DOI: 10.7860/JCDR/2013/6635.3732

Original Article

Biochemistry Section

Role of Reactive Oxygen Species and Antioxidants in Atopic Dermatitis

N. SIVARANJANI[1], S. VENKATA RAO[2], G. RAJEEV[3]

ABSTRACT

Background: In humans, oxidative stress is involved in many diseases such as atherosclerosis, Parkinson's disease, heart failure, myocardial infarction, Alzheimer's disease, Fragile X syndrome and chronic fatigue syndrome. Atopic dermatitis (AD), also known as atopic eczema, is a non-contagious, relapsing inflammatory skin disease which is characterized by eczema and pruritus. The skin reacts abnormally to irritants, food and environmental allergens and it becomes very itchy, which leads to scratching, redness and flaky skin. Very little study has been done to find out the relationship between oxidative stress and Atopic dermatitis.

Aim: The aim of our work was to evaluate the status of oxidative stress in patients of Atopic dermatitis in comparison with healthy control subjects.

Material and Methods: Twenty five patients of known Atopic dermatitis and 25 normal healthy controls of same age group were included in the study. Estimations of oxidants like Malondialdehyde (MDA), enzymatic antioxidants like Superoxide dismutase (SOD), Catalase, Glutathione peroxidase (GPX) and non-enzymatic antioxidants like reduced Glutathione (GSH), Vitamin A, Vitamin E and Vitamin C were done to assess the oxidative stress.

Results: Atopic dermatitis patients were more prone to damage caused by Reactive Oxygen Species (ROS) or Oxidants, than controls, which was evident from an increase of Malondialdehyde and a decrease of enzymatic and non enzymatic Antioxidants.

Conclusion: Antioxidants may possibly be beneficial in the treatment of Atopic dermatitis, which must be substantiated by further studies.

Keywords: Reactive oxygen species (ROS), Oxidative stress, Antioxidants, Atopic dermatitis

INTRODUCTION

'Eczema or Atopic dermatitis' is a term which is broadly applied to a range of persistent skin conditions. The hallmarks of Atopic dermatitis are a chronic relapsing form of skin inflammation, a disturbance of epidermal-barrier function that culminates in dry skin, eruptions and an Ig E mediated sensitization to food and environmental allergens. The eruptions are characterized by one or more of these symptoms: itching, scratching which leads to redness, swelling, cracking, "weeping" clear fluid, and finally, crusting and scaling, which become excoriated and lichenified. In most of the cases, there are periods of time when the disease is worse (called exacerbations or flares), followed by periods when the skin improves or clears up entirely (called remissions) [1]. The cause of eczema is unknown, but it is presumed to be a combination of genetic and environmental factors [2]. Two hypotheses concerning the mechanism of Atopic dermatitis have been proposed; the first one prompts that the primary defect resides in an immunologic disturbance that causes an Ig E-mediated sensitization, with epithelial barrier dysfunction, while the other proposes that an intrinsic defect in the epithelial cells leads to the barrier dysfunction; the immunologic aspects are considered to be an epiphenomenon [3]. Atopic dermatitis affects males and females at about the same rate. Although it may occur at any age, it begins most often in infancy and childhood [4]. Atopic dermatitis can theoretically affect any part of the body; it tends to occur more frequently on the hands and feet, on the ankles, wrists, face, neck and upper chest. Atopic dermatitis can also affect the skin around the eyes, including the eyelids [5]. Most people develop the skin inflammation condition first, before any skin lesions become visible. Currently, there is no single test for diagnosing Atopic dermatitis [2]. The role of ROS has been studied in Eczema and other skin diseases to some extent, but its importance in Atopic dermatitis has rarely been investigated.

Free radical production in the animal cell is inevitable. Normally, there is an equilibrium between a free radical and ROS formation and endogenous antioxidant defense mechanisms, but if this balance is disturbed, it can produce oxidative stress. This state of oxidative stress can result in injuries in all the important cellular components like proteins, DNA and membrane lipids, which can cause cell death. Free radicals mediate lipid peroxidation, which is considered to be main mechanism of cell membrane destruction and cell damage. [6,7] Antioxidants are the substances that scavenge and suppress the formation of free radicals and which even oppose their activities [8].

