Changes of Lipid Peroxidation and Antioxidant System in Serum and Tissues of Patients With Alopecia Areata

Alopesi Areatalı Hastaların Doku ve Serumlarında Lipid Peroksidasyon ve Antioksidan Sistem Değişiklikleri

Şule GÜNGÖR, MD,[a]
Gülfer AKBAY, MD,[a]
Elmas ÖĞÜŞ, MD,[b]
Meral EKŞİOĞLU, MD,[a]
Doğan YÜCEL, MD[b]

Departments of [a]Dermatovenereology, [b]Biochemistry, Ankara Training and Research Hospital, ANKARA

Geliş Tarihi/*Received:* 12.06.2007
Kabul Tarihi/*Accepted:* 13.05.2008

This case was presented as a poster at XVI. European Academy of Dermatology and Venereology Congress in 16-20 May 2007.

Yazışma Adresi/*Correspondence:*
Şule GÜNGÖR, MD
Ankara Training and Research Hospital, Dermatovenereology Department, ANKARA
drsulegungor@hotmail.com

ABSTRACT Objective: Alopecia areata (AA), is an autoimmune inflammatory disease, characterized by the loss of the scalp hair, and its etiology and pathogenesis is still unknown. To search the role of oxidative stress in the etiopathogenesis, lipid peroxidation and antioxidant enzyme levels are studied in the serum and tissues of patients with AA. **Material and Methods:** The study was performed on 21 adult patients diagnosed as AA and 21 healthy volunteers. All subjects were nonsmokers, did not have any systemic disease and had not recieved any systemic or topical theraphy. Blood samples were obtained from both the patients and the control group, and tissue specimens were taken only from the alopecic and normal scalp tissues of the patient group. Malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GP) enzyme levels were studied. Student's t test and Mann Whitney U test were used for the statistical analysis. **Results:** Serum eritrocyte SOD and GP levels were significantly higher in the patients with AA when compared with the control group (p=0.025, p=0.033, respectively). Plasma MDA levels were higher in the patient group, but this was not statistically significant (p=0.109). SOD levels of alopecic tissues were significantly higher than normal tissues for the patients (p=0.018). There was not any difference for the levels of GP and MDA in the alopecic and normal tissues (p=0.458, p=0.660, respectively). **Conclusion:** Antioxidant enzyme levels (SOD and GP) were higher in the patient group compared with control group, furthermore for the patient group these levels were higher in alopecic tissues compared with normal tissues. Therefore it is thought that, as the result of oxidative stress and lipid peroxidation, antioxidant enzyme levels are increased. In conclusion it is decided that, lipid peroxidation and changes in oxidant-antioxidant systems may play an important role in the pathogenesis of AA.

Key Words: Alopecia areata; lipid peroxidation; oxidative stress

ÖZET Amaç: Alopesi areata (AA), etyolojisi ve patogenezi kesin olarak bilinmeyen, saç dökülmesi ile karakterize, inflamatuvar otoimmün bir hastalıktır. Hastalığın etyopatogenezinde oksidatif stresin rolünü araştırmak amacıyla AA'lı hastaların serumlarında ve dokularında lipid peroksidasyonu ve antioksidan enzim düzeyleri araştırıldı. **Gereç ve Yöntemler:** Çalışmaya AA tanısı almış, sigara içmeyen, herhangi bir sistemik hastalığı olmayan, sistemik ve topikal tedavi almayan 21 erişkin hasta ve aynı koşullardaki 21 sağlıklı gönüllü dahil edildi. Kan örnekleri hasta ve kontrol grubundan, doku örnekleri ise sadece hasta grubunun alopesik ve normal saçlı derilerinden alındı. Bu örneklerde malondialdehit (MDA), süperoksit dismutaz (SOD), glutatyon peroksidaz (GP) enzim düzeyleri araştırıldı. İstatistiksel değerlendirmede Student t testi ve Mann Whitney U testi kullanıldı. **Bulgular:** AA'lı hastalardaki serum eritrosit SOD ve GP düzeyleri, kontrol grubuna göre anlamlı derecede yüksek saptandı (sırasıyla p=0.025, p=0.033). Plazma MDA düzeyleri hasta grubunda kontrol grubuna göre yüksek tespit edilse de bu fark istatistiksel olarak anlamlı değildi (p=0.109). Hastaların alopesik dokularındaki SOD düzeyleri normal dokularındaki SOD düzeylerine göre istatistiksel olarak anlamlı derecede yüksekti (p=0.018). GP ve MDA düzeyleri açısından alopesik ve normal dokular arasında fark yoktu (sırasıyla p= 0.458, p= 0.660). **Sonuç:** Bu çalışmada antioksidan enzim (SOD ve GP) düzeyleri hasta grubunda kontrol grubuna göre; ayrıca hastaların alopesik dokularında normal dokulara göre yüksek bulundu. Bu durumda oksidatif stres ve lipid peroksidasyonu sonucunda antioksidan enzim düzeylerinde artış geliştiği düşünüldü. Sonuç olarak lipid peroksidasyonunun ve oksidan-antioksidan sistemdeki değişikliklerin AA patogenezinde önemli rol oynayabileceği kararına varıldı.

