Original Article / Özgün Araştırma

A Marker for Evaluation of Oxidative Stress in Patients with Alopecia Areta: Thiol-Disulphide Homeostasis

Alopesi Areata Tanılı Hastalarda Oksidatif Stresin Değerlendirilmesinde Bir Belirteç: Tiyol-Disülfid Homeostazı

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Abstract

Objective: Alopecia areata (AA) is a disorder characterized by non-scarring hair loss, whose etiology involves oxidative stress. We aimed to determine the role of thiol/disulphide levels in AA pathogenesis and to investigate if they can be used as its marker.

Materials and Methods: This prospective study included 100 AA patients who presented to dermatology outpatient clinic and 100 healthy controls without any systemic and/or inflammatory dermatological disorder. The control and study groups were compared with respect to native thiol, total thiol, and disulphide levels, and disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol ratios. The relationships between demographic and lesion characteristics, native thiol, total thiol, and disulphide levels, and disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol ratios were studied.

Results: The mean age of AA patients was 37.5 years. Fifty-eight (58%) patients were male, and the median body mass index was 24 kg/m^2. Median age was significantly higher in the AA group ($p<0.05$). The AA group had a significantly lower total and native thiol level, native thiol/total thiol ratio, significantly higher disulphide level, disulphide/native thiol ratio, and disulphide/total thiol ratio ($p<0.05$ for all comparisons). There was no correlation between the parameters of thiol/disulfide hemostasis and demographic and lesion characteristics ($p>0.05$).

Conclusion: The thiol/disulphide homeostasis shifted towards oxidative stress, and a decrease in thiols and an increase in disulphides were found in the AA patients. This finding may be responsible for diffuse destruction of hair follicle in the pathogenesis of AA.

Keywords
Alopecia areata, oxidative stress, thiol-disulphide homeostasis

Anahtar Kelimeler
Alopesi areata, oksidatif stres, tiyol/disülfid hemostazı

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Öz

Amaç: Alopesi areata (AA) etiyolojisinde oksidatif stresin rol aldığı, skarsız kıl kaybı ile karakterize bir hastalıktır. AA hastalarında tiyol/disülfid düzeylerine bakarak AA patogenezindeki rolünü ve AA patogenezinde bir belirteç göstergesi olup olmayacağının araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Bu kesitsel çalışma 27.07.2016-01.02.2017 tarihleri arasında dermatoloji polikliniğine başvuran AA tanısı alan 100 hasta ve herhangi bir sistemik ve/veya enflamatuvar deri hastalığı olmayan 100 gönüllü kontrol grubunda

gerçekleştirildi. Kontrol ve hasta grubu arasında nativ tiyol, total tiyol, disülfid düzeyi, disülfid/nativ tiyol, disülfid/total tiyol ve nativ tiyol/total tiyol oranları karşılaştırıldı. AA hastalarının demografik ve lezyon özellikleri ile nativ tiyol, total tiyol, disülfid düzeyi, disülfid/nativ tiyol, disülfid/total tiyol ve nativ tiyol/total tiyol oranları arasındaki ilişki incelendi.

Bulgular: AA'lı hastaların yaş ortalaması 34,9±10,4 yıl, %58'i erkek ve vücut kitle indeksi ortancası 24 kg/m^2 olup, kontrol grubu ile yaş ortalaması açısından farklılık saptanmadı (p>0,05). AA hastalarında total tiyol düzeyi, nativ tiyol düzeyi ve nativ tiyol/total tiyol oranında düzeylerinde anlamlı düşüklük saptanırken; disülfid düzeyi, disülfid/nativetiyol ve disülfid/total tiyol oranlarında anlamlı yükseklik saptandı (p<0,05). AA hastalarında lezyonun süresinin, lezyon paterninin, lezyonun genişliğinin ve rekürrens sıklığının; total tiyol, nativ tiyol, disülfid düzeyleri, disülfid/nativ tiyol, disülfid/total tiyol, nativ tiyol/total tiyol oranlarıyla arasında istatistiksel olarak anlamlı bir ilişkiye rastlanmadı (p>0,05).

Sonuç: AA hastalarında mevcut mekanizmalardan biri oksidatif stres artışıdır. AA grubunda tiyol/disülfid hemostazı oksidatif stres lehine bozulmuş olup, bu hastalarda tiyollerde azalma, disülfid miktarında artma belirlenmiştir.

