

## Evaluation of oxidative stress indicators in lymphocytes of patients with alopecia areata

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### الخلاصة

داء الثعلبية هو تساقط الشعر على شكل بقع دائرية ملساء ومن الممكن أن تكون غير ملحوظة . يعتقد إن داء الثعلبية هو من الأمراض المناعية التي تشمل تجمع الخلايا المناعية حول بصيلة الشعر وقد اظهرت الدراسات الحديثة احتمالية وجود دور الجذور الأوكسجينية الحرة في أمراض داء الثعلبية الجزئي . لذا صممت هذه الدراسة السريرية لتقييم الإجهاد التأكسدي في الخلايا اللغفاوية وتقييم مانعات الأوكسدة (فيتامين A ,E and C) حيث اظهرت نتائج هذه الدراسة ارتفاع ملحوظ في المستوى الأساسي للمالوندايلديهيد (كموشر زناخة الدهون) , زيادة في حساسية الخلايا للتأكسد باستخدام بيروكسيد الهيدروجين المحدث في المختبر , قلة ملحوظة في مستوى الكلوتاتايون (مانع الأوكسدة الرئيسي) ومانع الأوكسدة الكلي في الخلايا اللغفاوية لمرضى داء الثعلبية الجزئي . وقد أدى شهرين من العلاج باستخدام مانعات الأوكسدة [فيتامين A (5000 وحدة دولية / يوم) , فيتامين E (400 ملغم / يوم) وفيتامين C (500 ملغم / يوم) ] إلى إرجاع المالوندايلديهيد إلى مستواه الطبيعي ورفع محتوى الخلايا من الكلوتاتايون ومانع الأوكسدة الكلي بصورة ملحوظة بالإضافة إلى قلة تحسس الخلايا للتأكسد المختبري باستخدام بيروكسيد الهيدروجين . وتقتصر الدراسة الحالية أن إعطاء المريض مانعات الأوكسدة تأثير إيجابي مباشر وغير مباشر على الجهاز المناعي حيث إن التأثير المباشر ربما يكون من خلال تنشيط الجهاز المناعي أو التأثير الغير مباشر من خلال إخماد التأثير الهدام للجذور الحرة المتكونة من عملية البلعمة للجهاز المناعي من خلال إمداد الخلايا المناعية بالكلوتاتايون الذي يقوي الجهاز المناعي و بالتالي يزيد من سرعة نمو الشعر .

### ABSTRACT

Alopecia areata is a nonscarring hair-loss condition that often has a patchy distribution and can be quite unpredictable. Its is believed to be an autoimmune disease involving T lymphocytic infiltrate around the hair follicle. Recent study reported a potential role of oxygen free radicals (OFR) in pathogenesis of alopecia. This clinical study was designed for further evaluation of oxidative stress in lymphocytes and evaluate the antioxidant effect of nutrient antioxidants (vitamin A,E&C). This study revealed a significant increase in basal malondialdehyde (MDA) level and increase of lymphocyte susceptibility to invitro challenge using hydrogen peroxide. Also there is significant decrease in lymphocyte glutathione content (GSH) and total antioxidant status (TAS) in patients with alopecia areata. Two month treatment with nutrient antioxidant combination [vitamin A (5000I.U./day); vitamin E (400mg/day) and vitamin C (500mg/day)] normalized MDA levels , decreased susceptibility to in vitro challenge with hydrogen peroxide , increased GSH content and TAS level in lymphocytes of patients with alopecia areata. The present study suggests that supplementation with nutrient antioxidant have direct and indirect positive effect on immune system. The direct effect may be through the antioxidant immunoenhancing / immunostimulant effect of vitamins on immune system and the indirect effect by counteracting damaging effects of oxygen free radicals (OFR) and utilization of GSH in neutralizing phagocytes-induced free radicals. So replenishment of GSH within natural killer (immune) cells strengthens the immune system and increases the rate of hair growth.

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## INTRODUCTION:

Alopecia areata is an auto-immune, non-scarring, multifocal disorder of hair growth characterized by circular bald areas, which occur on any hair bearing site of the body<sup>(1-5)</sup>.

The exact cause of alopecia areata is unknown. A positive family history is present in 10-30% of patients and it appears to be inherited as an autosomal dominant trait with variable penetrance, although a polygenic inheritance is not excluded<sup>(1-5)</sup>.

Since the exact cause of alopecia areata is unknown till now, the management of patients is obviously not restricted to the prescription of a treatment inducing hair growth<sup>(6)</sup>. It requires thorough exploration (history of hair loss, treatments and concomitant pathologies), detailed clinical examination of the integument and palpation of the thyroid.