Anahtar Kelimeler: Alopesi areata; lipid peroksidasyonu; oksidatif stres

Turkiye Klinikleri J Dermatol 2008;18:141-145

Alopecia areata (AA) is an autoimmune inflamatory disease, characterized by hair loss, and its etiology and pathogenesis is not known definetely.[1-3] The clinical exposition of AA according to the pattern of hair loss may be classical, total (characterized by hair loss covering the whole scalp) and universal (characterized by the loss of all body hair).[1,2] Immunological factors (autoimmunity, apoptosis, cellular factors), genetic predisposition, atopic state, emotional stress, neurologic factors, viral infections (cytomegalovirus), oxidative stress are thought to play role in the etiology of the disease.[1-8]

The presence of oxidative stress, arising as the result of imbalance between oxidant and antioxidant systems is shown by evaluation of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GP) and lipid peroxidation products such as malondialdehyde (MDA). SOD is the major antioxidant enzyme that reduces the superoxide radicals in the cell and also it is the first defence mechanism in the body for free oxygen radicals. GP, inhibits lipid peroxidation. MDA is a product of lipid peroxidation and is the best indicator of oxidative lipid peroxidation and so the oxidative stress in the plasma.[9-11]

The presence of anti-MDA antibodies is shown in systemic lupus erythematosus models and determined that oxidative stress may play a role in the development of autoimmune diseases.[12] Besides it is shown that oxidative stress may cause apoptosis.[13] Therefore it is thought that oxidative stress may play a role in development of AA, which is an autoimmune disease and apoptosis is accused in formation.

The aim of this study is to search the role of oxidative stress in the aetiopathogenesis of AA by studying the levels of lipid peroxidation and antioxidant enzymes in blood and tissues of patients with AA.

MATERİAL AND METHODS

Twenty-one patients with AA and twenty-one healthy volunteers as control subjects were included in the study. The study protocol was approved by the local Ethical Commitee and all participants signed informed consents prior to sample collection. All patients were nonsmokers, did not have any systemic disease and were not receiving systemic or local treatment for three months. Haemoglobin values, fasting plasma glucose, vitamin B12 levels, thyroid function tests and autoantibodies were researched for all patients. Patients with any abnormality for these tests were excluded from the study. Blood samples were obtained from both groups. On the other hand tissue samples were studied only from the patients with AA and from the alopecic and normal scalp tissues. Scalp tissue samples were obtained under local anesthesia by 4 mm punch biopsy technique.

Eritrocyte/tissue SOD and GP, plasma/tissue MDA levels were assigned using methods defined by Sun et al, Paglia et al, Hunter et al, Uchiyama et al, respectively.[14-17] Student's t test and Mann Whitney U test were used for statistical analysis. Results were expressed in mean±standard deviation (for Student t test) or median ±interquartile range (for Mann Whitney U test). p values ≤0.05 were considered statistically significant.

