ABSTRACT

**Background:** Thiols are antioxidant, organic compounds containing sulfhydryl group on their active sites. They have important roles on preventing oxidative stress. The thiol disulfide homeostasis has a vital importance in organism. Thiol disulfide imbalance is an early indicator of oxidative stress. Alopecia areata is an autoimmune skin disorder characterized with scar-free hair loss. Its pathogenesis is still unclear. Thiol disulfide homeostasis in these patients has not been studied at all.

**Objective:** To evaluate the thiol disulfide homeostasis in alopecia areata patients and healthy controls.

**Methods:** Fortysix alopecia areata patients and 41 healthy controls were included in the study. Native thiol, disulfide, total thiol levels were performed with a new and automatic spectrophotometric method. Rations of disulfide/total thiol, disulfide/native thiol and native thiol/total thiol were calculated as percentages.
Results: No statistically significance were detected between the values of native thiol, disulfide and total thiol in both two groups (p>0.05).

Conclusion: The dynamic thiol/ disulfide homeostasis is balance in patients with alopecia areata according to our findings. That is, the patients aren’t affected by oxidative stress. Furthermore we are suggesting that more studies with wider series should be performed.

Keywords: Alopecia areata; oxidative stress; thiol disulfide homeostasis.

1. INTRODUCTION

Alopecia areata; is an autoimmune skin disorder characterized with scar-free hair loss [1]. Its pathogenesis is still controversial [2-4]. Most considered factors are; genetic tendency, autoimmune and environmental factors [1,2]. The association with Human leukocyte antigen (HLA) DR and HLA DQ alleles supports the autoimmunity and act of T cells [5]. Recently, publications indicating the role of oxidative stress (OS) in pathogenesis are available [3,4,6-12].

Reactive oxygen species which occur during normal cellular metabolism, are critically harmful for the organism and have beneficial effects in low amounts and production of reactive nitrogen species are controlled by antioxidant defense mechanisms, thus redox homeostasis is being maintained. Over production of these or deficiencies in defense mechanism cause oxidative stress [13].

Dynamic thiol- disulfide homeostasis has also important roles on redox homeostasis. Thiols are antioxidant, organic compounds containing sulfhydryl group on their active sites. They have important roles on preventing OS [14-16]. Most of the plasma thiols are composed of its albumin and protein thiols, and much less part are composed of thiols with low molecular weight (such as cysteine, glutathione) [14,17]. Total thiols are composed of intracellular and extracellular thiols. Intracellular thiols such as cysteine, glutathione and thioredoxin contribute to the maintenance of high reduction potential of the cell [16,18]. Extracellular thiols are the disulfide proteins binded to a protein [18].

Plasma thiols are powerful antioxidants eliminating the free radicals physiologically. They stimulate the redox condition with glutathione mediated antioxidant enzymes [19]. However, it has been reported that elements of Thiol redox buffer systems are originated from cysteine/cystine (Cys/CysS), glutathione/glutathione disulfide (GSH/GSSH) redox pairs and thiol disulfide oxireductases (thioredoxin, glutaredoxin, peroxiredoxin), protein thiols are presented in much more ratios and they have important roles on intracellular redox homeostasis [16,20].

Cysteine residues of thiols are oxidized in presence of oxidants and disulfide bonds are established between protein thiols and protein thiols or thiols with low molecular weight. Such reaction is reversible, disulfide bonds may degrade to thiols groups again, if needed. This situation provides the maintenance of dynamic thiol disulfide homeostasis. The dysfunctions in this homeostasis may be caused from various diseases [14].

As far as we know, there is no study available which indicates the thiol- disulfide homeostasis in patients with alopecia areata. In present study, both directions of the balance will be measured in patients with alopecia areata with a new and easy method developed by Erel and Neselioglu and we aimed to evaluate the thiol/ disulfide homeostasis which has a vital importance.

