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INVESTIGATIVE REPORT

# Urinary 9α,11β-Prostaglandin $F_2$ in Children with Atopic Eczema/Dermatitis Syndrome: An Indicator of Mast Cell Activation?

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**To study the role of mast cell activation in children with atopic eczema/dermatitis syndrome (AEDS), we measured levels of urinary 9α,11β-prostaglandin $F_2$ (U-9α,11β-$PGF_2$) by enzyme-linked immunoassay in 88 children (mean age 44 months, range 3–135) with mild ($n=32$), moderate ($n=34$) or severe ($n=22$) AEDS, as well as in 72 non-atopic healthy controls. Fifty-eight of the children with AEDS were sensitized to common allergens (atopics) and 30 were not (non-atopics). Levels of U-9α,11β-$PGF_2$ were higher in children with severe AEDS (median 324 μg/mmol creatinine, quartiles 220–593) than in controls (198, 102–389, $p<0.001$), whereas levels of U-9α,11β-$PGF_2$ in moderate and mild disease were similar to controls. U-9α,11β-$PGF_2$ levels were similar in atopic and non-atopic children, but in severe AEDS those with atopy had higher levels than those without atopy ($p<0.05$). The results suggest a role for mast cell activation in children with severe AEDS. Exacerbation of AEDS caused by allergen triggering may involve mast cell activation, and U-9α,11β-$PGF_2$ may serve as a marker of this process.** ***Key words: atopy; mast cells; prostaglandins; urine.***

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Atopic eczema/dermatitis syndrome (AEDS) is common in childhood, and affects 10–15% of children in western countries (1, 2). The major immunopathogenic abnormality in AEDS is thought to involve T lymphocytes with hyperstimulatory activity and an increased expression of Th2 cytokines such as interleukin (IL)-4, IL-5 and IL-13 (2). There is further strong evidence that eosinophils are involved in the pathogenesis of AEDS, especially in chronic lesions (2, 3), whereas the role of mast cells is less clear (2, 4). In acute lesions of AEDS mast cells are present in normal numbers, but in various stages of degranulation, whereas in chronic lesions the mast cells are increased in number, but are generally fully granulated (2).

Prostaglandin $D_2$ ($PGD_2$) is the major cyclooxygenase product of mast cells (5). Few cells are capable of producing $PGD_2$ in comparable quantities to the mast cell, making it a good marker for mast cell activation (5–10). In the lung, the NADPH-dependent enzyme 11-ketoreductase converts $PGD_2$ to 9α,11β-prostaglandin $F_2$ (9α,11β-$PGF_2$), which is excreted intact into the urine (5). Recently, measuring urinary (U)-9α,11β-$PGF_2$ by direct enzyme immunoassay has been demonstrated to be sensitive and sufficiently specific to monitor activation of the $PGD_2$ pathway, thereby providing a valuable clinical tool to assess the status of mast cell activation *in vivo* (6). U-9α,11β-$PGF_2$ has been measured as a marker of mast cell activation in children and adults with asthma (7–10), but its value as a marker of mast cell activation in AEDS has not been studied.

The aim of this study was to evaluate the role of mast cell activation in AEDS in children by measuring levels of U-9α,11β-$PGF_2$, and to evaluate whether these levels may reflect disease activity and allergic sensitization in AEDS.

## MATERIALS AND METHODS

### *Study subjects*

Eighty-eight children with AEDS diagnosed by the criteria of Hanifin & Rajka (11) were included in the study (Table I). The children had no history of cough or wheeze indicative of asthma, and no history of allergic rhinitis or conjunctivitis. Assessment of disease severity was measured by a simple grading system suggested by Rajka & Langeland (12). The grading includes the extent of skin involvement, the course of the disease and intensity of itching. According to this grading, 32 children were assessed to have mild disease, 34 moderate disease and 22 severe disease.

Fifty-eight of the children were atopic and 30 were non-atopic. Atopic status was defined as having at least one positive skin prick test (SPT) for common allergens, including egg, milk, pea, hazelnut, peanut, cod, shrimp, *Cladosporium herbareum, Dermatophagoides pteronyssinus*, cat, dog, grass pollen and tree pollen (Soluprick, ALK, Denmark). We used histamine 10 mg/ml as the positive control and 0.9% saline as the negative control. A wheal of at least 3 mm in diameter was defined as a positive result.

Levels of urinary eosinophil protein X (U-EPX) (13) and urinary leukotriene E4 (U-LTE4) from the same children were observed earlier (unpublished observations), and possible correlations between these parameters and U-9α,11β-$PGF_2$ were calculated.