RESULTS

Table 1 shows the epidemiological and clinical characteristics of both groups. There was not any difference for sex and age between the groups. All of the patients had classical AA. The comparison of laboratory values for AA and control groups are shown in Table 2. Serum eritrocyte SOD and GP levels were significantly higher in the patients with AA. Serum MDA levels were higher in the patient group, but this was not statistically significant. SOD, GP ve MDA levels in alopecic and normal tissues for patient group are shown in Table 3. Tissue SOD levels were significantly higher in the alopecic tissues when compared with normal tissues of the patients (p=0.018). This significant difference was not found for GP and MDA levels. Figure 1 shows SOD, GP ve MDA levels in blood samples for patients and control group and Figure 2 shows SOD, GP ve MDA levels in alopecic and normal tissues for patient group.

TABLO 1: Epidemiological and clinical characteristics of patient and control group.

	Sex	Age (years)	First episode	Last episode	Type of AA
Patients	12 male (57%)	18-62	1week-144 months	1week-12months	100% classical AA
	9 female (43%)	(33.8±11.6)	mean12.90 months	mean 6.6 weeks	(<10% BSA)
Controls	11 male (52%)	25-50			
	10 female (48%)	(32.6±6.2)			

AA: Alopecia areata

BSA: Body Surface Area

TABLO 2: SOD, GP and MDA levels in blood samples of both groups.

Blood	Eritrocyte SOD (U/gr Hb)	Eritrocyte GP (U/gr Hb)	Plasma MDA (nmol/ml)
Patients	1248.3±544.1	57.90±15.77	8.57±2.41
Controls	972.7±312.8	49.70±12.15	7.75±1.80
p	0.0254*	0.033*	0.109*

SOD: Superokside dismutase, GP: Glutathione peroxidase, MDA: Malondialdehyde

*Student t test

TABLO 3: SOD, GP ve MDA levels in alopecic and normal tissues for patient group.

Tissue	SOD (U/gr tissue)	GP (U/gr tissue)	MDA (nmol/gr tissue)
Alopecic	5914.039±3774.706	511.050±410.275	126.440±286.095
Nonalopecic	3969.183±1583.337	427.940±269.105	145.830±208.570
p	0.0184*	0.458**	0.660**

SOD: Superokside dismutase, GP: Glutathione peroxidase, MDA: Malondialdehyde

* Student t test

**Mann Whitney U test

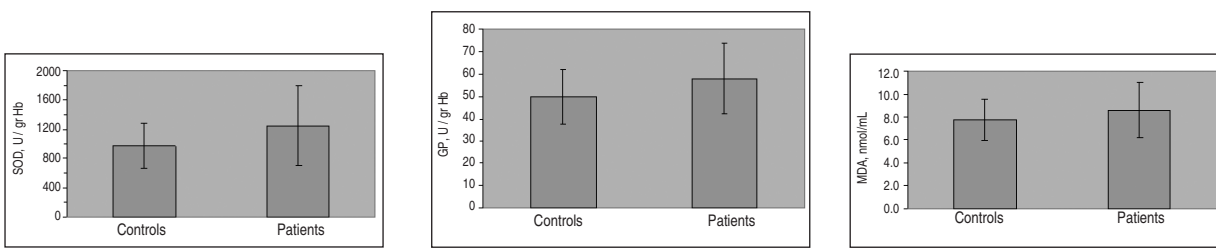

FIGURE 1: SOD*, GP* and MDA* levels in blood samples for patients and control group shown on the schemes.

SOD: Superokside dismutase, GP: Glutathione peroxidase, MDA: Malondialdehyde

*Student t test

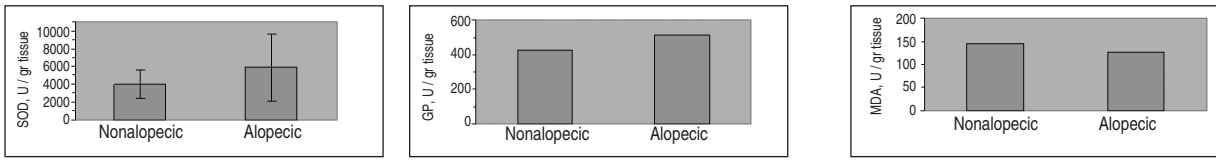

FIGURE 2: SOD *, GP** and MDA** levels shown on the schemes in alopecic and nonalopecic tissues for patient group