2. MATERIALS AND METHODS

Fortysix patients who were diagnosed with alopecia areata, non-smoker, did not use any drugs, has no additional diseases and applied to our polyclinic between the dates of January-September 2015; and 41 healthy, non smoker volunteer group were included in this present prospective case control study.

Study was performed in accordance with Good Clinical Practices and Helsinki declaration. Permission was granted from local ethics committee and written informed consent was collected from both of the patient and control groups.

Age, gender of the patients, onset duration of the disease, number and location of the lesions, recurrence and familial history of the patients were recorded. Severity of the disease was classified according to the rating system of Kavak et al. [2]:
1. **Mild**: 3 or less patches or eyebrow and eyelash involvement with a diameter of 3 cm or less

2. **Moderate**: >3 of alopecic patches or presence of patch with a diameter of wider than 3 cm.

3. **Severe**: alopecia totalis or universalis.

Venous blood samples which were collected from patients on an empty stomach, were stored in -80 Celsius in deep freezer until the analysis, and centrifuged in 1500 rpm for 10 minutes. Thiol/disulfide homeostasis assays were performed with a new and automatic spectrophotometric method described by Erel and Neselioglu [14]. Firstly, the free functional thiol groups were extracted by reduction of the disulfide bonds with sodium borohydride. Unused reduced sodium was eliminated to prevent the reduction of borohydride 5,5'-dithiobis-(2 nitrobenzoic) (DTNB). Total thiol groups containing reduced and native thiol groups were determined after reaction with DNTB. Dynamic disulfide amount was obtained by dividing the difference between total and native thiols by two. Ratios of disulfide/total thiol, disulfide / native thiol and native thiol/ total thiol were calculated as percentages.

Total protein and albumin was measured in automatic Roche- Hitachi Cobas c501 analyzer with a calorimetric method as g/dl.

The conformity of continuous variables used in this study into normal distribution was examined with Shapiro Wilk test. Variables conformed to normal distribution were presented as mean± standard deviation(mean±sd), variables which are not conformed with normal distribution were represented as median (IQW: Interquartile Width), and categorical variables are represented as number (%).

Depending on the distribution of variables in the comparison of values with continuous variables and whether the groups are balanced, t test and Mann Whitney U test were employed in distinct groups. The evaluation in accordance with gender distribution of patient and control groups, were performed with Yates Chi Square test. Test statistics and p value were provided in results of the analysis. Pearson R coefficient was calculated for the relationships between thiol and disulfide values with variables conformed to normal distribution, Spearman rho coefficient was calculated for the contrary case. The relations between consecutive variables with thiol and disulfide values were evaluated with Poliserial rho coefficient. Statistical significance level was accepted as p<0.05.

IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) software and “polycor” library of R package were used for the statistical analyses and calculations.

### 3. RESULTS

The mean age of 46 patients with alopecia areata and 41 healthy volunteers were calculated as 33.54±9.56 years and 34.88±10.49 years respectively and no statistically significant difference was detected between the groups by means of age (t=-0.621, p=0.536, Table 1). 65.2% (n=30) of the patient group and 48.8% (n=20) of control group were male individuals. It was determined that the gender distribution between patient and control groups were similar ($\chi^2$=1.771, p=0.183).

Median onset duration of disease was 2.00 months (IQW=4.00) in patient group (Table 2). Only 2 patients (4.3%) were in severe stage of the disease, and 3 patients (6.5%) also had familial history of alopecia.

No statistically significance were detected between the values of native thiol, disulfide and total thiol in both two groups (p>0.05) (Table 3).

Native thiol median of thirty male patient and 16 female patient was obtained as 488.40 (69.50) and 448.95 (91.60) respectively. Native thiol values of female individuals were significantly less than the men (Z=1.960, p=0.050).

When the thiol disulfide values of recurrent 13 patients were compared to 33 patients with no recurrence, two patient groups were similar (p>0.05). According to the lesion number, no statistically significance were determined by means of thiol disulfide values between patients with single or multi lesions and patients which have all three types of disease severity (mild, moderate and severe) (p>0.05).

