

Serum Copper, Zinc and Oxidative Stress in Patients with Psoriasis

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Abstract

Background Psoriasis is a chronic inflammatory skin disease characterized by well-demarcated erythema and scaly plaques. The pathogenesis of psoriasis still remains unclear. An increased reactive oxygen species (ROS) and insufficient antioxidant activity associated with the pathogenesis of psoriasis lesions.

Objective To evaluate the link between oxidative stress parameters and some trace elements like zinc (Zn) and copper (Cu) ions with the pathogenesis of psoriasis disease.

Method Fifty patients with psoriasis were included in this study, 32 patients with localized psoriasis, and 18 with general psoriasis, another fifty healthy controls were included in this study. We measured serum malondialdehyde (MDA), super oxide dismutase (SOD), vitamins E and A, and Zn and Cu in patients and control subjects.

Results Serum MDA in total psoriasis patients (1.8 ± 0.2 nmol/ml) was significantly higher than those of control (0.6 ± 0.19 nmol/ml; $p < 0.001$). The SOD activity (9.1 ± 1.0 U/ml) in serum of total psoriasis patients was significantly lower than that of controls (10.8 ± 0.3 U/ml). serum vitamin A and E patients (56.8 ± 3 , 9.0 ± 1.0 µg/ml respectively) were significantly lower than control (59.0 ± 1.3 , 9.7 ± 0.9 µg/ml correspondingly $p < 0.0001$), for trace elements the level of Zn in serum patients (79.0 ± 9.1 µg/ml) was significantly lower than control (83.3 ± 5.8 µg/ml), while for Cu statistically significant higher levels were noted in patients (111.8 ± 14.2 µg/ml) as compared with control (106.0 ± 9.0 µg/ml).

Conclusion Our results suggest that lipid peroxidation of cellular membrane of keratiocytes by free radicals and decreased antioxidants may associate the pathogenesis of psoriasis lesion. In addition, there was a possible benefit of an enriched diet or of a supplement of vitamins A, E and Zn in treatment of psoriasis diseases.

Key words Psoriasis, Zinc, Copper, Oxidative Stress, malondialdehyde, super oxide dismutase

Introduction

Psoriasis is a common inflammatory and proliferative skin disease of unclear etiology and is known to affect about 2–4.8% of the global population⁽¹⁾. Typical psoriatic lesions are erythematous papules which form plaques characterized by sharp borders and increased scaling⁽²⁾. Recently, oxidative stress has been implicated in the etiopathology of

psoriasis^(3, 5). Significant abnormalities of antioxidant mechanisms have been demonstrated in the blood and plaques of psoriatic patients⁽⁶⁾. An insufficient antioxidant system, together with increased levels of reactive oxygen species (ROS) has been suggested to be important in the pathogenesis of this disease⁽²⁾.

ROS can react with all macromolecules, such as lipids, proteins, nucleic acids, and carbohydrates, particularly polyunsaturated fatty acids on cell membrane. After the beginning of an initial reaction with ROS, a continuing chain reaction is started and cell injury and ultimately cell death occurs⁽⁷⁾.

Oxidative stress can result from deficiency of trace elements such as zinc Zn, copper Cu and selenium Se.

Trace elements and their compounds have been used since ancient times for their therapeutic as well as cosmetic effects on the skin^(8,9). The unique process of keratinization and melanin formation is enzyme-dependent and therefore could be influenced by trace element deficiencies or excesses as trace elements are involved in enzymatic activities and immunologic reactions⁽¹⁰⁾. Studies have also shown that essential trace elements like iron (Fe), copper (Cu), chromium (Cr), and vanadium (V) undergo redox cycling and have physiological significance, while nonessential toxic elements like cadmium (Cd), mercury (Hg), nickel (Ni) and lead (Pb), deplete glutathione and protein-bound sulfhydryl groups, resulting in the production of reactive oxygen species (ROS) like superoxide ion, hydrogen peroxide, and hydroxyl radical⁽¹¹⁾.