SOD: Superokside dismutase, GP: Glutathione peroxidase, MDA: Malondialdehyde

* Student t test

**Mann Whitney U test

DISCUSSION

Oxidative stress is accused in the aetiopathogenesis of many cutaneous and systemic diseases.[9-11,18,19] It is shown that oxidative stress plays role in psoriasis, vitiligo, acne, rosacea and photo-ageing.[9-11,20-25] But there are a few studies searching the presence oxidative stress in patients with AA. It is the first study that oxidative stress is evaluated in patients with AA both in blood and tissue at the same time, moreover normal and alopecic tissues are compared contemporaneously in the same patient for the first time. The most important advantage of using the same patient's normal tissue as control group for the tissue study is that both tissues are effected at the same level from any kind of exogen and endogen factors effecting oxidant-antioxidant systems. So the only reason of different enzyme levels between tissues is the clinical existanse of AA.

Eritrocyte SOD and GP levels were statistically significantly higher in the patient group. Serum MDA levels were higher in the patients but this was not statistically significant. Higher levels of antioxidant enzyme levels in the patient group may be a sign of oxidative stress and it is thought that antioxidant enzyme levels are increased as an answer to oxidative stress. Increases in the levels of antioxidant enzyme levels may decrease MDA levels which is the sign of lipid peroxidation. Tissue SOD levels were significantly higher in the alopecic tissue. Tissue GP levels were higher in the alopecic tissue than normal tissue but this was not statistically significant. Tissue MDA levels were lower in the alopecic tissue than normal tissue but this was not statistically significant. SOD is the first line enzyme to defence against oxidative stress and we thought that it may cause the higher tissue SOD levels in our early onset AA patients. High levels of SOD and GP in the tissue like blood samples show that antioxidant system is working sufficiently in AA and adequate response is given to oxidative stress.

Unlike this study Naziroğlu et al. and Koca et al. have reported lower levels of antioxidant enzymes and higher levels of lipid peroxidation product levels in the blood samples of patients with AA.[5,6] Similar with the results of this study, Akar et al. found higher tissue antioxidant enzyme levels but different from us they also reported higher tissue lipid peroxidation products levels in patient group.[4] The reason of the different results between the studies may be due to the difference of the number of the patients ,or type of AA of patients, or type of control group. Half of the patients were total or universal AA in Akar's study but all of our patients were classical AA, or Akar et al. used different individuals' tissues as control group but we used patients' normal tissues as control group.

Various hypothesis are claimed to explain the role of oxidative stress in the aetipathogenesis of AA. Oxidative stress causes lipid peroxidation and MDA is produced. MDA forms covalent bond with endogen proteins and causes the formation of new antigens by producing structural changes in the proteins. Autoimmune mechanisms are triggered by formation of new antigens.[12]

Another theory is that, oxidative stress triggers apoptosis and contributes to development of AA. As a result of the damage occurred in the mitochondrial membrane because of oxidative stress, caspase enzymes that play role in apoptosis are secreted and apoptosis develops in the hair follicle.[13] Although these two theories support the initiating role of oxidative stress in the aetipathogenesis of AA, it is still not known if oxidative stress is initiator for AA or if it develops as the result of inflammation.

With this study we concluded that oxidative stress plays role in the aetiopathogenesis of AA and adequate antioxidant response occurs due to oxidative stress in classical AA.

Because all of the patients were classical AA in the study and because less than 10% of body surface area were held, these questions should be answered. Do these patients have adequate reply to oxidative stress and so they have classical AA? If adequate reply to oxidative stress should not be performed, would all these patients develop total or universal AA?

More comprehensive studies including patients with total and universal AA or repetative tissue samples from studies on animal models from

the same model during the disease should help to clarify the relation between antioxidant answer and prognosis. This knowledge will clarify the effectiveness of antioxidant therapy in AA.

