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Original Article

Eosinophil Cationic Protein and Skin Prick Tests in Children With Steroid-Responsive Nephrotic Syndrome

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ABSTRACT

Background: Childhood nephrotic syndrome (MCNS) has often been associated with allergic symptoms. The association between atopy and nephrotic syndrome may have a causal or non-causal basis.

Objectives: To assess the atopic state of patients with SRNS.

Methods: Serum ECP levels were measured by chemiluminescent enzyme immunometric assay and skin prick tests were done in 32 children with SRNS and 10 age- and sex-matched healthy children without evidence of atopy. Out of the nephrotic patients, 19 children had active disease (Group I) and 13 were in remission (Group II). Among group I, 7 children were frequent relapsers (FR) while 12 were infrequent relapsers (IR) or non-relapsers (NR).

Results: We found that 37.5% of our patients had positive skin prick tests. Serum ECP levels were elevated in group I patients [median = 25.3 & Interquartile range (IQR) = 13.8-33.6 ng /ml] and group II patients [median = 14.2 & IQR = 12.0-20.2 ng/ml] compared to controls [median = 9.1 & IQR = 7.2-13.5 ng/ml, $p < 0.0001$ & 0.006 respectively]. Similarly, patients with negative skin prick tests in group I and group II had higher ECP levels compared to controls ($P = 0.007$ & 0.07 respectively). Among group I, ECP levels were higher in patients with positive skin prick tests to those with negative tests ($p < 0.0001$) and in FR compared to IR and NR ($p = 0.05$). Moreover, there was an association between the development of frequent relapses and positivity of skin prick tests (Fisher's Exact = 0.07 relative risk = 6.4 & confidence interval = 1.0-41.2).

Conclusions: Serum ECP levels are elevated in children with active SRNS. ECP could be considered as one of the neutralizing cations involved in the pathogenesis of proteinuria in these patients. Atopy could be assumed as a risk factor for the development of frequent relapses, so the value of a course of non-steroidal anti-inflammatory drug (as ketotifen) in frequently relapsing nephrotic children should be evaluated.

INTRODUCTION

Childhood nephrotic syndrome (MCNS) has often been associated with allergic symptoms such as bronchial asthma, atopic dermatitis, allergic rhinitis and elevated IgE levels and referred to involve immune dysfunction. In one study, boys with nephrotic syndrome were found to have three times higher incidence of bronchial asthma than the general population. Both boys and girls had about three times more allergic rhinitis and ten times more atopic dermatitis than the general population[1].

Activated eosinophils release granular proteins such as eosinophil cationic protein (ECP)[2]. Increased serum levels of ECP is related to the presence and activity of atopic disorders[3]. To date, there is only one reference that studies blood ECP levels in nephrotic children[4].

The association between atopy and nephrotic syndrome may have a causal or non-causal basis. The aim of this work is to

study serum eosinophil cationic protein (ECP) levels in children with steroid-responsive nephrotic syndrome (SRNS) and assess the correlation between atopy and course of the disease.

AIM OF THE WORK

To assess the atopic state of patients with SRNS.

SUBJECTS AND METHODS

This study was conducted on 32 children with SRNS, and 10 age-and sex-matched healthy children without evidence of atopy. Out of nephrotic patients, 19 children had active disease (Group I) and 13 children were in remission for 3 months after discontinuation of steroids (Group II). Among group I, 7 children were FR while 12 children were NR or IR, while all patients in group II were NR or IR. The diagnosis of these patients was done according to the criteria of International Study of Kidney Disease in Children[5]. Patients were recruited successively from Pediatric Nephrology Unit, Mansoura University Children's Hospital, Mansoura, Egypt. The characteristics of these patients are shown in Table 1.

Skin prick tests were done to all patients in Allergy and Clinical Immunology Unit, Mansoura University Children's Hospital. Six allergens were used: house dust (HD), house dust mite (HDM), cotton dust (CD), mixed pollens (MP), straw (St) and hay dust (Hay D). Serum ECP was measured using IMMULITE ECP Kit (Diagnostic Products Corporation, Los Angeles, CA, USA). IMMULITE ECP is a solid-phase two-site, chemiluminescent enzyme immunometric assay[6].

Statistical analysis was done using SPSS computer package (version 9). Normality of data was tested using Kolmogorow-Simirnovof Fit Test. Data were expressed as median and interquartile range. Non-parametric tests were applied. The significance of association between the nephrotic state and positivity of skin prick tests was tested by two-tail Fisher's Exact test. The relative risk was calculated by Odds ratio.

RESULTS

Table 1 shows that 12 patients (37.5%) have positive skin prick tests, nine of them have reaction to HDM, 2 to MP and one

Table 1: Characteristics of the studied children

	Group I (n = 19)	Group II (n = 13)
Male / Female	13/6	8/5
Age (mean ± SD)	5.7 ± 2.2 years	5.9 ± 2.8 years
Positive skin test	7 (36.8%)	5 (38.9%)
Bronchial asthma	4 (21.1%)	4 (30.8%)
Atopic dermatitis	2 (10.5%)	0
Allergic rhinitis	1 (5.3%)	1 (7.7%)

patient has reaction to HDM, MP and St. Bronchial asthma is found in 8 patients (25%) while 2 patients (6.2%) have atopic dermatitis and 2 patients (6.2%) have allergic rhinitis.