The aim of our work was to evaluate the status of oxidative stress in Atopic dermatitis patients. Oxidants include ROS, reactive nitrogen species (RNS) and Sulphur containing radicals, etc., which are controlled by various cellular defense mechanisms. These include enzymatic (Glutathione peroxidase, Catalase, Superoxide dismutase) and non enzymatic (Vitamins A, E and C) antioxidants. A lipid peroxidation product such as Malondialdehyde (MDA) is used as an indicator of lipid peroxidation. Such lipid hydroperoxides decompose under physiological conditions in the presence of iron or copper ions, to generate highly cytotoxic aldehydes. Among such aldehydes, MDA receives the most attention; yet it is now known to be relatively and poorly toxic [9,10].

Vitamin C is a water soluble vitamin; Vitamin E and Vitamin A are lipophillic antioxidants. Vitamin E acts as a chain breaking antioxidant, which removes free radicals and prevents their peroxidative effects on unsaturated lipids of cell membrane [11]. Vitamin C is needed for its regeneration. Vitamin C neutralizes free radicals by donating H+ ions to free radical R, while vitamin C becomes an ascorbate radical by itself [12]. Vitamin C not only neutralizes hydroxyl, alkoxyl and peroxyl radicals by H+ donation; ascorbate can also neutralize the radical forms of other antioxidants such as GSH and Vitamin E [13]. Beta carotenes are effective scavengers of Alkoxyl and Peroxyl radicals [14]. Hence, the present study was designed, to assess the

MDA extent and status of dietary antioxidants (Vitamin A, E and C) in Atopic dermatitis.

MATERIAL AND METHODS

This pilot study was conducted in Department of Biochemistry and Department of Dermatology, Dhanalakshmi Srinivasan Medical College and Hospital, Perambalur. 25 patients of known Atopic dermatitis (n=25), with an average age of 35 years (10-60 years), were selected for the study. Twenty five normal healthy controls of same age group were compared with patients of Atopic dermatitis. Control subjects were healthy, with no smoking or alcoholic habits and they were free from any other skin and systemic diseases like Diabetes mellitus or Hypertension. Fasting venous blood samples were collected in vials with EDTA, in plain vials (without anticoagulant) and in heparinized vials for the study of various parameters. Samples were used for the estimations of oxidative parameters like MDA and antioxidant parameters like Vitamin A, Vitamin E , Vitamin C, SOD, Catalase, GSH and GPX. Chemicals were obtained from Sigma Chemical Co., USA, Merck Ltd, India and Sis Co Research Laboratory, India.

Estimation of Malondialdehyde– Burge and Aust [15]. MDA, which is a stable end product of fatty acid peroxidation, reacts with TBA at acidic conditions, to form a complex that has a maximum absorbance at 532 nm.

Estimation of Vitamin C– Dichlorophenolindophenol method [16]. 2,6-Dichlorophenolindophenol is red in acidic solutions and on titration with a solution of ascorbic acid, it is reduced to a colourless leucobase, the ascorbic acid being oxidized to dehydroascorbic acid.

Estimation of GSH (reduced glutathione)– Buetler method [17]. The method is based on the development of a relatively stable yellow colour when 5,5' –dithiobis-(2-nitrobenzoic acid) is added to sulfhydril compounds.

Estimation of SOD (superoxide dismutase)– This is the method of Mark Lund S, Mark Lund G (1974) which was modified by Nandi et al., [18]. This method is based on ability of SOD to inhibit autoxidation of pyrogallol under specific conditions. The reading was taken at 420 nm.

Estimation of Catalase– Beers et al., [19]. This method is based on disappearance of peroxide and it was measured spectrophotometrically at 240 nm.

Estimation ofGlutathione Peroxidase (GPX)– Paglia's Method µ/gm of Hb [20]. Hydrogen peroxide is used and the rate of disappearance of NADPH at 37°C was recorded spectrophotometrically at 340 nm.

Estimation of Vitamin E (Alpha Tocopherol)– Baker and Fran (1968:172) [21]. This is based on reduction of ferric to ferrous ions by tocopherol to form a red coloured complex with 2,2' bipyridyl. It was read at 520nm.

