Role of Serum Melatonin and Oxidative Stress in Childhood Atopic Dermatitis: A Prospective Study

Abstract

**Background:** Many factors have been implicated in the pathogenesis of atopic dermatitis (AD) and recently the role of oxidative damage has been postulated. **Objectives:** To study the levels of oxidants and antioxidants including melatonin in the blood of children with AD and their association with the severity of AD. **Methods:** Thirty patients with atopic dermatitis, aged 6 months to 12 years, and equal number of age and sex-matched controls were included. Clinical characteristics and baseline severity assessment using SCORAD (scoring atopic dermatitis) severity index were noted. Blood superoxide dismutase, blood glutathione peroxidase, serum malondialdehyde, and serum melatonin levels were measured in cases and controls and results were compared. **Results:** The serum levels of malondialdehyde and melatonin were significantly higher among the cases compared to controls. The blood levels of superoxide dismutase and glutathione peroxidase were higher in cases but the difference with controls was not statistically significant. There was no significant correlation between these markers and the severity of the disease. **Conclusions:** Oxidative stress was increased in cases of childhood AD compared to the control group in this study. No correlation between oxidative stress and the severity of the disease was found. Larger studies are warranted.

**Keywords:** Atopic, children, dermatitis, eczema, oxidative stress, melatonin

Introduction

Atopic dermatitis (AD) is an itchy, chronically relapsing inflammatory skin disorder that often starts in early childhood (mostly before 2 years of age).[1] Various diagnostic criteria for AD exist and Hanifin and Rajka[1] are the most commonly used. There are also various scoring systems for the severity with SCORAD[2] (scoring atopic dermatitis) being one of the most commonly used systems.

The pathogenesis of AD is multifactorial with a damaged skin barrier, abnormal immune responses, genetic and environmental factors being implicated.[3] The role of oxidative stress in the pathogenesis of AD has been postulated.[3,4] Oxidative stress is known to promote tissue inflammation through upregulation of genes that code proinflammatory cytokines and in turn, inflammatory cells release free radicals when activated.

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) are intracellular enzymatic antioxidants and their levels are used as major parameters representing oxidative damage.[7] SOD is the primary enzyme clearing free oxygen radicals by converting superoxide anions to hydrogen peroxide.[6] GPx is the key enzyme converting hydrogen peroxide to oxygen and water.[7] One of the main mechanisms of tissue damage by oxidative stress is lipid peroxidation at cell membranes.

Malondialdehyde (MDA) is one end-product of lipid peroxidation and the MDA level in erythrocytes and plasma has been used as a marker of tissue damage resulting from in vivo free radicals.[8]

Melatonin is produced by the pineal gland and the skin expresses melatonin receptors (MT1 and MT2). A key regulator of circadian rhythm homeostasis in humans, melatonin is also a direct radical scavenger and an indirect antioxidant that stabilizes cell membranes, which makes them more resistant to oxidative damage.[1,5,9] There is still a controversy whether it decreases or increases in AD.[3,10,11]

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Since there is a paucity of studies on the role of oxidative stress in childhood AD and on the role of serum melatonin in pathogenesis of atopic dermatitis, this study was undertaken.

Materials and Methods

Study population

A case-control study was undertaken. Thirty patients aged from 6 months to 12 years of either sex with AD visiting the outpatient department of dermatology at a tertiary care center in New Delhi, India, between October 2016 and December 2017 were selected as cases by consecutive sampling and 30 age and sex-matched controls were recruited. The lower age limit was taken as 6 months because circadian rhythm is established around 3 months of age.\(^{12}\)

Approval was obtained from the institutional review board and informed consent was taken from all participants of the study. Those suffering from infections/severe systemic illness/chronic disease, those who have received nutritional supplementation (folic acid, glutathione, Vitamin E, Vitamin C, selenium, zinc, iron) in the past three months were excluded. Additionally, those suffering from alopecia areata/seborrheic dermatitis/vitiligo/acro/roacea/skin cancer/psoriasis/asthma were excluded from controls.

Study tools

A predevised questionnaire for history and clinical examination was used. Severity was calculated using SCORAD with the following categories: mild <25, moderate 25-50, and severe >50.\(^{13}\)

For the assessment of oxidative stress, 3 ml of venous blood was collected at 9 am from cases and controls to estimate SOD, GPx, MDA, and melatonin levels.

