibits hair follicles with the effect of neurohormones such as substance P and nerve growth factor and this causes TE. Psychological stress could also play a primary role or could aggravate provocative diseases. Another result obtained in our study is that in patient group with TE, psychological stress history was 96.2% (n = 50); moreover, emotional stress history could trigger TR and also causes an increase in OS due to the person getting stressed.

Another finding determined in our study is that native and total thiols decrease in TE with age. This supports the relation between aging and OS. At the same time, hair loss and hair whitening are also related to OS in aging.

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CONCLUSION

TE is a multifactorial disease of which reason is not known completely. In this study which we made about OS oriented for etiopathogenesis, in accordance with data obtained, we could say that thioldisulfide homeostasis is effected in TE, but the balance is not damaged. Advanced studies must be done in this issue to support these findings. We think that new and comprehensive studies could enlighten pathogenesis of TE.

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Conflicts of interest

There are no conflicts of interest.

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