Al-Jaff et.al<sup>(7)</sup>, reported a significant increase in basal malondialdehyde (MDA) levels, as a biomarker of lipid peroxidation, and a significant decrease in glutathione (GSH) level, as a major antioxidant, in erythrocytes of alopecic patients compared to their normal control, suggesting the role of oxidative stress in pathogenesis of alopecia.

This clinical study was aimed for further evaluation of oxidative stress in lymphocyte tissue of patients with alopecia and evaluate the antioxidant effect of nutrient antioxidant vitamins (A,E and C).

## SUBJECTS AND METHODS:

A-Control group: comprised of 20 normal subjects (mean age  $28.1 \pm 8.01$  years). They were non-smokers, non-alcoholics and free from any apparent disease.

B-Patient group: twenty one patients (mean age  $29.33 \pm 8.15$  years), 4 females and 17 males) with alopecia were included in this study, they were non-smokers, non-alcoholics and free from apparent other diseases. Patients involved in this study were under a dermatologist supervision. The duration of disease ranged from (1 month - 18 years).

Treatment schedules included a combination of antioxidants [vitamin A (5000I.U./day), vitamin E (400mg/day) and vitamin C (500mg/day)] with traditional treatments which includes [corticosteroids, diuretics (spironolactone), local irritant, phototherapy and tonics]. The treatment with nutrient antioxidants for alopecic patients included in this study continued for two months.

C-Samples: heparinized venous blood samples were collected from alopecic patients as well as from controls using plastic disposable syringes. Fresh blood samples were used for MDA and GSH measurements in lymphocytes and RBC. Human lymphocytes were separated from fresh blood according to boyum method using lymphoprep solution<sup>(8)</sup> and frozen for up to 14 days for total antioxidant status (TAS) measurement using commercial assay kit obtained from Randox<sup>(9)</sup>.

Measurement of lymphocytes MDA (which is a by product of lipid peroxidation), was carried out using the modified method of Stocks and Dormandy<sup>(10)</sup> as described by Gilbert et al<sup>(11)</sup>. The results were expressed as OD/mg protein. Lymphocytes GSH content was determined to the method of Godin et al.<sup>(12)</sup>. Amounts of GSH were expressed as OD/mg protein.

## RESULTS:

Baseline mean of lymphocytes MDA concentration in patients with alopecia was significantly higher than that of controls. Treatment for 2 months with antioxidants (vitamin A,C and E) normalized MDA levels in lymphocytes (Table 1).

Furthermore the baseline mean lymphocytes GSH and TAS levels were significantly lower in patients with alopecia than those of controls. Treatment with antioxidant combination did significantly elevate GSH content and TAS levels in patients with alopecia after one and two month of treatment (Table 1).

**Table 1 . Markers of oxidative stress in the lymphocytes of patients with alopecia areata and their aged matched controls**

	Control N= 20	Patients with alopecia areata N= 21		
		Pretreatment	Treatment period in months	
			1	2
MDA ( OD/ mg)	0.042 ±0.008	0.152± 0.031*	0.108±0.038*†	0.046±0.017†
GSH ( OD mg)	1.621 ± 0.197	0.791 ± 0.126*	1.066 ± 0.148*†	1.301± 0.152†
TAS (mMol/mg)	1.175 ± 0.424	0.478±0.113*	0.916±0.198†	1.367±0.281†

Values are expressed as mean ± standard deviation.

\* Significantly different from control (P< 0.05).

† Significantly different from pretreatment (P< 0.05).

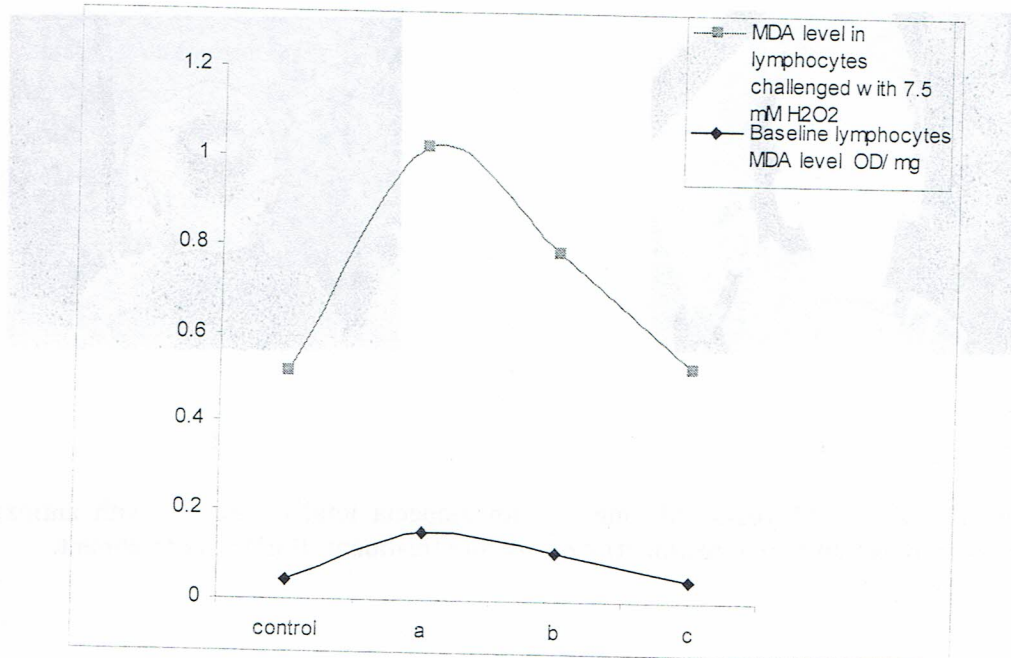
MDA: Malondialdehyde.