Estimation of Vitamin A– Sing et al., 14(4) [22]. Read at 525nm and the results were expressed as µg/dl.

STATISTICAL ANALYSIS

Data was described as Mean ± Standard deviation. The results were analyzed by using Students "t" test for unpaired data. For all inferential statistical tests, a two tailed p–value of < 0.05 was considered to be significant.

RESULTS

Our study revealed increased oxidative stress and decreased enzymatic and non–enzymatic antioxidants as the important contributing factors in the pathogenesis of Atopic dermatitis. The lipid peroxidation status which was assessed in terms of MDA levels was increased in Atopic dermatitis patients than in controls. The difference in the means was statistically significant, as shown in [Table/Fig–1]. The enzymatic antioxidant parameter like SOD,

Parameter	Control n=25	Patients n=25	t–value	p–value
MDA (n mol/ml)	3.46 + 0.11	4.38 + 0.13	-21.046	0.001
SOD (IU/gm of Hb)	680.96 + 12.25	584.22 + 19.10	18.620	0.001
Catalase(IU/gm of Hb)	8.42 + 0.10	6.11 + 0.03	11.628	0.001
GPX (IU/gm of Hb)	1.50 + 0.13	0.75 + 0.11	13.266	0.001
GSH(µmol/L)	77.58 + 1.14	51.75 + 1.05	15.52	0.001
Vitamin A (µg/dl)	40.02 + 1.013	25.96 + 0.735	11.62	0.001
Vitamin E (mg%)	1.46 + 0.14	0.94 + 0.24	12.385	0.001
Vitamin C (mg%)	1.33 + 0.05	0.63 + 0.04	13.702	0.001

[Table/Fig-1]: Statistical analysis of oxidative and antioxidative parameters of Atopic dermatitis patients and controls

Catalase and GPX were decreased in patients than in controls. The non enzymatic antioxidant parameters like GSH, Vitamin A, Vitamin E and Vitamin C were also decreased in patients than in controls. The difference in the means of antioxidant parameter was statistically significant, as shown in [Table/Fig–1].

DISCUSSION

The involvement of ROS in the pathogenesis of eczema, such as Atopic dermatitis, has been suggested but it has not yet been thoroughly verified experimentally [23]. Higher values of oxidative stress in Atopic dermatitis were caused due to increase in lipid peroxidation and decreased levels of antioxidants. These data suggest that oxidative stress plays some role in the pathogenesis of Atopic dermatitis. Our study reports supported the findings of Hirokazu Tsukahara et al., who stated that oxidative stress and altered antioxidant defenses were involved in the pathophysiology of acute exacerbation of Atopic dermatitis [24].

CONCLUSION

Along with corticosteroids and immunosupressants which are used in the treatment of Atopic dermatitis, supplementation of natural antioxidants like vitamins A, C and E can potentially be a valuable addition to the conventional therapy, in the management of this disease. To the best of our knowledge, this is the first study which was done to evaluate the role of oxidative stress in Atopic dermatitis in the south Indian population. Since these are preliminary data with relatively small number of subjects, it warrants observation in a larger, well defined population with Atopic dermatitis.