Serum ECP levels are significantly elevated in group I patients ($p < 0.0001$) and group II patients ($p = 0.006$) compared to controls (Table 2). No significant difference in ECP levels is present between group I and group II patients ($p = 0.16$).

Nephrotic patients with negative skin prick tests during activity of the disease or in remission have higher serum ECP levels compared to controls ($p = 0.007$ & 0.07 respectively) as shown in table 2. No significant difference in ECP levels is noticed between the 2 groups ($p = 0.27$).

Figure 1 shows that among group I patients, serum ECP levels are elevated in patients with positive skin prick tests compared to those with negative tests ($p < 0.0001$) and in FR compared to IR and NR ($p = 0.05$).

Table 3 demonstrates the association between the positivity of skin prick tests and development of frequent relapses (Fisher's Exact = 0.07, relative risk = 6.4 & confidence interval = 1.0 - 41.2.

Table 2: Serum ECP levels (ng/ml) in all patients, patients with negative skin prick tests during active disease (Group I) and remission (Group II) and controls

All patients				
	n	**Median**	**IQR[a]**	**p[b]**
Group I	19	25.3	13.8-33.6	<0.0001
Group II	13	14.2	12.0-20.2	0.006
Patients with negative skin prick tests				
	n	**Median**	**IQR[a]**	**p[b]**
Group I	12	14.4	12.2-22.8	0.007
Group II	8	12.6	10.1-14.2	0.07
Controls				
	n	**Median**	**IQR[a]**	**p[b]**
	10	9.1	7.2-13.5	

[a] *IQR Interquartile range.* [b] *P Group I or II versus controls.*

Table 3: Association between the positivity of skin prick tests and the nephrotic state

	Skin test		
Nephrotic state	**Negative**	**Positive**	**Total**
Infrequent and non-relapsers	18	7	25
Frequent relapsers	2	5	7
Total	20	12	32

Fisher's Exact = 0.07
Relative risk = 6.4, confidence interval = 1.0 - 41.2

DISCUSSION

This study demonstrates that 37.5% of children with SRNS have associated atopy. Yap et al.[7] have found higher incidence of atopy in SRNS children than general population. Also, positive skin prick tests were found in 37.5% of our patients. Radio absorbent skin prick tests scores for dermatophagoides farina and dermatophagoides ptenonyssinus were found to be positive in some patients with adult onset minimal change nephrotic syndrome[8].

Eosinophil activation accompanies a wide range of inflammatory conditions, including bronchial asthma, atopic dermatitis, rhinitis and autoimmune diseases. Activated eosinophils degranulate to release four highly basic proteins into the surrounding tissue. Among the four basic granule proteins ECP has proven to be a useful monitor for many active inflammatory diseases[2].

We found a significant increase in serum ECP levels in children with active SRNS compared to controls. After remission, ECP did not significantly decrease compared to children with active disease and still significantly higher than that in controls. Araujo et al.[4] found that serum blood levels were elevated in nephrotic children independently of simultaneous atopy.

The increased serum ECP levels during the activity of the disease may reflect the active participation of ECP in the pathogenesis of the disease rather than the associated atopic state alone since patients with negative skin prick tests still had elevated ECP levels compared to controls. The increased ECP levels may point to the occurrence of allergic inflammation with eosinophil activation as one of the pathogenic mechanisms underlying SRNS. Levin et al.[9] postulated that a generalized loss of glomerular basement membrane (GBM) negative charges occurs in nephrotic syndrome and that it is due to neutralization rather than absence of anionic groups. Other studies demonstrate the presence of cationic substances that bind to and neutralize the negative charge on GBM leading to proteinuria[10, 11]. On these bases we may suggest that ECP is at least one of these neutralizing cations involved in pathogenesis of proteinuria in these patients.

The persistence of elevated ECP levels after remission in our cases may be due to sampling of blood early in remission when eosinophils are still active and producing ECP. However long term follow up is required to confirm this assumption. A similar result is found in patients with allergic rhinitis who received immunotherapy for two years and inspite of clinical improvement still have significantly higher serum ECP levels than non-atopic controls[12].

This study also reveals that serum ECP levels are significantly higher in patients with positive skin prick tests compared to those with negative tests and in FR compared to IR and NR. Patients with positive skin prick tests are 6.4 more times liable to develop frequent attacks of nephrotic state than those with negative skin prick tests. So we could assume that atopy predisposes children with nephrotic syndrome to develop frequent relapses. In previous studies the prevalence of atopy was higher in steroid-dependent nephrotic

syndrome[13], and in children with frequent relapses[14].

In conclusion, serum ECP levels are elevated in children with active SRNS. ECP could be considered as one of the neutralizing cations involved in the pathogenesis of proteinuria in these patients. Atopy could be assumed as a risk factor for the development of frequent relapses, so the value of a course of non-steroidal anti-inflammatory drug (as ketotifen) in frequently relapsing nephrotic children should be evaluated.

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