There is no comprehensive study on the levels of trace elements, oxidants and antioxidants defence mechanism and their inter-element relationships in psoriasis. In the present study, we have analyzed the levels of 2 elements in serum samples of localized and generalized psoriasis patients and also measured malondialdehyde (MDA) and the status of antioxidant enzymes such as superoxide dismutase (SOD), and, non-enzymatic antioxidants like vitamins E and A.

Methods

A case-control study was conducted in the department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University. It included 50 cases, 32 were with localized plaque psoriasis (LPP), and 18 patients with

generalized plaque psoriasis (GPP) who were attending the department of Dermatology, Al-Khadymia Teaching Hospital, and another 50 age- and sex-matched healthy individuals were included as controls.

Patients and controls with diabetes mellitus, thyroid disease, autoimmune disorder or concomitant dermatological diseases, or had taken systemic or topical treatment within three months before the present study, or had a history of smoking or alcoholism or taking drugs for any other reason or taking antioxidant or vitamins were excluded.

All blood specimens were taken after 10-12 hours fast in the morning between 9- 11 hours. The blood from forearm vein was collected in a plain tube and allowed to clot at room temperature for 30 minutes and centrifuged for 15 minutes at 3000rpm (755xg). The serum was divided into proper aliquots and frozen at -20°C until used for measuring of Zn, Cu, MDA, SOD, vitamins A, and E.

All the precautions were taken in accordance with the Clinical and Laboratory Standards Institute criteria⁽¹²⁾ to eliminate metal contamination while collecting and storing the samples

Serum Zn and Cu concentrations were estimated in the samples using flame atomic absorption spectrophotometry (AAS). The samples were diluted (1:10). Standards (prepared in deionized water) were run in the range of 10–40 µg/dl for Cu and Zn.

Serum MDA levels were determined by the method of Draper and Hadley⁽¹³⁾ based on the reaction of MDA with thiobarbituric acid (TBA) at 70°C. In the TBA test reaction, MDA and TBA react to form a pink colour with maximum absorption at 532 nm.

For SOD activity determination we used (RANSOD kit, from Randox. The principle of the determination of xanthine by xanthine oxidase, and reduction of iodophenyl-nitrophenol-phenyltetrazolium (I.N.T) by the H₂O₂ produced.

Serum vitamins A and E were evaluated by high performance liquid chromatography (HPLC), in

brief for vitamin A serum was first deproteinized by 15% 5-sulphosalicylic acid, mixed and centrifuged, the sample was diluted and analyzed by HPLC system using (column C-18). The mobile phase was acetonitrile (100%) at a flow rate of 1ml/min and wavelength of 290 nm, while for vitamin E the mobile phase used was absolute ethanol-water (95:5 v/v) at a flow rate of 1ml/min and the wavelength of 229 nm.

Statistical analysis

All data were given as mean ± standard deviation (SD). Statistica version-6 for windows

was used for statistical analysis. Levels of Zn, Cu, MDA, SOD, vitamin A and vitamin E in sera of patients and control subjects were compared by paired student’s *t*-test. The differences were considered to be significant when the *p* value was less than 0.05.

Results

The study included 50 patients with psoriasis (29 women and 21 men) of age varied from 17-53 years, and 53 controls (27 women and 23 men) with age of between 17-53 years (Table 1).

Table1: MDA, SOD, VE, VA, Zn, and Cu in total psoriasis patients and normal control subjects

	No.	AGE	MDA (nmol/ml)	SOD (U/ml)	VA (ug/ml)	VE (ug/ml)	Zn(ug/dl)	Cu (ug/dl)
Control	50	34.20±10.7	0.6±0.19	10.8±0.3	59.0±1.3	9.7±0.9	83.3±5.8	106.0±9.0
Psoriasis	50	33.8±10.8	1.8±0.2	9.1±1.0	56.8±3.0	9.0±1.0	79.0±9.1	111.8±14.2
P value		0.85	0.001	0.000	0.0000	0.000	0.006	0.01

Table 2 show the mean values of total lipid peroxidation (MDA), plasma antioxidants (SOD enzymes, Vit. A, and Vit. E), and the levels of trace elements (Zn and Cu), in both patients and controls.