GSH: Glutathione.

TAS: Total antioxidant status.

RBC: Erythrocytes

The susceptibility of lymphocytes to in – vitro challenge of 7.5 mMol of H<sub>2</sub>O<sub>2</sub> was studied in alopecic patients and their aged – matched controls. The response of lymphocytes MDA concentrations was noticeably higher among the pretreated patients compared to normal controls with increased oxidant challenge, (figure 1).



**FIG 1 . SUSCEPTIBILITY OF LYMPHOCYTES FROM CONTROLS, PRETREATED AND ANTIOXIDANT TREATED PATIENTS TO IN- VITRO CHALLENGE WITH 7.5 MMOL H<sub>2</sub>O<sub>2</sub>**

The response of lymphocytes MDA levels in patients treated with antioxidant, in general, was obviously lower than that of pretreatment level after one and two months of treatment. Clinically, there was an obvious improvement in rate of hair growth after 1 and 2 months of treatment with antioxidants and traditional treatment as shown in the following pictures.

A- Pretreated alopecic patients

B- Pretreated alopecic patients after one month treatments with nutrient antioxidants

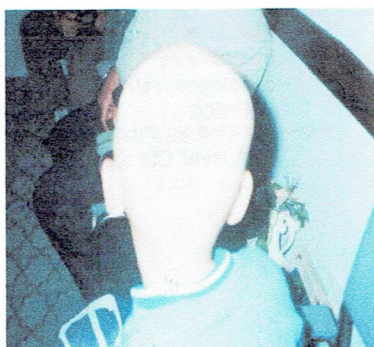
C- Pretreated alopecic patients after two month treatments with nutrient antioxidants



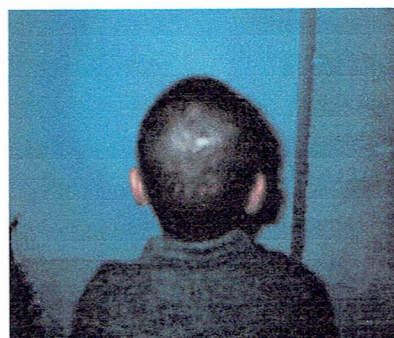
A



B

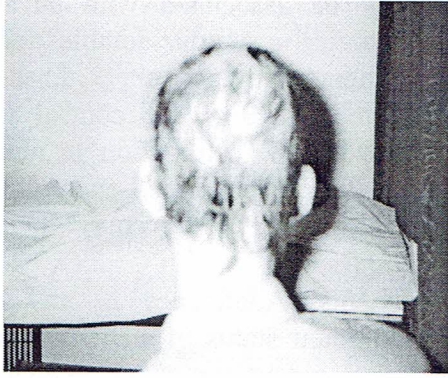


C



D

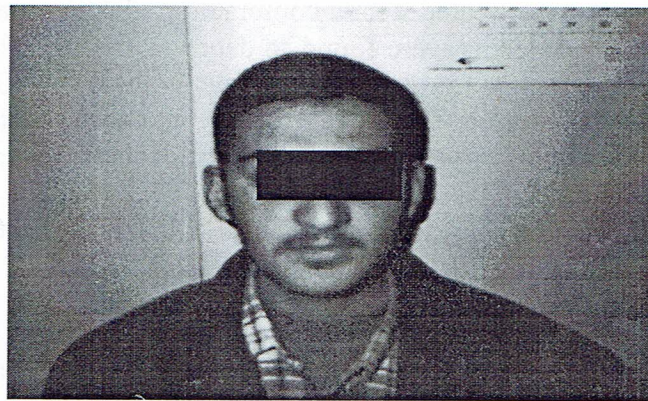
Patient (1) : Z.S a 17 years old male with alopecia totalis (treated with antioxidant combination plus traditional treatment) A&C before treatment B&D after treatment.