There were negative significant relations between age with native and total thiol (Spearman rho=-0.475, rho=-0.449, p<0.001 respectively).
Table 1. The demographic characteristics of the patients

|                          | Patients (n=46) | Controls (n=41) | Test statistics | p   |
|--------------------------|----------------|----------------|----------------|-----|
| Age [mean±Sd]            | 33.54±9.56     | 34.88±10.49    | t=-0.621       | 0.536|
| Gender [n (%)]           |                |                |                |     |
| Male                     | 30 (65.2)      | 20 (48.8)      | χ²=1.771       | 0.183|
| Female                   | 16 (34.8)      | 21 (51.2)      |                |     |

Yates Correction, Sd: standard deviation

Table 2. Disease duration, severity, number of lesion, recurrence and family history in alopecia areata patients

| Statistics | Disease duration [month] | Min – Max | Lesion count [n (%)] | Statistics |
|------------|--------------------------|-----------|----------------------|------------|
|            | 2.00 (4.00)              | 1.00 – 84.00 | Single | 22 (47.8) |
| Disease severity [n (%)] | mild | 31 (67.4) | Multiple | 24 (52.2) |
|                     | moderate | 13 (28.3) | Recurrence [n (%)] | 13 (28.3) |
|                     | severe | 2 (4.3) | Family history [n (%)] | 3 (6.5) |

Table 3. Thiol and disulfide levels in patients and controls

| Statistics | Patients [mean±Sd] median (IQW) | Controls [mean±Sd] median (IQW) | Test statistics | p |
|------------|---------------------------------|---------------------------------|----------------|---|
| Total Protein (g/dl) | 7.39±0.36 | 7.54±0.42 | t=-1.796 | 0.076 |
| Albumin (g/dl) | 4.85±0.27 | 4.80±0.27 | t=0.946 | 0.347 |
| Native Thiol (mmol/L) | 477.61±56.62 | 467.27±50.91 | t=0.892 | 0.375 |
| Disulfide (mmol/L) | 21.73±6.38 | 20.93±8.40 | t=0.501 | 0.617 |
| Total Thiol (mmol/L) | 521.07±56.78 | 509.31±55.19 | t=0.976 | 0.332 |
| %Disulfide/total thiol | 0.04 (0.02) | 0.04 (0.03) | Z=0.387 | 0.698 |
| %disulfide / native thiol | 0.04 (0.02) | 0.04 (0.02) | Z=0.924 | 0.356 |
| %native thiol/ total thiol | 0.92±0.02 | 0.92±0.03 | t=0.387 | 0.700 |

Sd: standard deviation, IQW: Interquartile Width

4. DISCUSSION

There are two theories available related with OS pathway in alopecia areata pathogenesis: OS causes lipid peroxidation and malondialdehyde (MDA) formation as a final product. MDA establishes covalent bonds with proteins, causes new antigenic formations and contributes to the initiation of autoimmune mechanisms. OS also induces the apoptosis and may act in the formation of alopecia areata. It is still controversial whether the OS induces the disease or forms the inflammation in disease [21].

