Retinol Levels in Serum and Chronic Skin Lesions of Atopic Dermatitis

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Abstract

Background: Atopic dermatitis (AD) is a common childhood dermatosis and a distressing cause of morbidity. The pathogenesis of AD is known to be associated with disorders of immune response and defect in antioxidant defense, genetic predisposition, environmental triggers, psychosomatic factors, and other mechanisms. Retinol has immunomodulatory and antioxidant effects, thus may have a protective role in AD. Objective: The objective of this study was to evaluate the correlation of retinol levels in skin lesions and serum, with AD. Materials and Methods: The study was a hospital-based, case–control study. Punch biopsy from the skin and venous blood of 86 participants (including 43 cases and 43 controls) were assayed for retinol levels by a reversed-phase high-performance liquid chromatography method. Analysis of data was performed using appropriate statistical methods. Results: Skin and serum retinol levels were highly significantly decreased in patients in respect to that of controls. Conclusion: Retinol levels were decreased in AD. Retinol estimation may be used as a promising parameter for the elaboration of treatment strategy and monitoring.

Key Words: Antioxidant, atopic dermatitis, immune response, retinol, serum, skin

Introduction

Atopic dermatitis (AD) is a common dermatological disorder causing significant morbidity and manifested by diffuse symmetrical eczematous eruption. It is characterized by a personal and/or family history of allergic syndromes of asthma or rhinitis. It usually occurs in infants and young children and affects the flexural aspects of the body.1

In chronic AD, there is skin infiltration of inflammatory dendritic epidermal cells, eosinophils, and macrophages, which, induced by interferon (IFN)-γ, produce interleukin (IL)-1, IL-6, IL-12, IL-11, IL-23, and tumor necrosis factor-alpha (TNF-α).2,3 These cytokines bind to receptors on vascular endothelium, activating cellular signaling including the nuclear factor kappa B pathway and inducing the expression of vascular endothelial cell adhesion molecules. These events initiate the process of tethering, activation, and adhesion of inflammatory cells to the endothelium followed by extravasation. Once the inflammatory cells have infiltrated into the tissue, they respond to chemotactic gradients established by chemoattractant cytokines and chemokines, which emanate from sites of injury or infection.4

Pathogenesis of AD also involves environmental pollution, solar radiation, and endogenous pro-oxidant-mediated increased reactive oxygen species (ROS) production. For example, monocytes from patients with AD are primed to generate ROS in response to zymosan, a toll-like receptor 2 ligand, suggesting that Staphylococcus aureus may damage lesional skin by the production of ROS. Mast cells generate mainly intracellular ROS following the aggregation of FcεRI.5 These ROS may, among other effects, induce oxidative protein damage in the stratum

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corneum, leading to the disruption of barrier function and exacerbation of AD.[6]

Retinol has important immunomodulatory effects.[7] Furthermore, retinol is an effective antioxidant.[8] Thus, monitoring retinol levels in serum and skin lesions of AD patients, as an indicator of the immune response and antioxidant defense could be important for the clinicians’ treatment strategy.

Indian literature on AD is limited, probably due to lower prevalence, and milder disease.[9] Dhar et al. had found, among other findings, 39%, 38%, 20%, and 3% of Indian patients had flexor, extensor, face, and both flexor and extensor involvement.[10] There is a paucity of data available in the literature regarding serum and skin lesion retinol levels in AD patients, particularly from our country. The present study was designed to determine whether AD is accompanied by a change in serum and skin lesion retinol levels.

