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Eosinophil-derived neurotoxin as a biomarker for disease severity and relapse in recalcitrant atopic dermatitis

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ABSTRACT

Background: Eosinophils are encountered in many skin diseases, but the role of eosinophils in atopic dermatitis (AD) remains uncertain.
Objective: To examine the role of serum eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), and total IgE as a biomarker of disease severity and relapse in severe recalcitrant AD.
Methods: We enrolled 99 patients with AD: 37 with severe recalcitrant AD, 20 with severe AD, and 42 with mild to moderate AD. We examined the difference in serum level of total IgE, ECP, and EDN between the groups and whether any correlation existed between disease severity and ECP or EDN. Lastly, difference in levels of ECP or EDN between those who experienced relapse was examined in the severe recalcitrant group.
Results: Serum levels of total IgE, ECP, and EDN were significantly higher in the severe recalcitrant AD group and severe AD group compared with the mild to moderate AD group. No significant difference was found in serum levels of total IgE, ECP, and EDN between the severe recalcitrant group and severe group. EDN had a significant positive correlation with the SCORing Atopic Dermatitis. No significant correlation was found between EDN and ECP. In the severe recalcitrant group, 29.7% of patients experienced relapse, and EDN was significantly higher in those who experienced relapse. The cutoff value of EDN for predicting relapse was 64.5.
Conclusion: EDN correlated with the disease severity of AD. EDN may predict relapse in severe recalcitrant AD. The EDN serum level could be considered a candidate molecule as a clinical biomarker for evaluating AD disease activity and a predictor of relapse.

Introduction

Eosinophils are encountered in many skin diseases, but their functional role remains largely unclear. There is evidence that eosinophils might contribute to pathogen defense, regulate inflammatory responses, and induce fibrosis/remodeling in skin disease.[1,2]

In atopic dermatitis (AD), tissue eosinophilia is a typical finding, often associated with increased blood eosinophil levels and correlating with disease severity.[3] In the skin, eosinophils as part of the perivascular inflammatory infiltrate and extracellular granule protein deposits have been identified.[4,5]

Eosinophils can serve as major effector cells, inducing tissue damage and dysfunction by releasing granule proteins, including eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), inflammatory lipid mediators, and mitochondrial DNA.[5] ECP and EDN are known to reflect the activity of eosinophils.[2] In this regard, AD is characterized by blood and tissue eosinophilia and increased ECP that correlates with the disease activity in patients.[6]

So far, the activity of eosinophils in AD remains uncertain. It seems possible that eosinophils contribute to host defense against invading microbes through the defective skin barrier by generating extracellular DNA traps, regulating the immune response, and/or remodeling.[7–9]

Studies have focused on eosinophil and serum ECP as reflecting disease severity in AD, but studies on eosinophils, serum ECP, and serum EDN in severe recalcitrant AD that requires systemic immunosuppressant are lacking.[3,6] In this study, we aimed to examine the level of eosinophil granule protein, ECP, and EDN, which are known to reflect activity of eosinophils in AD.

Methods

Patients

Ninety-nine children with AD (mean [SD] age, 6.0 [4.9] years), defined according to the Hanifin and Rajka criteria, were included in the study. Severity of AD was assessed by SCORing Atopic

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Dermatitis (SCORAD) on visiting the clinic. Patients were classified as follows: patients with SCORADs greater than 50 and those who needed systemic immunosuppressive treatment because of poor response to topical steroids were defined as having severe recalcitrant AD, patients with SCORADs greater than 50 and those who had good response to topical steroids were defined as having severe AD, and those with SCORADs less than 50 were defined as having mild to moderate AD. No patients had SCORADs less than 50 and required systemic immunosuppressive treatment. We included 37 consecutive patients who were treated with cyclosporine for severe recalcitrant AD, 20 consecutive patients with severe AD, and 42 consecutive patients with mild to moderate AD, who were treated with topical steroids. Twenty individuals were included as a healthy control group, selected from patients who visited the well-child clinic and those who visited Uijeongbu St Mary's hospital for minor surgery with no history of AD and available measured serum total IgE levels. Patients with a history of treatment with systemic corticosteroids, cytotoxic agents, or phototherapy within 2 weeks before entry, previous treatment with cyclosporine, abnormal renal or liver function, and hypertension were excluded. Blood samples were obtained before initiation of treatment. This research was approved by the Uijeongbu St. Mary's Hospital Institutional Review Board. Written informed consent was obtained from parents or guardians; assent was obtained from invited children.

