Int J Clin Exp Med 2014;7(4):1089-1093
www.ijcem.com /ISSN:1940-5901/IJCEM0000083

Original Article

Evaluation of total antioxidant status, total oxidant status and oxidative stress index in patients with alopecia areata

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Received February 24, 2014; Accepted March 22, 2014; Epub April 15, 2014; Published April 30, 2014

Abstract: Objectives: In this study, we aimed to evaluate total oxidative stress and total antioxidant capacity in serum samples from patients with Alopesia Areata (AA) in our laboratory conditions. Methods: In this study, 46 subjects with AA (26 females, 20 males) and the control subjects of 36 (20 females, 16 males) age- and sex-matched healthy volunteers from our hospital staffs were enrolled (the mean age was 23.7 ± 11.0 years). Blood samples were obtained following an overnight fasting state, and were collected on ice at 4°C. The serum samples were separated from the cells by centrifugation at 3000 rpm for 15 min and were stored at -80°C and used for the analysis of the Total Antioxidant Status (TAS) and Total Oxidant Status (TOS). Results: Total Antioxidant Status (TAS) and Total Oxidant Status (TOS), Oxidative Stress Index (OSI) (TOS/TAS) levels of AA patients were 1.4777 ± 0.1986; 9.7490 ± 6.0445; 0.6593 ± 0.4069 respectively. TAS; TOS; OSİ (TOS/TAS) levels of controls were 1.4028 ± 0.1687; 9.4627 ± 4.2781; 0.6875 ± 0.3232 respectively. TAS, TOS and OSİ levels showed no significant difference between the control and AA group ($p > 0.05$). Conclusion: Future studies about AA pathogenesis should be based not only on oxidant/antioxidant balance but also on several other factors. Because it was observed that the disease showed recurrence in different situations. Since the selection criteria of patients is affected from disease severity and environmental and genetical factors, multicentric studies with better sampled patient population and higher patient number is required.

Keywords: Alopecia areata (AA), TAS, TOS, OSI, oxidative stress

Introduction

Alopecia areata (AA) is a recurrent and autoimmune inflammatory disease presenting with non-scarring hair loss. AA has an incidence of 0.1-0.2% in the general population while this rate is approximately 2% in new patients attending to dermatology outpatient clinics [1-3]. It is still unknown how AA triggers T-cell-mediated inflammation in hair follicle [4, 5]. The exact etiology and pathogenesis of AA is unknown and many factors including autoimmunity, genetic predisposition, family history, environmental factors, infections agents, drugs, trauma, infection, oxidative stress and possible emotional stress have been proposed to be responsible in the pathogenesis of AA [1, 6, 7].

There exists a balance between oxidative damage and antioxidant protection in normal aerobic cells. Insufficient antioxidant protection or excessive production of reactive oxygen species (ROS) generates a condition known as oxidative stress, which is thought to play an important role in skin cancers, cutaneous aging and many inflammatory skin diseases such as AA [8-12]. Enzymatic (glutathione peroxidase, catalase and superoxide dismutase) and non-enzymatic (antioxidant a-tocopherol, ubiquinone, beta-carotene, ascorbate, glutathione) antioxidant systems play an important role in normal homeostasis of the skin [13, 14].

AA, an autoimmune inflammatory disease, affects the oxidant-antioxidant balance has been

Table 1. The demographic data in patients with alopecia areata

	n	%
Sex		
Female	26	56.5
Male	20	43.5
SALT		
S1	30	65.2
S2	8	17.4
S3	3	6.5
S4	3	6.5
S5	2	4.2

suggested in some studies [2, 8, 9]. In this study we aimed to evaluate total oxidative stress and total antioxidant capacity from the blood of our patient groups in our laboratory conditions.

Methods

Subjects

This study was conducted in the Department of Dermatology, Faculty of Medicine, Mustafa Kemal University. In this study, 46 subjects with AA (26 females, 20 males) and the control subjects of 36 (20 females, 16 males) age- and sex-matched healthy volunteers from our hospital staffs were enrolled. The average age of the patients ranged from 5 to 48 and the mean age was 23.7 ± 11.0 years. In our study, 13 patients had thyroid disease, 12 had asthma and five had allergic rhinitis, 31 had diabetes mellitus, one had Lupus erythematosus, one had atopic dermatitis, one had vitiligo, four had psoriasis, one had rheumatoid arthritis, and two had a family history of Down syndrome. The patients had not received any systemic or topical therapy. Global severity of AA was evaluated by combination of hair loss and hair density. According to SALT (Severity of Alopecia Tool) scores, diseases are divided into 5 categories, as S1, S2, S3, S4 and S5. The demographic characteristics of the subjects with AA and controls are shown in **Table 1**. The AA subjects were selected among adults who visited the Department of Dermatology because of dermatological diseases.

The patients had not received any systemic therapy such as corticosteroids minoxidil, anthralin within the last two months or topical therapy that affects cellular immunity or photo chemotherapy. This study was approved by local ethical committee.

Blood samples

Blood samples were obtained following an overnight fasting state, and were collected on ice at 4°C. The serum samples were separated from the cells by centrifugation at 3000 rpm for 15 min and were stored at -80°C and used for the analysis of the Total Antioxidant Status (TAS) and Total Oxidant Status (TOS).

Analysis of blood samples

Serum TAS and TOS were determined with kits (Rel Assay Diagnostics kit; Mega Tıp, Gaziantep, Turkey) developed by Erel and Oxidative Stress Index (OSI) values were calculated.

Measurement of the TAS: Serum TAS levels were determined using a novel automated measurement method, developed by Erel. In this method, the antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The results are expressed as μmol Trolox Eq/L [15].

