The Ability of Nutrient Antioxidants to Influence Oxidative Stress and Lower the Dose of Prednisolone in Patients with Alopecia Areata

AshwaqN. Al- Jaff * , Salim A.Humadi * , Saleh.A. Wohaieb**

Received 9-2-2003 Accepted 14-12-2003

ABSTRACT

Alopecia areata is a common disorder, hypothesized to be autoimmune in etiology. Cortisone taken orally may stimulate new hair growth. Prednisone (orally administered steroid) has proved effective for patients with alopecia areata, but its potential side effects include weight gain, metabolic abnormalities, acne and menstrual problems.

This clinical study was designed to assess the clinical significance of the nutrient antioxidants (vitamin A, vitamin E and vitamin C) in reducing the dose of corticosteroids (prednisolone), and as a consequence, their side effects in patient with alopecia. The results of this study reveal the potential clinical significance of the therapy for two months with these antioxidants in reducing the dose of prednisolone from 100mg to 10 mg administered each other day and improving the rate of hair growth by attenuating free radicals damaging effect on immune system, thereby decreasing the immune complex deposition. According to the results of this study, the use of nutrient antioxidants may have an important role in protecting the immune system, and decreasing the dose and side effects that result from the use of high dose of corticosteroids.

الخلاصة

يعتبر داء الثعلبة من الأمراض الشائعة و التي يعزى أسبابه إلى الجهاز المناعي. يؤدي الكورتيزون المعطى عن طريق الفم إلى تحفيز نمو الشعر حيث اثبت فعليا فعالية البردنزولون لمرضى داء الثعلبة الجزئي مع تأثيره الجانبي المؤثر والذي يتضمن زيادة الوزن ,اضطرابات في العمليات الأيضية ,انتشار الحبوب و اضطراب الدورة الشهرية.

صممت هذه الدراسة السريرية لتقيم الأهمية لسريرية للعلاج لمدة شهرين بمانعات الأكسدة فيتامين(أ,ه ,ج) في تقليل جرعة البردنزولون وبالتالي تقليل تأثيره الجانبي الناتج من الجرعة العالية المعطاة للمرضى المصابين بداء الثعلبة.

أضهرت نتائج هذه الدراسة الأهمية السريرية العالية لمانعات الأكسدة في تقليل جرعة البردنزولون من 100 ملغ إلى 10 ملغ بين يوم و آخر وتحسين سرعة نمو الشعر عن طريق إنهاء التأثير الهدام للجذور الحرة على الجهاز المناعي و بالتالي تقليل ترسب المعقدات المناعية. وتبعا لذلك فأن هذه الدراسة تبين أهمية دور مانعات الأكسدة في حماية الجهاز المناعي مما يؤدي إلى تقليل الحاجة إلى جرع عالية من الكورتيزونات (البردنزولون) و بالتالي تقليل التأثير الجانبي الناتج من الجرع العالية.

INTRODUCTION

Alopecia areata is a common, unpredictable, non-scarring form of hair loss [1,2,3,4]. This disorder affects all age groups , with a higher prevalence in children and adolescents [4]. The cause is unknown but it is associated with an alteration in the immunological system [5,6]. Current treatment is not, at this point , directed at the etiology of alopecia areata but rather at the resulting inflammatory infiltrate and (presumably) the growth inhibitory factors produced by this response [5,6]

The use of nutrient antioxidants in alopecic patients revealed a significant decrease in basal and H2O2 induced MDA (biomarker of oxidative stress) level in RBC and plasma, increase glutathione level (major antioxidant) in both RBC and plasma, increase total protein and finally increase catalase activity. These effects suggest the important role of nutrient antioxidants in protecting the body(immune system from the oxidative damage produced by the disease) and may influence the severity of the disease[7]. Research has shown that the disease responds to a variety of immunomodulating treatments , that patients with alopecia areata may have a higher incidence of circulating antibodies against other body organs or tissues , and that family members have a higher incidence of autoimmune disease [3, 5, 6].

Departments of clinical pharmacy, College of pharmacy, university of Baghdad, Baghdad- Iraq
Department of Pharmcology and Toxicology,College of Pharmacy, University of Baghdad, Baghdad – Iraq .

Research has shown that the disease responds to a variety of immunomodulating treatments , that patients with alopecia areata may have a higher incidence of circulating antibodies against other body organs or tissues , and that family members have a higher incidence of autoimmune disease [3, 5, 6].