Treatment With Cyclosporine

All patients given a dose of 5.0 mg/kg of cyclosporine (Sandimmun Neoral soft gelatine capsules or oral solution, Novartis Pharmaceuticals Ltd, Seoul, Korea) per day, which was reduced to 1.5 to 3.0 mg/kg after 4 weeks according to response. Treatment was stopped at week 12 after a 4-week dose-tapering period, and treatment was discontinued in all patients who had not already had treatment discontinued. Treatment was restarted at 5.0 mg/kg when patients relapsed or when the investigator or patient believed that further systemic therapy was required. Patients were followed for up to 1 year. Use of topical corticosteroids was allowed throughout the study, with no restriction on the potency. The dose of cyclosporine was reduced by 25% in the following cases: those with an increase of serum creatinine of 30% to 50% above baseline, those with significant hypertension after 2 consecutive visits, and those with an increase of more than 3 times the upper normal limit for any liver function parameter. The dose of cyclosporine was reduced by 50% if the increase in creatinine was 50% to 100% greater than baseline, and the patient was withdrawn from the trial if the increase exceeded 100%.

Measurement of Blood Eosinophils, Serum ECP, and EDN

The NE-8000 system (Sysmax, Kobe, Japan) was used to count white blood cells and eosinophils automatically in peripheral blood. Blood samples for determination of serum ECP and EDN were drawn into vacuum tubes (Vacutainer SST, Becton Dickinson, Mountain View, California) and allowed to clot at room temperature for 60 minutes, followed by centrifugation at 1300*g* for 10 minutes at 4°C. Each sample was aliquoted into a fresh plastic tube and stored at −20°C until the assays for serum ECP and EDN were performed. Serum ECP was measured with the CAP radioallergosorbent technique (UniCAP. Pharmacia and Upjohn, Uppsala, Sweden). Sensitivity for ECP was 2.00 ng/mL, and the normal serum ECP range was 4 to 20 ng/mL. Serum EDN was measured with an enzyme-linked immunosorbent assay kit (MBL, Nagoya, Japan) according to the manufacturer's instructions. Briefly, 100 μL of each sample was transferred to a microwell coated with antihuman EDN antibody and incubated at room temperature for 1 hour. After the sample was rinsed with wash solution, 100 μL of conjugate solution was added to the wells, and incubation was continued at room temperature for an additional hour. After another washing, 3,3', 5,5;-tetramethylbenzidine chromogen solution (100 μL per well) was added and allowed to incubate for 10 minutes at room temperature. Enzyme reactions were stopped with 0.2 M sulfuric acid (100 μL per well) and the absorbance measured at 450 nm with a microplate reader within 30 minutes of stopping. According to the manufacturer's data sheet, the sensitivity for EDN was 0.62 ng/mL, and the linear range was 0.62 to 40 ng/mL. Values that exceeded the linear range were calculated after dilution. All assays were performed in duplicate for each sample, with the mean values reported here.

Measurement of Total IgE

Total IgE levels were measured with the ImmunoCAP immunofluorimetric assay (Thermo Fisher Scientific, ImmunoDiagnostics, Uppsala, Sweden). Measures of total IgE levels were expressed in international units per unit volume (1 IU = 2.4 ng). The measuring range of total IgE was 2 to 5,000 IU/mL.

Statistical Analysis

Statistical analyses were performed using SPSS software, version 20.0 (IBM Inc, Armonk, NY). All continuous variables are expressed as mean (SD) and categorical variables as number (percentage). The Shapiro-Wilk test was used to verify normality of nonparametric variables, The Bonferroni method was used for post hoc analysis. A linear regression analysis was applied for correlations between SCORAD and laboratory variables of the patients. The *t* test was used to compare the differences in laboratory findings in those who experienced relapse and those who did not. A receiver operating characteristic (ROC) curve was constructed, and the area under the curve for each parameter was compared with 0.5 to estimate the optimal cutoff levels for detecting relapse of severe recalcitrant AD. Sensitivity, specificity, positive predicted value, and negative predicted value were calculated. Statistical significance was defined at $P < .05$ in a 2-tailed test.

Results

Characteristics of Patients

A total of 119 patients were included in the study, and their characteristics are summarized in Table 1. There were 37 patients in the severe recalcitrant group, 20 patients in the severe group, 42 patients in the mild to moderate group, and 20 in the control group. Sex, age, and white blood cell count showed no significant difference, but SCORAD was significantly higher in the severe group compared with the mild to moderate group. Total IgE was significantly higher in the severe group compared with the control group. In regard to the number of eosinophils and eosinophil granule protein levels, the number of eosinophils was significantly higher in the severe group and mild to moderate group compared with control group. EDN was significantly higher in the severe group compared with mild to moderate group and control group. Likewise, ECP was significantly higher in the severe group and mild to moderate group compared with the control group (Table 1).

Correlation of Initial Total IgE, Blood Eosinophil Counts, ECP, and EDN With Disease Severity

SCORAD had significant correlations with EDN ($r = 0.365$, $P < .001$). Total IgE levels, blood eosinophil counts, and ECP failed to show significant correlations with SCORAD ($r = 0.154$, $P < .001$, $r = .081$, $P = .004$, and $r = 0.087$, $P = .003$, respectively) (Fig 1).

Correlation of EDN With ECP

EDN and ECP failed to show a significant correlation with each other ($r = 0.131$, $P < .001$) (Fig 2).