Introduction

Alopecia areata (AA) is a disease characterized by non-scarring hair loss, which constitutes for 0.7-3.8% of all dermatoses (1,2). Although its prevalence varies between countries, it is estimated to be between 1.7% and 2% (3,4). Whereas its etiology and pathogenesis are not entirely clear, it has been reported that many factors such as autoimmunity, genetic tendency, family history, environmental factors, infectious agents, drugs, trauma, infection, oxidative stress, and psychological stress are responsible at various degrees.

The antioxidant properties of sulphur containing amino acids are variable (5). Thiols are both antioxidant and pro-oxidant molecules. The level of oxidative stress in an organism determines thiols' antioxidant and pro-oxidant effects. This balance has a dynamic status and reflects the overall condition of an organism (6,7).

Plasma thiols are organic compounds that contain sulfhydryl groups composed of hydrogen, sulphur, and carbon atoms. They are strong antioxidants that physiologically eliminate free radicals (8). Reactive oxygen species (ROS) forming in an organism cause oxidation and form disulphide bonds by transferring excess electrons to thiols. Those bonds are reversible and, depending on the oxidant-antioxidant balance, electrons may return back to thiols (6,9,10). The antioxidant effect of thiol-disulphide homeostasis has a critical role in signal transduction, enzymatic reactions, transcription, detoxification, apoptosis reaction, regulation of enzymatic activation, and cellular signal mechanisms. Under normal circumstances the thiol-disulphide homeostasis has a dynamic pattern and is affected by disease states resulting from oxidative stress (6,9). According to a widely accepted view, the changes in thiol-disulphide homeostasis would provide important clues about various abnormal biochemical processes in pathological conditions causing oxidative stress (6). In the present study we aimed to investigate the role of thiol-disulphide homeostasis in AA pathogenesis and to find out if it can be used as its marker.

Materials and Methods

This prospective case-control study was conducted by the Department of Dermatology at Muğla Sıtkı Koçman University Training and Research Hospital (Muğla, Turkey) and Department of Biochemistry at Ankara Yıldırım Beyazıt University Training and Research Hospital (Ankara, Turkey) between 27.07.2016-01.02.2017. Ethical approval (date: 27.07.2016, no: 13/III) was obtained from Muğla Sıtkı Koçman University Local Ethics Committee and written informed consent was obtained from all patients and controls before the start of the study. It involved 100 patients with AA who presented to the dermatology outpatient clinic and 100 healthy volunteers who free of any systemic or skin disorder. Our study was performed in accordance with the guidelines of good clinical practice and the Helsinki declaration.

Persons with any systemic disorder (coronary artery disease, liver failure, renal failure, malignancy etc.), inflammatory skin disorders, smoking/alcohol consumption, and systemic/topical drug use were excluded, as were those who were pregnant, breastfeeding, and younger than 18 years of age. Age, sex, AA duration, lesion size, and recurrence status were recorded in all cases. Native thiol, total thiol, disulphide levels and disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol ratios were compared between the patient and control group's sera. The correlations between AA lesion

properties and total thiol, native thiol, and disulphide levels as well as disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol ratios were studied. The samples were centrifuged at 3600 rpm for 10 minutes at the biochemistry laboratory and kept at -80 degrees celsius until the final biochemical analysis. All samples were thawed simultaneously, and serum thiol-disulphide parameters were studied at Yıldırım Beyazıt University, Ankara Training and Research Hospital Biochemistry Laboratory, with a Roche Hitachi Cobas c501 automatic analyzer and using the automatic measurement method recently developed by Erel and Neselioglu (6).

Statistical Analysis

SPSS for Windows 18.0 software package was used for statistical analyses. The Kolmogorov-Smirnov test was used to test the normality of the distribution of continuous variables. The descriptive statistics included mean and standard deviation for normally distributed quantitative variables and median and interquartile range (IQR) for non-normally distributed quantitative variables. Categoric variables were presented as number and percentage (%). Variables with non-normal distribution were compared using the Mann-Whitney U test, and categoric variables were compared using the Pearson's chi-square test. Pearson correlation test was used for comparison of normally distributed quantitative variables. A p value of less than 0.05 was considered statistically significant.

Results

The median age of AA patients was 37.5 (IQR: 11) years, and 58 (58%) of them were male. The median body mass index (BMI) was 24 kg/m^2. The mean age was significantly higher in the AA group ($p<0.05$). No statistically significant difference was found between the groups with respect to gender and BMI ($p>0.05$) (Table 1).

The AA group had a significantly decreased native thiol level ($p<0.05$) (Table 2). The AA group had a significantly decreased native thiol/total thiol ratio and a significantly increased disulphide level, disulphide/native thiol, and disulphide/total thiol ratios ($p<0.01$) (Table 2). The total thiol level was similar between the AA and control groups ($p>0.05$) (Table 2).