When compared with the control group, total psoriasis patients showed significantly higher values for MDA (*p*<0.001), and significantly

lower values for SOD (*p*<0.0001), Vit. A (*p*<0.0001), and Vit. E (*p*<0.0001). Furthermore, trace elements Zn level show a significantly lower value (*p*<0.0001) in serum of psoriasis groups compared to the control, while for Cu, patients group showed significantly higher concentration (*p*<0.01) as compared with the control group.

Table2: MDA, SOD, VE, VA, Zn, and Cu in LP, and GP patients and normal control subjects.

	AGE	MDA (nmol/ml)	SOD (u/ml)	VA (µg/ml)	VE (µg/ml)	Zn (µg/dl)	Cu (µg/dl)
Control (n=50)	34.20±10.7	0.6±0.19	10.8±0.3	59.0±1.3	9.7±0.9	83.3±5.8	106.0±9.0
LPP (n=32)	34.7±11.3	1.8±0.1 ^{a***} P=0.00	9.0±1.0 ^{a***} P=0.000	56.5±2.8 ^{a***} P=0.000	9.1±1.1 ^{a**} P=0.01	78.1±8.6 ^{a**} P=0.001	110.8±14.6 ^a P=0.07
GPP (n=18)	32.3±9.9	1.8±0.2 ^{a**} P=0.00 ^b P=0.60	9.4±1.1 ^{a**} P=0.000 ^b P=0.15	57.2±3.3 ^{a**} P=0.00 ^b P=0.46	8.7±0.9 ^{a***} P=0.000 ^b P=0.25	80.6±10.0 ^a P=0.17 ^b P=0.36	113.6±13.7 ^{a***} P=0.01 ^b P=0.51

LPP = localized plaque psoriasis, GPP = generalized plaque psoriasis

^at-test: comparison of the LP, GP groups with control

^bt-test: comparison of the LP patients with GP groups.

We have also compared the values according to the type of psoriasis (LPP and GPP); we found no significant differences for all values studied.

Discussion

Oxidative stress is now considered to be important in the pathogenesis of psoriasis^(4,6). Under normal physiological conditions, free radical-induced oxidative stress is combated by a complex antioxidant defence system. In humans there are two main defence systems against oxidative stress, the first involves mineral-dependent enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), the second defence system consists of non-enzymatic substances. Albumin, uric acid, and ascorbic acid account for over 85% of the total antioxidant capacity in human plasma⁽¹⁴⁾. This predominance is due largely to their high concentrations relative to those of other antioxidants in blood, e.g. α -tocopherol, β -carotene, and bilirubin. Although individual antioxidants play a specific role in the antioxidant defence system, the above antioxidant molecules may act cooperatively *in vivo* to provide synergistic protection against oxidative damage⁽¹⁵⁾. Measuring the levels of specific antioxidant molecules can yield valuable information, and low levels of such antioxidants provide suggestive, but not definitive evidence of oxidative stress.

The oxidative destruction of polyunsaturated fatty acids (PUFAs) of phospholipids, known as lipid peroxidation, can be in fact considered as a hallmark of oxidative stress. MDA an end product of lipid peroxidation induced by ROS is well correlated with the degree of lipid peroxidation⁽¹⁶⁾. There are different reports about MDA level in the literature. Yildirim et al⁽⁴⁾ found no significant difference in the serum MDA levels between controls and psoriasis groups, while they found statistically significant increased tissue levels of MDA in psoriasis group, but Petronila et al⁽¹⁷⁾ reported that the level of thiobarbituric acid (TBA) in plasma was significantly higher in patients with psoriasis

than in controls, also Arpita et al⁽¹⁸⁾ found that the MDA levels were significantly higher in psoriasis patients as compared with normal volunteers; furthermore Vijaykumar et al⁽¹⁹⁾ found a positive correlation between increased MDA, and NO in sera of psoriasis patients with severity of psoriasis. In our study, the significantly higher levels of serum MDA support previous finding and indicate that lipid peroxidation may have a role in the pathogenesis of psoriasis.