(A)



(B)



(C)

Patient (2): R.M 27 years old male with alopecia areata treated with a combination of antioxidant plus traditional treatment ; A& B before treatment; C after treatment.

## DISCUSSION:

In our study, we found alterations in oxygen free radical scavenging process in lymphocytes of alopecic patients manifested by increases in both basal and  $H_2O_2$  – induced MDA levels, and decreases in lymphocytes GSH content and total antioxidant status. It seems that antioxidant remain high in normal individual, keeping lipid peroxidation under control. However, in alopecic patients total antioxidant status defenses are significantly lowered, thus exposing lymphocytes to the damaging effects of lipid peroxides.

The endogenous basal MDA levels in lymphocytes of alopecic patients were significantly higher than those of controls (Table-1). This may be due to the effect of alopecia, as a disease, where the already formed immune complex resulted in phagocytes – derived free radicals. As a consequence, lipid peroxidation occurred which was demonstrated by the significant increase in endogenous basal MDA levels. Lymphocytes from alopecic patient's also showed a higher MDA levels in response to exogenous  $H_2O_2$  challenge than controls, suggesting the presence of increased susceptibility to oxidative stress which, in turn, may indicate the presence of increased free radicals generation associated with the accelerated oxidation of lymphocytes membrane lipids.

GSH, a major scavenger of oxygen reactive intermediates, protect cells against the effect of free radical and related intermediates (e.g. peroxides) that are formed endogenously<sup>(15)</sup>. Therefore, a reduction in cellular GSH content may be associated with an increased susceptibility to oxidant stress or reflect a response to it<sup>(16)</sup>. A considerable decrease in lymphocytes GSH level has already been reported in patients with alopecia<sup>(17)</sup>. In the present study, the total GSH content in patients decreased to 48.8% of the control values in lymphocytes (Table-1). This may be due to the increased GSH utilization in neutralizing phagocytes – derived free radicals which can damage both the source cell and cells in close apposition to stimulated phagocytes. These changes in GSH level may increase the susceptibility of lymphocytes to endogenous oxidative stress.

The antioxidant defence system has many components. A deficiency in any of these components can cause a reduction in the overall antioxidant status of an individual. Total antioxidant status enables assessment of the integrated antioxidant system which encompasses all biological components with antioxidant activity. Reduction in total antioxidant status has been implicated in several disease states, such as cancer and heart disease and patients with poor nutritional status<sup>(13,14)</sup>.

In this study alopecic patients were treated with a combination of antioxidants which include, vitamin A, C and E. These antioxidants were found to decrease the basal level of MDA, and the susceptibility of lymphocytes to oxidative challenge with H<sub>2</sub>O<sub>2</sub>. Optimum effect of treatment was obtained after 2 months. This could be due to the fact that supplementation with vitamin A, C and E may elevate these antioxidants to levels that successfully attenuate the increased oxidative insult.

The antioxidant treatment led to increase lymphocytes GSH content, and total antioxidant status. This may be due to a direct and/or an indirect scavenging activity of these antioxidants, a fact that may in turn lead to inhibition the oxidation of protein and a decrease in the utilization and damage of GSH and proteins by free radicals.

The most important point is the clinical significance of antioxidants in improving the hair growth response of alopecic patients. This may be due to direct and/or indirect scavenging activity of these antioxidants, possibly by decreasing the damage, and utilization of GSH in neutralizing phagocytes induced free radicals. So, replenishment of GSH within natural killer (immune) cells strengthens the immune system and increases the rate of hair growth.

The combination of therapy affects positively the immune system. The traditional treatment (especially prednisolone) affects the immune system by decreasing the immune complex deposition, while the nutrient antioxidants act by inhibiting the negative effect of ROS on immune system thus increasing rate of hair growth. In one study, 30 elderly patients were supplemented with vitamins A, C, and E or placebo. Following 28 days of supplementation with these vitamins, there was a distinct improvement in cell-mediated immune function. In particular, the number of T cells, T4 cells and the T4:T8 ratio increased significantly. In contrast, no significant change was noted in the immune function of the placebo group". It also showed that a combination of nutrients may be synergistic even though most published studies focus only on a single nutrient, hormone or drug to boost immunity.

In conclusion, the protective effect of vitamins combinations on tissue antioxidant status, in addition to the clinical improvement in the rate of hair growth, suggest the use of antioxidants plus traditional treatment for the management of alopecic patients.

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