Seventy-two children without a history of AEDS or any other inflammatory disease and without a history of infection

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Table I. *Study subjects and levels of urinary 9α,11β- prostaglandin $F_2$(U-9α,11β-$PGF_2$) in children with atopic eczema/dermatitis syndrome (AEDS) (results are expressed as median and quartiles)*

| Group | No. | Atopy (yes/no) | Sex (M/F) | Age in months (mean, range) | U-9α,11β-$PGF_2$ (μg/mmol creatinine) |
|---|---|---|---|---|---|
| AEDS | 88 | 59/29 | 51/37 | 44, 3–135 | 219, 136–344 |
| Mild | 32 | 20/12 | 16/16 | 48, 6–122 | 193, 113–322 |
| Moderate | 34 | 23/11 | 22/12 | 41, 4–135 | 204, 106–322 |
| Severe | 22 | 16/6 | 13/9 | 42, 3–110 | 324, 220–593*† |
| Atopics | 58 | | 33/25 | 50, 3–99 | 219, 123–332 |
| Non-atopics | 30 | | 15/15 | 41, 4–135 | 216, 155–353 |
| Controls | 72 | 0/72 | 36/36 | 45, 2–152 | 198, 102–389 |

*$p<0.001$ versus controls.
†$p<0.01$ versus mild and moderate disease.

in the last 2 weeks served as controls (Table I). These children had no parental history of allergy, AEDS or asthma nor a history of any allergic disease such as asthma or wheezing, food allergy, or allergic rhinoconjunctivitis.

### *Study design and ethics*

Children with AD were consecutively included, as they were examined at the outpatient clinic as part of their routine examination. The controls were recruited at the outpatient clinic among children attending for non-inflammatory conditions, or from a baby clinic. The parents of all children answered a questionnaire about atopy and smoking habits in the family, and a history including infections in the last 2 weeks was taken.

The study was approved by the regional ethical committee, and signed statements of informed consent were obtained from all parents.

### *Laboratory analyses*

Urine was collected at home in the morning and kept in a refrigerator until aliquoting and freezing (−20°C) within 6 h.

U-9α,11β-$PGF_2$ was analysed by a specific enzyme-linked immunoassay (Cayman Chemical, MI, USA). The detection limit in the assay was <5 ng/l and the inter-assay variation was <12%. Urine creatinine levels were measured by Vitros 250 system (Ortho Clinical Diagnostics, USA). All measurements were done in duplicate. U-9α,11β-$PGF_2$ levels are presented as μg/mmol creatinine.

### *Statistical methods*

Non-parametric tests were used in comparative analysis. The Kruskal-Wallis analysis of variance was first applied to the groups. When this was significant, each pairing was analysed by the Mann–Whitney U-test. Results are presented as median and quartiles. Relationships between variables were evaluated by the Spearman rank correlation coefficient.

Data were analysed using the SPSS 11.5 statistical package (SPSS Inc., Chicago, USA). Tests were two-tailed and probabilities of <5% were considered significant.

## RESULTS

Levels of U-9α,11β-$PGF_2$ in different groups of children with AEDS and controls are presented in Table I. The levels of U-9α,11β-$PGF_2$ were higher in children with severe AEDS than in controls ($p<0.001$), and higher than in children with mild and moderate AEDS ($p<0.01$ for both). Levels of U-9α,11β-$PGF_2$ in children with mild and moderate AEDS were similar to those in controls. There was considerable overlap between all groups of children with AEDS and controls (Fig. 1).

Children with AEDS and atopy (positive SPT) had levels of U-9α,11β-$PGF_2$ similar to those who were non-atopic. No differences in U-9α,11β-$PGF_2$ were observed between children with a positive SPT to one allergen and those with a positive SPT to more than one allergen, or between those who had a positive SPT to aeroallergens and those with positive SPT only to food allergens. In children with severe AEDS, those with atopy had higher levels of U-9α,11β-$PGF_2$ than those without atopy ($p<0.05$) (Fig. 2), and levels of U-9α,11β-$PGF_2$ in the non-atopic children were similar to the controls.

Levels of U-9α,11β-$PGF_2$ were strongly correlated both to U-LTE4 ($r=0.58$, $p<0.001$) and to U-EPX ($r=0.52$, $p<0.001$). There was a negative correlation between U-9α,11β-$PGF_2$ and age ($r=-0.277$, $p<0.01$).

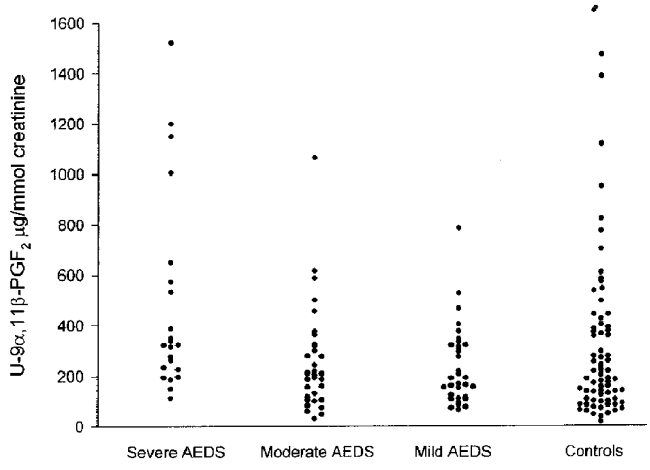


*Fig. 1.* Urinary 9α,11β-prostaglandin $F_2$ (U-9α,11β-$PGF_2$) in children with atopic eczema/dermatitis syndrome (AEDS) with severe ($n=22$), moderate ($n=34$) and mild disease ($n=32$) and in controls ($n=72$).