Systemic steroids are reserved for use in rapidly progressive or extensive alopecia areata [8,9,10]. Systemic steroids, particularly a short course (4-8 weeks) of tapering doses, are often used either alone or in combination with topical agents. A high dose up to 100 mg prednisolone daily has been recommended. In this setting acne and weight gain are commonly seen side effects [11]. Prednisolone doses as low as 20 mg per day may be associated with septic necrosis of the hip or severe gastrointestinal bleeding [12,13].

This study was designed to investigate the role of nutrient antioxidants (A, E, &C) in reducing the dose of prednisolone and as a consequence their side effects in patient with alopecia.

SUBJECTS and METHODS

1- Subjects

A-Study Group: comprised of total of 84subjects, 30 normal controls (mean age 25.97± 8.09 years) and 54 cases with alopecia (mean age 25.20 ±7.05 years). Patients involved in this study were under a dermatologist supervision who determined the severity of the disease according to number of the patches they have, and according to progression of disease [2] they were non-smokers, non-alcoholics and free from apparent other diseases. The duration of disease ranged from (20 day- 18 years).

B-Patients: Fifty-four patients aged 10-40 years (26 females, 28 males) with alopecia(with no previous treatment) were included in this study. [Twenty seven of them received corticosteroids (100 mg prednisolone) each other day, and the other twenty seven receive (10 mg prenisolone) each other day]. Treatment schedules also included a combination of antioxidants [vitamin A (5000 I.U./day), vitamin E (100 mg/day) and vitamin C (500 mg/day)] given to both groups. 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 sample were used for MDA and GSH measurments.

2-Methods

- **Erythrocytes Malondialdehyde (MDA) Assay:**

Measurements of erythrocyte and plasma MDA (which is a by product of lipid peroxidation), based on the reaction of thiobarbituric acid (TBA) forming TBA-MDA adduct, were carried out using the modified method of Stocks and Dormandy[14] as described by Gilbert et al[15]. The results were expressed as nmole/g Hb and µmol/ L plasma based on the molar extinction coefficient of 10^5 $M^{-1}.CM^{-1}$. × MDA is 1.56

- **Glutatione Assay:**

Erythrocytes and plasma GSH contents were determined according to the method of Godin et al.(16). Known amounts of GSH were assayed by the same method and used for calculation of GSH quantities in erythrocytes. The statistical significance of the difference in mean was tested by student t-test.

RESULTS

A- Basal plasma and erythrocyte MDA levels in both groups of patients were significantly higher than those in controls. Treatment with either 10 or 100 mg prednisolone plus antioxidants normalized MDA levels in both plasma (Table 1) and erythrocytes (Table 2) as early as 1 month after treatment.

Furthermore, total plasma GSH content was significantly higher than controls (Table 3), and treatment of patients with both doses of prednisolone plus antioxidants slightly increased GSH patients, but did not normalize these values. On the other hand, erythrocyte GSH content was significantly lower in patients compared to controls, and that treatment with both doses of prednisolone plus antioxidants did significantly elevate GSH content in patients after 1 month of treatment, and normalized these values after 2 month's of treatment (Table 4).

B- Clinically there is lower incidence of prednisolone side effects(acne and weight gain) among those patients taking prednisolone dose 10 mg each other day than those taking 100 mg each other day (Table 5 and 6).

Table (1): Effect of the addition of nutrient antioxidants (A, E, &C) to prednisolone therapy (10& 100 mg) on plasma MDA levels in patients with alopecia areata.

Group	MDA (μ mole /L)			
	Control N=30	Patients with alopecia		
		Pre-treatment N=27	*Months after treatment*	
			1 N=27	2 N=27
I- Antioxidants + 10 mg prednisolone	0.72±0.30	3.17±1.67*	0.87± 0.45†	0.66±0.27†
II- Antioxidants + 100mg prednisolone	0.72±0.30	2.73±1.66*	0.86± 0.46†	0.67±0.26†

Values are expressed as means ± SD.
*** Significantly different from control (p< 0.05).**
† Significantly different from pretreatment values (p<0.05).
N Number of subjects

Table (2): Effect of the addition of nutrient antioxidants (A, E, &C) to prednisolone therapy (10& 100 mg) on erythrocytes MDA levels in patients with alopecia areata.

Group	MDA (n mole /g Hb)			
	Control N=30	Patients with alopecia		
		Pre-treatment N=27	*Months after treatment*	
			1 N=27	2 N=27
I- Antioxidants + 10 mg prednisolone	5.98±1.04	28.38±18.60*	6.25±2.95†	5.44±2.48†
II- Antioxidants + 100mg prednisolone	5.98±1.04	28.40±18.84*	6.27±3.09†	5.46±2.60†

Values are expressed as means ± SD.
*** Significantly different from control (p< 0.05).**
† Significantly different from pretreatment values (p<0.05).
N Number of subjects

Table (3): Effect of the addition of nutrient antioxidants (A, E, &C) to prednisolone therapy (10& 100 mg) on plasma glutathione levels in patients with alopecia areata.