Demographic properties of the patients are shown on Table 1. No correlation was found between the

Table 1. Comparison of demographic and clinical characteristics of the study groups

		AA patients	Control	p
Age, mean ± SD		37.5 (11)	32 (16)	0.002
Gender	Male, n (%)	58 (58)	47 (47)	0.119
	Female, n (%)	42 (42)	53 (53)	
BMI, median (IQR)		24 (0)	24 (1)	0.214
Disease duration	6 months >, n (%)	28 (28)	-	-
	6 months <, n (%)	72 (72)	-	-
Disease pattern	Mono AA, n (%)	42 (42)	-	-
	Poli AA, n (%)	55 (55)	-	-
	AA Totalis, n (%)	3 (3)	-	-
Lesion size	S1 (<%25), n (%)	69 (69)	-	-
	S2 (%25-49), n (%)	26 (26)	-	-
	S3 (%50-74), n (%)	2 (2)	-	-
	S4 (%75-90), n (%)	2 (2)	-	-
	S5 (%100), n (%)	1 (1)	-	-
Disease recurrence	First, n (%)	68 (68)	-	-
	1. Recurrence, n (%)	24 (24)	-	-
	2. Recurrence, n (%)	5 (5)	-	-
	3. Recurrence, n (%)	3 (3)	-	-

AA: Alopecia areata, SD: Standard deviation, IQR: Interquartile range

Table 2. Comparison of thiol-disulphide homeostasis parameters of the study groups

	AA patients (n=100) median (IQR)	Controls (n=100) median (IQR)	p
Native thiol	404.1 (94.2)	424.7 (87.9)	0.006
Total thiol	441 (103.4)	456.5 (90.8)	0.071
Disulphide	20.7 (8.1)	15.65 (7.2)	<0.001
Disulphide/native thiol	5.3 (2.2)	3.7 (1.7)	<0.001
Disulphide/total thiol	4.8 (2.1)	3.4 (1.4)	<0.001
Native thiol/total thiol	90.3 (4.3)	93.1 (2.9)	<0.001

AA: Alopecia areata, IQR: Interquartile range

total thiol level, native thiol level, and native thiol/total thiol ratio and significantly increased disulphide level, disulphide/native thiol, and disulphide/total thiol ratios levels as analyzed by BMI, lesion duration, disease pattern, and recurrence frequency (p>0.05) (Table 3).

Discussion

AA is an autoimmune disorder characterized by the destruction of hair follicles by T cells (2,3). AA generally starts at a young age and it adversely affects quality of life by its psychological burden (11,12). It is of debate whether oxidative stress develops primarily or as a result of T cell infiltration (13). It has been shown that oxidative stress is augmented, and oxidative balance is impaired in disorders with inflammatory or autoimmune reactions, such as AA, atopic dermatitis, pemphigus, psoriasis, allergic contact dermatitis, acne vulgaris, and vitiligo (2,14-16). Although AA has a well-known close association with autoimmunity, the role of oxidative stress in its pathophysiology has not been clearly demonstrated (2,13,17,18). It has been reported that lipid peroxidation may play a role in AA pathogenesis and may increase total oxidant capacity/total antioxidant capacity (5,19). ROS is activated by abnormal lymphokine release, eicosanoid metabolism, fatty acid metabolism, and inflammatory cells surrounding the hair follicle (20).

We detected significant decreases in total thiol level, native thiol level, and native thiol/total thiol ratio, but significant increases in disulphide level, disulphide/native thiol ratio, and disulphide/total thiol ratio in the AA patients.

Kilinc et al. (21) reported that the disulfide/native thiol and native thiol/total thiol ratios and native thiol, total thiol, and disulfide values were similar in AA patients and the control group. Akbas et al. (8) reported that disulphide/thiol ratio increased in chronic urticaria and increased oxidative stress reduced disulphide ratio in chronic stage.