## DISCUSSION

In this study we have demonstrated for the first time that levels of the prostaglandin metabolite 9α,11β-PGF$_2$ in urine were elevated in children with severe AEDS, whereas children with mild or moderate AEDS had levels similar to controls. The results support a role for activation and degranulation of mast cells in children with AEDS, at least in those with severe disease.

A pathophysiological role for mast cells in AEDS has been suggested by the observation of mitosis of mast cells in lesions of AEDS (14). However, the histological features of AEDS and the activity of different cell types may depend on the acuity of the skin lesions (2). In acute lesions T lymphocytes dominate a perivenular inflammatory cell infiltrate in the dermis, whereas in chronic lesions eosinophil infiltration and degranulation probably play a major role (2, 3). Immunohistological studies of AEDS have demonstrated degranulation of mast cells in acute lesions of AEDS, and increased numbers of mast cells in chronic lesions (2, 15). In our study, all children with severe AEDS had a continuous course of the disease, suggesting that activation of mast cells plays a role in chronic lesions. However, in children with severe disease, the grading system did not separate those with a possible acute exacerbation in addition to a continuous course. Hence, the study design does not allow us to rule out if any involvement of mast cells during an acute exacerbation may have contributed to increased levels of U-9α,11β-PGF$_2$ in severe AEDS.

The role of allergy and the production of IgE in AEDS are not clear. Recent classifications tend to separate children with AEDS into two relative distinct groups. For the majority, there is an association with sensitization to environmental or food allergens. For a minority no such association seems to exist (4), but the association may be age-dependent (16). Immunodermatological investigations show differences in T-cell cytokine secretion and immunohistology between the two types of AEDS (4, 17). In our study, in children with severe disease only those with atopy demonstrated increased levels of U-9α,11β-PGF$_2$, suggesting a differential role for mast cell activation in the atopic and non-atopic types of AEDS.

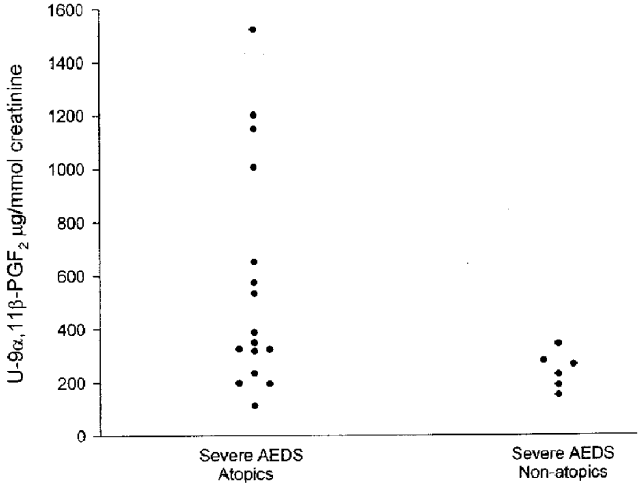


*Fig. 2.* Urinary 9α,11β-prostaglandin F$_2$ (U-9α,11β-PGF$_2$) in children with severe atopic eczema/dermatitis syndrome (AEDS) with atopy ($n=16$) and without atopy ($n=6$).

Differential levels of U-9α,11β-PGF$_2$ in atopic and non-atopic children with severe AEDS may reflect different immunohistological features between the two types of AEDS. However, it is also possible that triggering by allergens may have activated mast cells in the atopic children, reflected by higher levels of U-9α,11β-PGF$_2$. Recent studies suggest that cytokines expressed locally in the skin play several critical roles in AEDS (18). It has been demonstrated that in lesions of AEDS mast cells may be an initial source of IL-4, a cytokine which may drive the lesion T cells in a Th2 direction, and which is involved in allergen-specific triggering of AEDS (4). Furthermore, IL-13 is a T-cell-derived cytokine that shares several functions with IL-4, including the induction of IgE synthesis, and a recent study has suggested that mast cells are one of the major sources of IL-13 production in AEDS lesions (18). It is possible that expression of IL-4 and IL-13 by activation of mast cells may partly explain how mast cells are involved in the pathogenesis of AEDS in the atopic children with AEDS.

Levels of U-9α,11β-PGF$_2$ were negatively correlated to age, and it is possible that this marker may be age-dependent in early childhood; this should be considered when measuring this marker in young age. However, age dependency should not interfere with the results of the present study as age did not differ significantly between groups.

We have recently demonstrated that in children with severe AEDS, levels of both U-EPX (13) and U-LTE4 (unpublished observations) were higher in atopic than in non-atopic children. In the present study, we found a strong correlation between levels of U-9α,11β-PGF$_2$ and both U-EPX and LTE4. U-EPX is a marker of eosinophil degranulation (19), whereas U-LTE4 is a marker of cystinyl-leukotriene production from eosinophils and other inflammatory cells, including mast cells (20). The positive correlations may suggest that activation of both eosinophils and mast cells are involved in the same mechanisms in atopic children with severe AEDS, possibly after triggering by specific allergens.

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