Superoxide dismutase (SOD) is a group of metalloenzymes that protects cells from the toxic effects of superoxide radicals are produced as endogen. We found significantly lower levels of serum SOD activity in patients with psoriasis compared to healthy controls and this is in agreement with Yildirim et al⁽⁴⁾ They found significant decreased levels of erythrocyte SOD and GP activities were noted in psoriatic subjects, and with Vijaykumar et al⁽¹⁹⁾ their study showed that the serum SOD activity was observed to be decreased significantly from mild to moderate and from moderate to severe psoriasis patients. Superoxide and hydroxyl radicals are the most important radicals in lipid peroxidation. Decreased SOD activity could be responsible for the increase of superoxide radicals, which may explain the increased level of MDA⁽⁶⁾.

There is an association between the antioxidant enzymes such as glutathione peroxidase and superoxide dismutase and trace elements including selenium, zinc, copper, and manganese⁽²⁰⁾. A deficit of those elements may result in the decrease of antioxidant enzyme activity and the increases of oxidative stress induce cell damage.

We measured copper and zinc in order to illuminate the possible role of trace metals in the pathogenesis of psoriasis. This approach appears reasonable because copper and zinc are known to be among the constituents of the skin and to play essential roles in maintenance of its function in association with the enzyme systems activated by trace metals⁽²¹⁾.

Essentiality of zinc is related mainly to its

function as the metal moiety of important enzymes. Zinc is considered as an antioxidant because the extracellular enzyme superoxide dismutase is zinc- dependent, it plays a vital role in the protection against free radical damage. Trace elements including zinc catalyze the rearrangement of dopachrome to form 5,6 - dihydroxy indole – 2 carboxylic acid (DICA) in the process of melanogenesis⁽²²⁾.

It was reported in literatures that both normal and psoriasis skin show no significant differences in Zn^(21, 23, 24) while Bhatnagar et al⁽²⁸⁾ found in their study on active and remissive phases of psoriasis an increased in serum Zn level, our results indicated that Zn concentrations in psoriasis groups show a decreasing trend and are consistent with other studies^(9, 25-27). A reduction in blood Zn concentration with increasing surface area involvement in psoriatic may be due to Zn depletion secondary to loss of Zn through exfoliation⁽²⁹⁾. An alternative possibility is that disturbance in the blood Zn status might actually be resulting in greater surface area involvement⁽⁹⁾.

Our results show increased concentration of serum Cu in psoriasis groups. This is in accordance with few studies where elevated levels of serum Cu have been reported in psoriasis and other skin diseases^(25, 26, 30). Increased Cu may be attributable to inflammation associated with the disease.

We also evaluated some natural liposoluble antioxidants vitamins, namely vitamins A and E. These vitamins, being supplied by diet, may allow the control of their plasma levels by an enriched diet or even by a therapeutic vitamin supplementation^(31, 32). Early studies on the relationship between a vitamin A and E deficient diet and disturbances in epidermal growth and differentiation suggest the importance of those compounds for the maintenance of skin homeostasis⁽²⁶⁾. It is known that moderate and severe inflammatory reactions lead to a decrease in the blood retinol and beta-carotene content, whereas lipid peroxidation increases in inflammatory

reactions⁽²⁰⁻³⁵⁾. In this study vitamin A and vitamin E were significantly lower in patients with psoriasis than in healthy controls and this is in agreement with Petronila et al⁽¹⁷⁾ they found decreased vitamins A and E levels in (inactive and active) psoriasis patients as compared with the control group. Our observed low vitamin A and E in the psoriasis patients might be explained by the inflammatory conditions since the Psoriasis is a chronic and recurrent inflammatory skin disease.

In conclusion, we observed that there is impairment in the antioxidant system in psoriasis, leading to free-radical mediated damage to skin cells. Our finding revealed that this oxidative stress is not a localized phenomenon but a generalized process and may be one of the reasons for the progressive nature of the disease. In view of these findings, the levels of MDA increased in patients with psoriasis by peroxidation whereas serum SOD and vitamins A and E and Zn element decreased. Antioxidant treatments such as vitamins A and E and Zn in patients with psoriasis may be of use in treatment of psoriasis in two respects: a- to decrease the inflammation in skin tissues by virtue of the inactivating effect of free radicals and b- to confirm stability on cell membranes by a positive effect on membrane stabilization and repair.

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