Our study suggested that thiols are reduced in AA as a result of increased oxidative stress. An important step in hair formation is the thiol-disulphide conversion that occurs simultaneously with the process of hair formation (22). It is thought that thiol transfer is the basic mechanism underlying the non-enzymatic thiol-disulphide conversion (23). In the enzymatic reaction, on the other hand, it has been shown that the enzyme catalyzing thiol-disulphide conversion of cystine and glutathion or other low molecular weight compounds is actually a two-substrate mechanism involving thiol transfer (23). Histochemical examination of hair has shown that in the trichilemmal sac encircling hair the germinal cells rich in free thiol groups are abundant, and disulphide bonds limit themselves (24). In cases of failed hair formation or absent hair follicle, on the other hand, thiol ratio decreases and sulphide content increases (22). Previous studies have indicated increased oxidative activity and decreased antioxidant activity in the scalps of AA patients (13,14,18,25). Free radicals originating from normal metabolism or pathological processes and increased oxidative stress break off one proton from various molecules including thiols and fatty acids and form new radicals, ultimately leading to cellular injury (8,26,27). Degradation of thiol balance may explain cellular damage (28). In our study, we believe that in AA thiols are consumed as a consequence of increased oxidative stress, leading to the conversion of this thiol-disulfide into AA pathophysiology.

Table 3. Correlations of thiol/disulphide hemostasis parameters with demographic and clinical characteristics of the study patients

	BMI		Disease duration		Lesion size		Lesion pattern		Recurrence	
	r	p	r	p	r	p	r	p	r	p
Native thiol	0.023	0.819	0.005	0.957	0.037	0.712	0.084	0.408	-0.117	0.248
Total thiol	0.021	0.832	-0.010	0.925	0.028	0.783	0.067	0.509	-0.121	0.232
Disulphide	-0.019	0.853	0.115	0.256	0.056	0.582	0.025	0.803	-0.070	0.491
Disulphide/native thiol	-0.027	0.791	-0.088	0.385	-0.100	0.324	-0.157	0.118	0.046	0.651
Disulphide/total thiol	-0.028	0.785	-0.099	0.328	-0.103	0.308	-0.171	0.088	0.057	0.571
Native thiol/total thiol	0.023	0.821	-0.120	0.233	0.084	0.405	0.153	0.127	0.039	0.701

BMI: Body mass index

It has been previously reported that while total thiol levels are correlated to oxidative stress and disulphide levels in oxidative stress, native thiol ratio is not necessarily correlated to disulphide levels (8,29). This can be attributed to a greater decrease in total thiol than native thiol in oxidative stress. Hence, our study showed a reduced disulphide/total-native thiol ratio but an increased native/total thiol ratio, which can be attributed to a lesser consumption of native thiol in AA.

Kilinc et al. (21) reported that thiol/disulfide values were not associated with gender, disease duration, number of lesions, or recurrence frequency in AA patients. Akar et al. (18) reported that oxidation was augmented in the acute stage compared to the chronic stage. Akbas et al. (8) showed that thiol/disulfides did not change in cases of acute urticaria but decreased in chronic cases, and there was a significant relationship between disease severity and thiol/disulfide level. Abdel Fattah et al. (13) reported a positive correlation between lesion size and oxidative stress factors. Heidarloo and Adışen (2) found no correlation between oxidant levels and AA lesion severity, and they attributed it to the majority of cases being S1. In our study, there no correlation observed between total thiol level, native thiol level, and native thiol/total thiol ratio and significantly increased disulphide level, disulphide/native thiol and disulphide/total thiol ratios levels as analyzed by BMI, lesion duration, disease pattern, and recurrence frequency. There was a shift of thiol-disulphide homeostasis towards oxidation, albeit statistically non-significant, in patients with a disease duration of less than 6 months, which may suggest that oxidation is more prominent in the acute stage of the disease. We believe the thiol-disulphide homeostasis had higher levels in patients with larger lesions and wider pattern but the low number of patients in this group possibly prevented the occurrence of statistical significance. We believe that this is related to disease process starting over each time rather than AA's attack frequency.

Conclusion

In conclusion, it can be suggested that oxidative stress is one of the possible mechanisms playing a role in the pathophysiology of AA. In the AA patients, the thiol/disulphide homeostasis shifted towards oxidative stress, and a decrease in thiols and an increase in disulphides were also observed. This condition may be related to thiol-disulfide conversion of thiol in case of oxidative stress. Large-volume studies are needed in order to fully elucidate these mechanisms.

Ethics

Ethics Committee Approval: Ethical approval (date: 27.07.2016, no: 13/III) was obtained from Muğla Sıtkı Koçman University Local Ethics Committee before the start of the study.

Informed Consent: The written informed consent was obtained from all patients and controls before the study.

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Authorship Contributions

Surgical and Medical Practices: S.D.P., E.T.A., Concept: S.D.P., G.D., E.T.A., Design: S.D.P., E.T.A., G.D., Data Collection or Processing: S.D.P., G.D., E.T.A., Analysis or Interpretation: S.D.P., S.N., Ö.E., Literature Search: S.D.P., Writing: S.D.P.

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