Materials and Methods
The study was a hospital-based case–control study conducted in a tertiary care center in West Bengal, India. The study was approved by the Institutional Ethical Committee. Before enrollment of the subjects, informed consent was obtained from the guardians of all the participants. The duration of the present study was 7 months and included forty-five chronically moderately severe AD patients attending the Dermatology outpatient department during the above mentioned period. These patients were further grouped into younger (<5 year of age) and older patients (more than 5 year of age). Forty five age- and sex-matched patients attending skin outpatient department (OPD), characterized by the absence of any sign of and of personal or family history of atopic disease, served as control. Consecutive patients attending the OPD and satisfying inclusion criteria were selected assuming that the patients attended OPD randomly. Hanifin and Rajka criteria were used to diagnose AD cases.[11] Exclusion criteria included participants who had cholestasis, unusual dietary habits, acute or chronic infections, fever, malabsorptive syndromes, oral supplements or drugs-containing vitamin A and steroids during the past 6 months and topical application of vitamin A, potent steroids or UV irradiation during the preceding 1 month. Complete history and physical examination of all cases and controls were undertaken.

Punch biopsy (from a representative skin lesion in cases and from corresponding sites in matched controls) and venous blood sample were collected from each case and each control after 12 h of fasting. All samples were coded and assayed in a blind fashion by an investigator who was unaware of the participant’s clinical status.

Serum and skin retinol levels were assayed using a reversed-phase high-performance liquid chromatography (HPLC) method.[12]

Statistical analysis of data was performed using SPSS software version 20 (IBM, New York, USA), and inferences were drawn. A value of $P<0.05$ was considered to be statistically significant.

Results
Of the 45 patients initially included in the study, two males and of the 45 controls two females did not complete the study protocol and as such were excluded from the analysis.

The age of patients ranged from 1 year 2 month to 8 year 11 month with a mean age of 4 year 7 month. The mean duration of the disease in the patients (including recurrences and remissions) was 2 years 3 months. Age and sex distribution of cases and controls are given in Table 1.

Skin retinol levels were highly significantly decreased in patients with respect to controls [Table 2]. Serum retinol levels were also highly significantly decreased in patients with respect to controls [Table 3].

When a comparison was made between two age groups of atopi patients, e.g., below and above 5 year of age,

### Table 1: Age and sex distribution of cases and controls

|          | Cases          | Controls        |
|----------|----------------|-----------------|
| Male, mean age (in years)±SD (n) | 4.7±0.39 (21) | 4.8±0.52 (22) |
| Female, mean age (in years)±SD (n) | 4.6±0.4 (20)  | 4.6±0.39 (21) |

$n$: Number of subjects, SD: Standard deviation

### Table 2: Two-sample difference of means: Retinol levels (ng/g) of skin in atopic dermatitis patients and in control subjects

|          | AD patients | Controls |
|----------|-------------|----------|
| t-statistic | df | $p$ (2-tailed) |
| Equal variance | −59.2711 | 84 | 0.00001 |
| Unequal variance | −59.2707 | 59 | 0.00001 |

SD: Standard deviation, AD: Atopic dermatitis, df: degree of freedom

### Table 3: Two-sample difference of means: Retinol levels (mcg/dl) in serum in atopic dermatitis patients and in control subjects

|          | AD patients | Controls |
|----------|-------------|----------|
| t-statistic | df | $p$ (2-tailed) |
| Equal variance | −37.6416 | 84 | 0.00001 |
| Unequal variance | −37.6418 | 75 | 0.00001 |

SD: Standard deviation, AD: Atopic dermatitis, df: degree of freedom
the skin and serum retinol levels were found to be significantly low in the younger patients in relation to the older ones (Tables 4 and 5).

There was no significant difference in serum/skin retinol levels between male and female patients.

**Discussion**

Retinol has a broad range of immunological effects. It inhibits IL-12 production from the antigen-presenting cells such as Langerhans cells, inflammatory dendritic epidermal cells, and macrophages; inhibits IFN-γ production from T and NK cells and decreases IgG levels.[13,14] It also inhibits IL-1-induced IL-6 production.[15] Finally, it decreases TNF-α levels.[16]

All these effects of retinol may help to reduce the inflammatory process in chronic AD.