REFERENCES

[1] Vida Feizy, Arefeh Ghobadi. Atopic dermatitis and systemic autoimmune diseases: A descriptive cross-sectional study. *Dermatology Online Journal.* 2006;12(3):3.
[2] Atopic dermatitis. National Institute of Health. http://www.niams.nih.gov/Health_Info/Atopic_Dermatitis/default.asp. Retrieved 27 May 2013.
[3] Thomas Bieber. Atopic dermatitis. *N Engl J Med.* 2008;358:1483-94.
[4] Rashmi Sarkar, Amrinder J Kanwar. Atopic dermatitis. *Indian Pediatrics.* 2002;39:922-30.
[5] Thestrup Pedersen K. Clinical aspects of Atopic dermatitis. *Clin Exp Dermatol.* 2000;25:535-43.
[6] Mitchell RN, Cotran RS. Cell injury, adaptation, and death. In: Kumar V, Cotran RS, Robbins SL, Editors. Robbins: Basic Pathology. 7th edition. New Delhi: *Harcourt (India) Pvt. Ltd*; 2003; 3-33.
[7] Sen CK. Oxygen toxicity and antioxidants: State of the art. *Indian J Physiol Pharmacol.* 1995;39:177–96.
[8] Dormandry TL. Free radical oxidation and antioxidants. *The Lancet.* 1978;311:647-50.
[9] Estertbaner H, Cheese Man KH. Determination of aldehyde lipid peroxidation products Malondialdehyde. *Method in Enzymology.* 1970;186:407-21.
[10] Esterbauer H, Cheesman KH, Dianzani MU, Poll G, Slater TF. Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe2 in rat liver microsomes. *Biochem. J.* 1982;208:129-40.
[11] Hamid AA, Aiyelaagbe O, Usman LA, Ameen OM, Lawal A. Antioxidants: Its medicinal and pharmacological applications. *African Journal of Pure and Applied Chemistry.* 2010;4(8):142-51.
[12] Hossain MA, Kozi Asada. Monodehydroascorbate Reductase from Cucumber is a Flavin Adenine Dinucleotide Enzyme. *The Journal of Biological Chemistry.* 1985;260(24):12920-26.
[13] Garry R Buettner, Beth Anne Jurkiewicz. Catalytic metals, Ascorbate and free radicals: combinations to avoid. *Radiation Research.* 1996;145:532-41.
[14] Debajyothi Das. Biochemistry. 13th edition. Kolkatta: *Academic Publishers*;

2008.
[15] Buege JA, Aust SD. Microsomal lipid peroxidation methods. *Enzymol*.1978; 52:302-10.
[16] Varely, Gowenlock, Bell. Practical Clinical Biochemistry, Vol 2 - Hormones, Vit, Drugs and Poisons. 5th edition, 1st Indian print. *William Heinemann Med books Pvt Ltd*; 1991; 253-55.
[17] Beutler E, Duron O, Kelly B M. Improved method for the determination of blood glutathione. *J Lab Clin Med.* 1963;61:882-88.
[18] Nishal HK, Sharma MP, Goyal RK, Kaushik GG. Serum superoxide dismutase levels in diabetic mellitus with or without microangiopathic complications. *JAPI.* 1998;46(10):853-55.
[19] Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem*. 1952;195:133–40.
[20] DE Paglia, WN Valentine, JG Dahlgren. Effects of Low-level Lead Exposure on Pyrimidine 5'-nucleotidase and Other Erythrocyte Enzymes: Possible Role of Pyrimidine 5'- nucleotidase in the Pathogenesis of Lead-induced Anemia. *J Clin Invest*. 1975;56(5):1164–69.
[21] Baker H, Frank O. Tocopherol. Clinical vitaminology, methods and interpretation. New York Inter Science Publisher, *John Wiley and Sons, Inc*.;1968; 172–3.
[22] Sing et al. Estimation of vitamin A. *Indian J Biochem Biophys.* 1978;14(4): 30-33.
[23] Cheeseman KH, Slater TF. An introduction to free radical biochemistry. *British Medical bulletin.* 1993;49:481-93.
[24] Tsukahara H, Shibata R, Ohshima Y, Todoroki Y, Sato S, Ohta N, et al. Oxidative stress and altered antioxidant defenses in children with acute exacerbation of Atopic dermatitis. *Life Sci*. 2003;72(22):2509-16.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Biochemistry, Dhanalakshmi Srinivasan Medical College and Hospital, Perambalur-621212, Tamil Nadu, India.
2. Professor and HOD, Department of Biochemistry, Dhanalakshmi Srinivasan Medical College and Hospital, Perambalur-621212, Tamil Nadu, India.
3. Tutor, Department of Biochemistry, Dhanalakshmi Srinivasan Medical College and Hospital, Perambalur-621212, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. N. Sivaranjani,
Assistant Professor, Department of Biochemistry, Dhanalakshmi Srinivasan Medical College and Hospital, Perambalur-621212, Tamil Nadu, India.
Phone: 07845226536, E-mail: ranjani_09@yahoo.co.in

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jun 05, 2013**
Date of Peer Review: **Aug 13, 2013**
Date of Acceptance: **Sep 06, 2013**
Date of Publishing: **Dec 15, 2013**