Group	GSH (μ mol/L)			
	Control N=30	Patients with alopecia		
		Pre-treatment N=27	*Months after treatment*	
			1 N=27	2 N=27
I- Antioxidants + 10 mg prednisolone	0.90±0.20	1.21±0.35*	1.27±0.55*	1.54±0.77*
II- Antioxidants + 100 mg prednisolone	0.90±0.20	1.23±0.38*	1.27±0.57*	1.58±0.79*

Values are expressed as means ± SD.
* Significantly different from control ($p < 0.05$).
N Number of subjects

Table (4): Effect of the addition of nutrient antioxidants (A, E, andC) to prednisolone therapy (10and 100 mg) on erythrocytes glutathione levels in patients with alopecia areata

Group	GSH (μ mole/gm Hb.)			
	Control N=30	Patients with alopecia		
		Pre-treatment N=27	*Months after treatment*	
			1 N=27	2 N=27
I- Antioxidants + 10 mg prednisolone	6.53±0.83	3.95±1.32*	5.43±1.39*†	5.98±1.25†
II- Antioxidants + 100 mg prednisolone	6.53±0.83	4.06±1.39*	5.44 ±1.42*†	5.99 ±1.29†

Values are expressed as means ± SD.
* Significantly different from control ($p < 0.01$).
† Significantly different from pretreatment values ($p<0.01$).
N Number of subjects

Table (5): body weight of control and age matched alopecic patients..

Group	Body weight (Kg)			
	Control N=30	Patients with alopecia		
		Pre-treatment N=27	*Months after treatment*	
			1 N=27	2 N=27
I- Antioxidants + 10 mg prednisolone	69.69 ±10.79	66.09±12.00	68.88±12.27	71.22±12.63
II- Antioxidants + 100 mg prednisolone	69.69 ±10.79	66.79±12.76	72.56±12.62	80.88±12.23

Values are expressed as means ± SD.
N Number of subjects

Table (6): severity of acne appearance in control and age matched alopecic patients.

Group	Presence of acne			
	Control N=30	Patients with alopecia		
		Pre-treatment N=27	*Months after treatment*	
			1 N=27	2 N=27
I- Antioxidants + 10 mg prednisolone	negative	negative	+	+
II- Antioxidants + 100 mg prednisolone	negative	negative	++	++++

Severity of the presence of acne determined by dermalogists.
N Number of subjects

DISCUSION

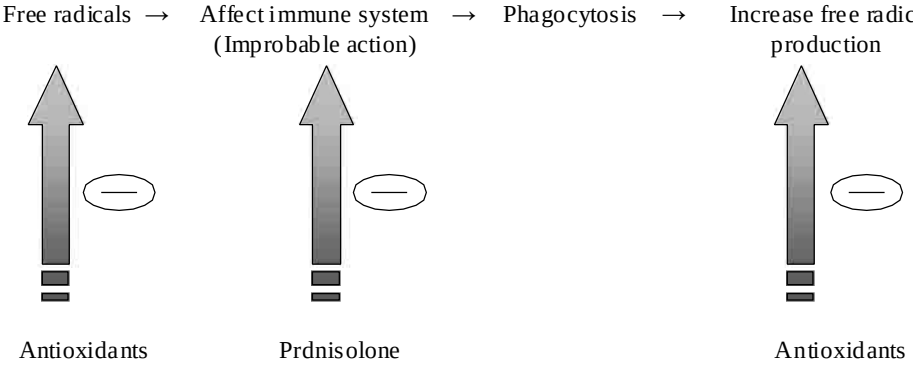
Corticosteroids are part of the treatment of many disorders in which inflammation is thought to be caused by excessive or inappropriate activity of the immune system like in Alopecia areata [17, 18, 19, 20, 21]. Given in high doses, corticosteroid drugs reduce inflammation by blocking the action of prostaglandins responsible for triggering the inflammatory response [16]. They also temporarily depress the immune system by reducing the activity of certain types of white blood cells. The extent of hair loss and the age of the patient are used to select an appropriate treatment for patients with alopecia areata [3]. For those with more than fifty percent scalp hair loss one may consider the use of systemic corticosteroids but the concern about long-term use and side effects of systemic corticosteroids must be taken into consideration.