Inconsistent studies about OS in patients with alopecia areata, are available [3,4,8,10,11,21]. Güngör et al. and Akar et al. were showed that the antioxidant defense is not defective in patients with alopecia areata [3,21]. Yenin et al., Neziroğlu et al. and Koca et al. also detected the lipid peroxidation in alopecia areata [6,10,11]. While Bilgili et al. found an increase in OS index (total oxidant capacity/total antioxidant capacity), Motor et al. reported that there is no difference between the indices of patient and control groups [9,22]. The organism has to control and stabilize the presence of oxidants and antioxidants and so maintain the balance between them. Because any disturbance in this balance is harmful to the organism. This balance known as redox potential which is specific to each biological environment and organelle, is critically important in sustain of cellular and biochemical functions. Increase of oxidants (oxidation power) causes OS in balance, increase of antioxidants (reduction power) causes reductive stress, as a summary, both two increases causes cellular damage [23]. Cells are continuously exposed to either exogenous oxidants or endogenous oxidants affiliated with normal metabolic activity. Another
cellular pathway of the response to oxidants is achieved via redox regulating proteins [24]. Thiols and disulfides acting important role on cellular redox control, are normally balanced. Thiol status is a control mechanism to maintain the redox homeostasis [25]. Thiol homeostasis is important for the survival of the cells [26]. Total thiol groups are hypersensitive to oxidation, are the first type of oxidants which are consumed by the cell in case of exposure to OS [18]. They constitute the most of the total plasma antioxidant capacity [19,27]. In presence of oxidants known as free radicals, proteins or low molecular weight thiol groups are oxidized and disulfide bonds are established reversibly [20]. Such disulfide bonds are essential for maintaining the structural stability of proteins. These disulfide bonds between thiol groups are reduced to thiol groups again and thus supports the maintenance of dynamic thiol-disulfide homeostasis. This recycling reaction are mediated with specific reductases using NADPH or NADH as an electron source [28]. This homeostasis plays important roles on antioxidant defense as well as detoxification, apoptosis, enzyme regulation, transcription and signal transduction [14]. Dominant for of cysteine which is a precursor in GSH is easily oxidized to disulfide cysteine in normal cells. GSH controls the important biological redox pairs. The reduction potential of GSH/GSSG redox pair is more than the Cys/Cyss [20]. In various studies, it has been shown that the increase of GSH/GSSG ratio causes the proliferation and increase of the same ration causes the apoptosis. GSH/GSSG and Cys/Cyss ratios decreases due to reasons such as diabetes, elderliness, myocardial perfusion defects.

Plasma disulfide values increase in degenerative diseases, while they decrease in proliferative diseases. While the GSH/GSSG and Cys/Cyss ratios were used in previous studies, recently, it is possible to determine the plasma thiol/disulfide ratio totally with a novel method developed by Erel and Neselioglu [14].

There are only two study published reporting the dynamic thiol/disulfide homeostasis in skin disease [29,30]. But, the dynamic thiol/disulfide homeostasis in alopecia areata patients have not been investigated.

Akbas et al. investigated the thiol/disulfide balance in patients with acute and chronic spontaneous urticaria and they did not find a significant difference in patients with acute urticaria, but showed that the balance shifted to disulfide side in patients chronic spontaneous urticaria [29]. Increased disulfide values indicate the presence of oxidative stress in chronic spontaneous urticaria. Demirseren et al. stated that native thiol values of patients with basal cell carcinoma are higher than the control group but disulfide values are lower than the control. They also suggested that the thiol/disulfide homeostasis plays a role in pathogenesis of basal cell carcinoma [30]. We also investigated this balance in patients with alopecia areata. When the native thiol, total thiol and disulfide values with disulfide/native thiol and native thiol/total thiol ratios of the patients were compared to the control group, no statistically significant difference was detected (p > 0.05). Native thiol values of female patients are lower than the male patients (p=0.05). No gender comparison was performed in prior studies. No relation between thiol/disulfide values and duration of disease, number of lesions, severity, recurrence (p > 0.05). We detected that the age is correlated with native and total thiol values. This finding suggests that as the age increases, the OS also increases.

5. CONCLUSION

Eventually according to the data obtained, the dynamic thiol/disulfide homeostasis is not distracted in patients with alopecia areata. Because most of our patient were comprised of mild alopecia areata, in order to determine whether this homeostasis is effected in case of progressive disease, more patients with alopecia totalis and universalis should be compared. Furthermore we are suggesting that more studies with wider series should be performed.

CONSENT

As per international standard or university standard, patient’s written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard, written approval of Yildirim Beyazit University Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.
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