Measurement of the TOS: Serum TOS values were determined using a novel automated measurement method, such as TAS, developed by Erel. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide (H_2O_2) and the results are expressed in terms of micromolar hydrogen peroxide equivalent per litre (μmol H_2O_2 Eq/L) [16].

Calculation of OSI: The OSI was defined as the ratio of the TOS level to TAS level. Specifically, OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq/L)/TAS (μmol Trolox Eq/L) [17].

Statistical analysis

Data was analyzed with a commercially available statistics software package (SPSS12 for Windows v. 16.0, Chicago, USA). Distributions of the groups were analyzed with a one-sample Kolmogorov - Smirnov test. Parametric variables were compared using Student's t-test. Nonparametric continuous variables were com-

Table 2. Oxidant and antioxidant parameters in alopecia areata and controls

	Patient (n = 46)	Control (n = 36)	*p*
TAS (μmol Trolox Eq/L)	1.4777 ± 0.1986	1.4028 ± 0.1687	0.0740
TOS (μmol H_2O_2 Eq/L)	9.7490 ± 6.0445	9.4627 ± 4.2781	0.8123
OSI (arbitrary unit)	0.6593 ± 0.4069	0.6875 ± 0.3232	0.7366

Values are mean ± SEM. TAS, total antioxidant capacity; TOS, total oxidant status; OSI, oxidative stress index.

pared with the Mann - Whitney U test. The results are presented as means ± SEM. *P* values less than 0.05 were regarded as statistically significant.

Results

TAS; TOS; OSI (TOS/TAS) levels of AA patients were 1.4777 ± 0.1986; 9.7490 ± 6.0445; 0.6593 ± 0.4069 respectively. TAS; TOS; OSİ (TOS/TAS) levels of controls were 1.4028 ± 0.1687; 9.4627 ± 4.2781; 0.6875 ± 0.3232 respectively. TAS, TOS and OSİ levels showed no significant difference between the control and AA group ($p > 0.05$). The TAS, TOS, OSI (TOS/TAS) levels of the subjects with AA and controls are shown in **Table 2**.

Discussion

Alopecia areata (AA), is an autoimmune inflammatory disease, characterized by the loss of the scalp hair. Etiology and pathogenesis of this disease not known precisely, but immunological factors, genetic predisposition, atopic status, emotional stress, viral infections neurological factors and oxidative stress is believed to play an important role in this disease [18, 19]. Some studies in the literature support that free radicals are associated with the pathogenesis of AA [2, 12]. In this study, we aimed to investigate oxidative stress via the measurement of TAS and TOS which could have been systematic reflection of oxidative stress, in subjects with AA rather than investigating local hair follicles.

Antioxidant enzymes SOD and GSH-Px, antioxidant parameters such as TAS, has been studied in patients with AA. Koca et al and Abdel Fattah et al. observed decreased SOD activities in the serum and erythrocytes [2, 20]. However, Akar et al. [12] investigated increased SOD activities in the scalp of patients with AA. Similarly, Güngör et al [18] reported increased SOD activities in both erythrocyte and tissue of subjects with AA compared with controls. Naziroglu et al [14] observed a significantly decreased erythrocyte and plasma GSH-Px activity and glutathione, substrate of GSH-Px in AA compared with control group. Akar et al [12] investigated double increased GSH-Px activity compared to control group in the early phase of disease compared to late phase lesions on the scalp biopsy specimen. In another study Güngör et al [18] found increased glutathione peroxidase activity in erythrocytes of subjects with AA, but there was no difference in terms of GSH-Px activity between the normal alopecic tissues.

Malondialdehyde (MDA), which is a lipid peroxidation product in plasma and erythrocytes has been the focus of attention in several studies. Naziroglu et al [14] found statistically significantly higher MDA levels, as Akar et al [12] who found significantly higher MDA levels which reacts with TBA (thiobarbituric acid) in scalp hair of patients with AA compared to healthy subjects. Koca et al [2] found increased MDA level in serum. Abdel Fattah et al. [20] observed statistically significantly higher MDA levels in plasma of subjects with AA compared with controls. However, Güngör et al. [18] reported that plasma MDA levels were no statistically significantly increased in the patient group compared to control.

In the present study, TAS and TOS levels showed no significant difference between the control and AA patient group. However, a study carried by Bilgili et al [21] with 39 AA and 39 healthy control group revealed that TAS levels in the patient group was significantly lower than the control group. Similarly to previous study, Kim et al. [22] found decreased antioxidant levels and increased free radical levels in AA.

The role of oxidative stress in the etiology of AA is still not clearly defined. Because there are significant differences among the oxidant/antioxidant results. We used the method of Erel for the measurement of TAS and TOS to evaluate the systemic effect of oxidative stress. In a study by Bilgili et al [21], no significant differences were found between the antioxidant levels concerning AA pathogenesis in age and sex matched groups. In several studies it was pro-

posed that oxidative stress played role in the pathogenesis of various skin disorders such as psoriasis vulgaris [23], lichen planus [24], Behçet's disease [25], vitiligo [26], and AA [19]. Future studies about AA pathogenesis should be based not only on oxidant/antioxidant balance but also on several other factors. Because it was observed that the disease showed recurrence in different situations [27]. Since the selection criteria of patients is affected from disease severity and environmental and genetical factors, multi-central studies with better sampled patient population and higher patient number is required.

Conclusions

The results of studies related to oxidative stress and oxidant/anti oxidant statutes in patients with AA show important discrepancies. Because the selection criteria for the patients are affected by many situations such as environmental and genetic factors, further multicenter studies are needed to evaluate disease pathogenesis.

Disclosure of conflict of interest

None.

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