The present study revealed the presence of endogenous oxidative stress in both groups of patients, as manifested by the increased MDA

levels and decreased GSH contents in erythrocytes.

This oxidative stress may result from phagocytes derived free radicals and the associated lipid peroxidation [22]. Data of the present study also indicated that, despite the difference in oral prednisolone dose (10 vs. 100 mg) between the two groups, addition of nutrient antioxidants to prednisolone therapy resulted in comparable and significant decrease in MDA levels and correction of GSH content in blood, as well as similar improvement in the rate of hair growth with less side effect (acne, weight gain and gastrointestinal disturbances) regardless the dose of prednisolone. Previous study in our lab showed that, without antioxidant therapy, the effect of 100 mg prednisolone was more effective than lower doses of prednisolone in improving hair growth in alopecic areata patients [7].

Therefore, the addition of nutrient antioxidants to corticosteroids attenuated the negative effects of oxidative stress on immune system and decreased the need for high dose of corticosteroid; thereby decreased the unwanted side effects associated with the prolonged use of high doses.



REFRENCES:

1. A.J.Papadopoulos, R.A. Schwartz and Krysicka Janniger.alopecia areata: emerging oncepts .dermatovenerologica.2000, vol 9, number 3.
2. Sharma VK, Dawn G, Kumar B. Profile of alopecia areata in northern India. Intern J Dermatol 1996; 35: 22-7.
3. Sharma VK, Kumar B, Dawn G. A clinical study of childhood alopecia areata in Chandigarh, India. Ped Dermatol 1996; 13: 372-7.
4. Safavi KH, Muller SA, Suman VJ, et al. Incidence of alopecia areata in Olmstead County, Minnesota, 1975 through 1989. Mayo Clin Proc 1995; 70: 628-33.
5. Coskey RJ, Drake LA, Hordinsky MK, Rosenberg EW, Solomon AR, Chancho Turner ML. Guidelines of care for alopecia areata. JAAD 1992; 26:247-50.
6. Shapiro J. Alopecia areata: update on therapy. Dermatologic Clinics 1993; 11:35-46.
7. AL-Jaff A.The role of oxidative stress in alopecia areata. MSc.thesis.college of pharmacy university of Baghdad.2001.
8. Shapiro J. Practical management for alopecia areata and outlook for the future.Exp-Dermatol.1999 Aug; 8(4): 298.
9. Dolecek-R. Treatment of alopecia with glucocorticoids and other hormonal preparations.parkt-Lek; 1969; 49(11); 423-431.
10. Sharma-VK; Muralidhar-S. Treatment of widespread alopecia areata in young patients with monthly oral cortico steroid pluse.Pediatr-Dermatol.1998 Jul-Aug; 15(4):313-7.
11. Winter-RJ; Kern-F; Blizzard-EM. Prednisolone therapy for alopecia areata. Arch-Dermatol. 1976; 22: 1549-92.

12. Fiedler VC. Alopecia areata. A review of therapy, efficacy, safety, and mechanism. Arch Dermatol 1992; 128: 1519-29.

13. Fiedler VC. Alopecia areata. A review of therapy, efficacy, safety, and mechanism. Arch Dermatol 1993 Jul; 129(7): 908-9.

14. Stocks-J and Dormandy-T.L. The autooxidation of human red blood cell lipids induced hydrogen peroxide. Brit-J-Haematol. 1971; 20:95-111.

15. Gilbert-H.S; Stump-D.D and Roth-E.F. A method to correct for errors caused by generation of interfering compounds during erythrocyte lipid peroxidation. Anal-Biochem.1984; 137:282-286.

16. Godin-D.V; Wohaieb-S.A; Garnett-M.E and Goumeniouk-A.D. Antioxidant enzyme alterations in experimental and clinical diabetes. Mole. And Cellular-Bioch. 1988; 84:223-231.

17. The british medical association new guide to medicines and drugs, 1995.

18. Price VH. Treatment of hair loss. N Engl J Med 1999; 341: 964-73.

19. Schwartz RA, Janniger CK. Alopecia areata. Cutis 1997; 59: 238-41.

20. Shapiro J, Price VH. Hair regrowth. Therapeutic agents. Dermatol Clin 1998; 16: 341-56

21. Sharma-VK; Gupta-S. Twice daily dexamethasone oral pluse in the treatment of extensive alopecia areata. J-Dermatol. 1999 Sep; 26(9); 562-5.

22. AL- Jaff A., Hamadi S, Wohaieb S. The role of oxidative stress in alopecia areata. Iraqi journal of pharmacy.2001; vol 1, No 1:34-45.