Estimation of blood SOD\(^{14}\) was carried out using the RANSOD kit (RANDOX, Randox Laboratories Ltd, 55 Diamond Road, Crumlin, Co Antrim, United Kingdom, BT29 4QY). Xanthine is oxidized in the presence of Xanthine oxidase into uric acid and superoxide free radical. In the presence of superoxide free radical, 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl tetrazolium chloride (I.N.T.) is reduced into a red formazan dye. Normally SOD scavenges these O2- free radicals, thus preventing the formation of formazan dye. Thus, SOD activity can be measured by the degree of inhibition of formazan synthesis and is proportional to it.

GPx levels in blood were assayed by the method developed by Paglia and Valentine, employing the RANSEL kit (RANDOX, Randox Laboratories Ltd, 55 Diamond Road, Crumlin, Co Antrim, United Kingdom, BT29 4QY).\(^{15}\) GPx catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), the oxidized glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to NADP+. This reduction in the concentration of NADPH correlates with a decline in absorbance of UV light at 340 nm, which can be measured by spectrophotometry to estimate the corresponding levels of GPx.

MDA is a lipid peroxidation product, which is measured as an index of oxidative stress. Estimation of MDA in serum is done by TBA-TCA-HCl assay.\(^{16}\) Trichloroacetic acid precipitates the protein present in plasma. Hydrochloric acid hydrolyzes the lipid peroxides from the protein to yield MDA. This MDA reacts with thiobarbituric acid (TBA) to produce MDA-TBA adduct. On boiling in a water bath, this gives a pink color which is measured at an absorbance of 535 nm using HCl as blank.

Melatonin is a powerful endogenous free radical scavenger. The assay is performed on the enzyme linked-immunosorbent assay (ELISA) kit supplied by Qayee-bio (for life science). It is a double antibody sandwich ELISA to assay the level of melatonin in samples.\(^{17}\)

Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 17. All quantitative variables were estimated using measures of central location (mean, median). For normally distributed data, variables were compared using the paired t-test. Qualitative variables were described with frequencies and proportions. Correlations were determined using the Pearson’s correlation coefficient and values interpreted as <0.20 very weak; 0.20-0.39 weak; 0.40-0.59 moderate; 0.60-0.79 strong; ≥0.80 very strong.

\(P\)-value <0.05 was considered significant.

Results

The demographic and clinical characteristics of the patients are presented in Table 1.

Thirty children with AD aged between 6 months and 12 years (mean 4.95 years/SD 3.5; 21 males/9 females) were included in the study, and equal number of age and sex-matched controls. The majority of the cases (53.3%) had mild disease as measured with SCORAD, 40% had moderate disease, and 6.7% had severe disease.

Table 2 shows the differences in the blood GPx, blood SOD, serum MDA, and serum melatonin between cases and controls and their correlation with SCORAD severity [Figure 1].

The mean levels of serum MDA in cases were 0.118, while in the control group the average level was 0.063. The mean serum melatonin levels in cases were found to be 7.03,
whereas in controls it was 3.98. The difference between the two groups was statistically significant ($P < 0.05$). No statistically significant difference in blood GPx and blood SOD was found between cases and controls. None of the parameters had a statistically significant correlation with SCORAD. Additionally, our multivariate analysis also showed that none of the parameters had a statistically significant correlation with age or sex (+0.1, +0.06, respectively).

**Discussion**

In this study, 46.67\% (14 cases) had onset in infancy and 100\% of the cases had onset in the first 6 years of life, similar to the findings of 94.4\% by Sarkar and Kanwar\textsuperscript{18} and 97.91\% by Dhar and Kanwar\textsuperscript{19} All our cases having onset in the first 6 years of life maybe because of the small sample size of our study. Our study has shown a marked male predominance of 2.3:1, which is in concordance with studies from North India\textsuperscript{18,19}. However, studies from other parts of the country have shown a female preponderance\textsuperscript{20,21}

Majority cases were of mild (53.3\%) and moderate (40\%) severity. It is a known fact that the severity of AD is lesser in India as compared to the West\textsuperscript{22}

Oxidative stress is postulated to be an important factor in the pathogenesis of AD. Measurement of various markers for oxidative damage and antioxidants helps to further clarify the role of oxidative stress in AD with possible future therapeutic implications. Recently, Sivaranjani et al. conducted a pilot study where levels of antioxidants SOD, GPx and catalase were lower and levels of MDA were significantly higher in adults and children with AD than healthy controls\textsuperscript{4} Amin et al. also demonstrated higher levels of MDA in cases\textsuperscript{23} However, others have shown no significant difference\textsuperscript{5,24} in serum MDA levels.