REFERENCES

1. Madani S, Shapiro J. Alopecia areata update. J Am Acad Dermatol 2000;42:549-66.
2. Sehgal VN, Jain S. Alopecia areata: clinical perspective and an insight into pathogenesis. J Dermatol 2003;30:271-89.
3. Schwartz RA, Janniger CK. Alopecia areata. Cutis 1997;59:238-41.
4. Akar A, Arca E, Erbil H, Akay C, Sayal A, Gür AR. Antioxidant enzymes and lipid peroxidation in the scalp of patients with alopecia areata. J Dermatol Sci 2002;29:85-90.
5. Naziroglu M, Kokcam I. Antioxidants and lipid peroxidation status in the blood of patients with alopecia. Cell Biochem Funct 2000;18: 169-73.
6. Koca R, Armutcu F, Altinyazar C, Gürel A. Evaluation of lipid peroxidation, oxidant/antioxidant status, and serum nitric oxide levels in alopecia areata. Med Sci Monit 2005;11: CR296-299.
7. Tobin DJ, Fenton DA, Kendall MD. Cell degeneration in alopecia areata. An ultrastructural study. Am J Dermatopathol 1991;13:248-56.
8. Norris DA, Duke R, Whang K, Middleton M. Immunologic cytotoxicity in alopecia areata: apoptosis of dermal papilla cells in alopecia areata. J Invest Dermatol 1995;104(5 Suppl): 8S-9S.
9. Öğüş E, Yılmaz FM, Yılmaz H, Duranay M, Yücel D. Hemodiyaliz ve periton diyalizi hastalarında serum malondialdehit düzeyleri ve oksidasyona yatkınlık. Turkiye Klinikleri J Med Sci 2004;24:316-22.
10. Briganti S, Picardo M. Antioxidant activity, lipid peroxidation and skin diseases. What's new. J Eur Acad Dermatol Venereol 2003;17:663-9.
11. Turk HM, Sevinc A, Camci C, Cigli A, Buyukberber S, Savli H, et al. Plasma lipid peroxidation products and antioxidant enzyme activities in patients with type 2 diabetes mellitus. Acta Diabetol 2002;39:117-22.
12. Khan MF, Wu X, Ansari GA. Anti-malondialdehyde antibodies in MRL+/+ mice treated with trichloroethene and dichloroacetyl chloride: possible role of lipid peroxidation in autoimmunity. Toxicol Appl Pharmacol 2001;170:88-92.
13. Kannan K, Jain SK. Oxidative stress and apoptosis. Pathophysiology 2000;7:153-163.
14. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:497-500.
15. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158-69.
16. Hunter MI, Nlemadim BC, Davidson DL. Lipid peroxidation products and antioxidant proteins in plasma and cerebrospinal fluid from multiple sclerosis patients. Neurochem Res 1985;10:1645-52.
17. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem1978;86:271-8.
18. Gunduz K, Ozturk G, Sozmen EY. Erythrocyte superoxide dismutase, catalase activities and plasma nitrite and nitrate levels in patients with Behçet disease and recurrent aphthous stomatitis. Clin Exp Dermatol 2004;29:176-9.
19. Köse K, Yazici C, Cambay N, Aşcioğlu O, Doğan P. Lipid peroxidation and erythrocyte antioxidant enzymes in patients with Behçet's disease. Tohoku J Exp Med 2002;197:9-16.
20. Oztas MO, Balk M, Ogüs E, Bozkurt M, Ogüs IH, Ozer N. The role of free oxygen radicals in the aetiopathogenesis of rosacea. Clin Exp Dermatol 2003;28:188-92.
21. Yildirim M, Baysal V, Inaloz HS, Kesici D, Delibas N. The role of oxidants and antioxidants in generalized vitiligo. J Dermatol 2003;30:104-8.
22. Koca R, Armutcu F, Altinyazar HC, Gürel A. Oxidant-antioxidant enzymes and lipid peroxidation in generalized vitiligo. Clin Exp Dermatol 2004;29:406-9.
23. Basak PY, Gultekin F, Kilinc I. The role of the antioxidative defense system in papulopustular acne. J Dermatol 2001;28:123-7.
24. Boisseau-Garsaud AM, Garsaud P, Lejoly-Boisseau H, Robert M, Quist D, Arveiler B. Increase in total blood antioxidant status and selenium levels in black patients with active vitiligo. Int J Dermatol 2002;41:640-2.
25. Kökçam I, Naziroğlu M. Antioxidants and lipid peroxidation status in the blood of patients with psoriasis. Clin Chim Acta 1999;289:23-31.