The antioxidant property of retinol is well known. It has a very high chemical reactivity or scavenging activity toward lipoperoxyl radicals.[17] Furthermore, there are synergistic interactions between all-trans-retinol and α-tocopherol against lipid peroxidation. Moreover, by limiting auto-oxidation of all-trans-retinol, α-tocopherol strongly promotes its antioxidant effectiveness.[18]

Reduced retinol levels in skin and in serum lead to reduced retinol activities that aggravate the inflammatory reaction so characteristic of AD. In this study, serum retinol levels were highly significantly decreased in patients with respect to that of controls [Table 2].

### Table 4: Two-sample difference of means: Retinol levels (ng/g) in skin in subgroups of atopic dermatitis patients

|               | Younger | Older |
|---------------|---------|-------|
| Sample mean±SD | 47±4.2  | 52±6.3|
| Sample size (n)| 19.0000 | 24.0000|
| t-statistic    |         |       |
| df            |         |       |
| p(2-tailed)   | <0.005  |       |

### Table 5: Two-sample difference of means: Retinol levels (mcg/dl) in serum in subgroups of atopic dermatitis patients

|               | Younger   | Older     |
|---------------|-----------|-----------|
| Sample mean±SD | 182.0000±12.7 | 227.0000±19.3 |
| Sample size (n)| 17.0000   | 26.0000   |
| t-statistic    |           |           |
| df            |           |           |
| p(2-tailed)   | <0.00001  | <0.00001  |

However, researchers found normal concentrations of carotenoids, retinol, and retinol-binding protein in serum, and increased dehydroretinol but decreased retinol in superficial shave biopsies, in AD patients compared to that in controls.[19] Similarly, reduced concentrations of retinol and all-transretinoic acid in affected and unaffected skin, and normal retinol and all-trans retinoic acid in serum were shown by Mihaly and coworkers.[20] In our study also, skin retinol levels were highly significantly decreased in patients with respect to controls [Table 3]. Further, the younger group of patients had significantly decreased levels of retinol in the skin and highly significantly decreased levels of retinol in serum compared to the older group of patients [Tables 4 and 5]. Thus, with increasing age, there is an increase in levels of the antioxidant retinol, which may help to counter oxidative stress, one of the factors in the pathogenesis of AD. We think that this finding is in concurrence with the well-known fact that in the natural course of AD there is gradual remission with increasing age.[21-23] The decrease of retinol in skin and serum might be due to increased oxidative stress in AD. Therefore, if retinol level can be increased, it might counter the decreased antioxidant defense and imbalance in immune response.

Increased oxidative stress and decreased antioxidant defense have been found in AD.[24] Other researchers have revealed reduced AD risk with increased serum levels of α-tocopherol and retinol.[25] Another study found an inverse association of retinol levels in infants with the risk of subsequent development of allergic symptoms.[26] Only further research in this area can show if there is a link between this finding and our findings.

This study has limitations that should be considered. To assess retinol, reversed-phase HPLC was used. Retinol can be estimated by various methods, but the present method was employed as it was standard and time-tested. Patients were taking a number of medications (other than steroid drugs) to control AD. However, these treatments are characteristic of patients with AD and do not affect serum retinol levels. Furthermore, the number of patients in the study group was not large. Thus, care should be taken while extrapolating the present findings to other populations. We conducted the present study in a tertiary care hospital. However, in our country, most people visit district, sub divisional, and lower-tier hospitals for treatment. Hence, results of our study might not reflect the true picture of the population as a whole. Probably, a multicentric study on a larger population would be better in revealing the actual statistics. Despite these limitations, we believe that our study results point toward using retinol estimation as an important, potential parameter for AD. As our findings point to a decrease in the antioxidant and immunoregulatory retinol, the problem of oxidative stress and immunity in pathogenesis of AD should be further investigated, and other similar
biological parameters to determine oxidative stress and immunoregulation should also be assessed.

**Conclusion**

The results of this study suggest that AD may be related to reduction in retinol levels in serum and skin. Thus, retinol estimation may be considered in AD for the elaboration of treatment strategy and monitoring.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

**What is new?**

- Atopic dermatitis may be related to reduction of retinol levels in serum and skin
- Retinol estimation may be used as a parameter for elaboration of treatment strategy and monitoring.

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