In our study, mean levels of GPx and SOD in blood were raised, although not significantly, in children with AD compared to controls. In a scenario of raised oxidative stress, levels of antioxidants increase to effectively scavenge free radicals. Also, our study showed mean serum MDA levels were higher in children with AD than in controls. This suggests increased oxidant load in AD.

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| Table 1: Characteristics of cases |
|----------------------------------|
| **Characteristic** | **Number (SD)*** | **Percentage** |
| No. of patients | 30 | 100 |
| Sex distribution | M: 21, F: 9 |  |  |
| Sex ratio | 2.3:1 |  |  |
| Age of presentation |  |  |  |
| Range | 0.5-12 |  |  |
| Mean | 4.95 (3.5) |  |  |
| Distribution |  |  |  |
| Infants (0-1 yr) | 3 | 10 |
| Toddlers (>1-3 yr) | 11 | 36.7 |
| Preschool children (>3-< 6 yr) | 3 | 10 |
| School children (≥6 yr) | 13 | 43.3 |
| Age of onset |  |  |  |
| Range | 0.1-5 |  |  |
| Mean | 1.5 (1.27) |  |  |
| Distribution |  |  |  |
| Infants (0-1 yr) | 14 | 46.67 |
| Toddlers (>1-3 yr) | 14 | 46.67 |
| Preschool children (>3-< 6 yr) | 2 | 6.66 |
| School children (≥6 yr) | 0 | 0 |
| Duration of disease |  |  |  |
| Range | 0.1-10 |  |  |
| Mean | 2.62 (2.75) |  |  |
| Distribution of lesions (>1 year) |  |  |  |
| Face | 25 | 67.57 |
| Extensors | 21 | 56.67 |
| Flexors | 14 | 51.85 |
| Seasonal exacerbation |  |  |  |
| No variation | 17 | 57 |
| Summer | 3 | 10 |
| Winter | 10 | 33.33 |
| SCORAD |  |  |  |
| Mean/SD | 24 (12.8) |  |  |
| Severity |  |  |  |
| Mild | 16 | 53.3 |
| Moderate | 12 | 40 |
| Severe | 2 | 6.7 |

\*SD - Standard deviation

| Table 2: Mean blood glutathione peroxidase (GPx), blood superoxide dismutase (SOD), serum malondialdehyde (MDA) and serum melatonin levels in cases and controls with their correlation coefficients with SCORAD and respective $P$ values |
|----------------------------------|
| **Mean±SD** (cases) | **Mean±SD** (controls) | **$P$** | **Correlation coefficient (Pearson’s correlation)** | **$P$** |
| Blood GPx | 246.52±90.76 | 209.60±54.21 | 0.061 | -0.03 | 0.877 |
| Blood SOD | 9.48±4.36 | 7.86±4.08 | 0.142 | +0.146 | 0.441 |
| Serum MDA | 0.118±0.095 | 0.063±0.015 | 0.003* | +0.0441 | 0.818 |
| Serum Melatonin | 7.03±7.43 | 3.98±2.04 | 0.034* | -0.063 | 0.741 |

*P<0.05 is statistically significant. **SD-standard deviation
Uysal et al.\(^3\) have reported higher serum melatonin levels among children with AD compared to controls. A study by Chang et al.\(^{25}\) showed that a reduced level of nocturnal urinary melatonin was found to be associated with sleep disturbance and greater severity of disease in children with AD. Another study by Chang et al.\(^{26}\) evaluated the effectiveness of melatonin supplementation for children with AD and showed significant improvement in the sleep-onset latency and disease severity in children and adolescents with AD. Our study showed mean serum melatonin levels were higher in children with AD than in controls. It may be hypothesized that this increased serum melatonin in AD may be a compensatory response to reduce skin inflammation by trying to alleviate oxidant overproduction.

Correlation of MDA with disease severity has been studied before only in one study, that of Uysal et al.\(^3\) where no significant association was found. Serum melatonin levels were negatively correlated with disease severity by Uysal et al.\(^3\) and lower serum melatonin levels in children with severe AD were noted than that in those with mild AD but there was no significant correlation between mild and moderate severity. To the best of our knowledge, correlations of GPx and SOD have not been studied previously in any other study.

In our study, there was no significant correlation with disease severity as measured with SCORAD.

However, the limitations of our study include a small sample size due to time constraints and the presence of less cases of severe AD, which may also affect the correlation findings.

To conclude, our study shows that a state of oxidative stress may exist in AD. Extensive multicentric studies should be conducted in both children and adults to further ascertain the correlation between markers of oxidative stress and the severity of AD.

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### Conflicts of interest
There are no conflicts of interest.

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