Serum Eosinophilic Cationic Protein (ECP) in Asthmatic Malaysian Children

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Introduction
The understanding of the pathophysiologic processes that result in bronchial asthma have led to the recognition of the many cellular and immuno-chemical components that are pivotal in the inflammatory cascade of this disorder. Eosinophil infiltration and activation in the airways is an important aspect of childhood asthma. Its activation is associated with the release of granule-derived proteins, the most cytotoxic being eosinophilic cationic protein (ECP) and major basic protein 1, both of which have been demonstrated to cause airway damage. Blood measurements of ECP can be done using a commercially available assay and may be used as a tool to reflect eosinophil activation in the lung. Nonetheless, its relevance and usefulness in a tropical environment and where eosinophilic activation may be due to previous worm infestation are less well documented. We therefore set out to evaluate serum ECP in 20 asthmatic Malaysian children compared to non-asthmatic healthy controls.

Materials and Methods
We recruited 20 children attending the Paediatric Asthma Clinic who had an established diagnosis

Summary
Eosinophilic inflammation in the airways is important in the pathogenesis of childhood asthma. Serum eosinophilic cationic protein (ECP), a marker of eosinophil activation was measured in 20 asthmatic children and 19 non-asthmatic controls. There was no difference in the socio-demography, passive smoke exposure, urinary cotinine levels and family history of asthma between the 2 groups. The median serum ECP in asthmatic children was 27.0 mcg/L (IQ1 8.8, IQ3 59.0), which was higher than in non-asthmatic controls (5.9 mcg/L (IQ1 3.0, IQ3 11.9), p=0.002).

An elevated serum ECP level can be helpful as supportive evidence in the diagnosis of bronchial asthma in Malaysia children.

Key Words: Childhood asthma, Serum eosinophilic cationic protein
of moderate to severe bronchial asthma and were receiving inhaled prophylaxis. Asthmatic children who received a course of oral corticosteroids in the preceding 4 weeks were excluded. Nineteen non-asthmatic healthy controls were recruited from children admitted for elective surgical procedures. Non-asthmatic healthy controls were excluded if they had any history of allergic rhinitis, food allergy or atopic eczema. All these children were aged between 5 and 15 years and did not have any previous history of an immunodeficiency disorder. Ethics committee approval was obtained for the study and parental informed consent was given before inclusion into the study. Each parent was interviewed individually to determine the socio-demographic profile and passive smoke exposure prior to collection of blood samples for serum ECP and urine cotinine levels. A peak expiratory flow rate (PEFR) was also performed 3 times for each subject and the best was taken into account at the time of recruitment.

Data collected was analyzed with SPSS version 7.0 (SPSS Inc., Chicago, IL, USA) using Windows 1998 operating system. Quantitative data was compared using the Mann-Whitney U non-parametric test and the student's t test where appropriate. Dichotomous variables were compared using the Fishers exact test. A p value of less than 0.05 was considered significant.

**Measurement of serum ECP**

Blood samples for the determination of serum ECP were collected by drawing venous blood and transferring 2 ml directly into a 2.5ml silicone-containing glass tube (Hemogard SST® tube; Becton Dickson Vacutainer Systems). The blood was then allowed to clot at 20 - 24°C for 90 minutes after which it was centrifuged at 1300 G for 10 minutes. The resultant serum was then collected and stored at -20°C till analysis. The serum concentrations of ECP were measured with a commercially available fluoro-immunoassay in accordance with the manufacturer's guide and instructions (ImmunoCAP ECP kit; Pharmacia CAP Diagnostics AB, Upppsala, Sweden).

### Table I: Socio-demography profile of 20 asthmatic children and 19 non-asthmatic controls

<table>
<thead>
<tr>
<th>Clinical Parameter</th>
<th>Asthmatic children (n=20)</th>
<th>Non-asthmatic controls (n=19)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>10.0 (IQ1 6.3, IQ3 14.0)</td>
<td>12.0 (IQ1 10.0, IQ3 14.0)</td>
<td>0.23*</td>
</tr>
<tr>
<td>Sex</td>
<td>Boys 10 (50%)</td>
<td>6 (32%)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>Girls 10 (50%)</td>
<td>13 (78%)</td>
<td></td>
</tr>
<tr>
<td>Family history of asthma</td>
<td>11 (55%)</td>
<td>8 (42%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Either parent smokes</td>
<td>9 (45%)</td>
<td>6 (37%)</td>
<td>0.85</td>
</tr>
<tr>
<td>Median urinary cotinine levels (ng/ml)</td>
<td>1.1 (IQ1 0.7, IQ3 2.7)</td>
<td>0.7 (IQ1 0.0, IQ3 1.2)</td>
<td>0.08*</td>
</tr>
</tbody>
</table>

*Mann-Whitney U non-parametric test*
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importantly, the test is a non-invasive measurement and can be readily done with commercially available kits with several useful clinical applications.

The diagnosis of asthma in children may not always be simple due to its heterogeneous presentation, as some children may have persistent but non-specific respiratory symptoms. In addition, very young children cannot perform the necessary pulmonary function tests required for the diagnosis. Situations like these make commitment to regular long term inhaled anti-inflammatory therapy solely on clinical suspicion difficult and illustrate the importance of having available tools like serum ECP to support a suspected diagnosis of childhood asthma.

Disease severity and symptomatology in asthma is determined by airway inflammation; for which treatment strategies adopted are directed towards reducing these inflammatory processes. Asthma symptoms and pulmonary function tests in children who can comply, presently guide managing airway inflammation and adjusting treatment in childhood asthma. Although these clinical parameters are important, they indirectly reflect airway inflammation; therefore normal lung function and symptom free asthma does not necessarily indicate that there is no ongoing airway inflammation. The measurements of immuno-chemical mediators like serum ECP have been shown to reflect disease severity and ongoing airway inflammation in asthmatic children. Our study subjects with PEFR of less than 70% had a tendency to have higher serum ECP levels also illustrates this relationship; however this observation failed to reach statistical significance possibly due to the small sample size. Serial monitoring of serum ECP may therefore provide a potentially important adjunct in optimizing and tailoring treatment in asthmatic children. It has been shown that using an elevated serum ECP of > 20 mcg/L as an indication of increasing the corticosteroid dose in asthma management improved pulmonary function, decreased acute exacerbations and improved

Figure 1: Serum ECP in 20 asthmatic children and 19 non-asthmatic controls

Results

There was no significant difference in the socio-demographic profile, family history of bronchial asthma, passive smoke exposure and urinary cotinine levels between the asthmatic children recruited and the non-asthmatic controls [Table I].

The median serum ECP in asthmatic children was 27.0 mcg/L (IQ1 8.8, IQ3 59.0); which was higher than in the non-asthmatic controls [5.9 mcg/L (IQ1 3.0, IQ3 11.9), p = 0.002] [Figure I].

Five children who had a PEFR of less than 70% of their usual best showed a higher serum ECP than those with better PEFR (45.5 ± 22.8 vs 34.6 ± 31.0 mcg/L, p = 0.523) but this difference failed to reach statistical significance.

Discussion

An elevated serum ECP as a reflection of eosinophil activation in bronchial asthma is a fairly consistent finding and provides a potential tool or marker for this disease. More

Figure 1: Serum ECP in 20 asthmatic children and 19 non-asthmatic controls

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asthmatic well-being scores in adults. These improvements were also associated with a falling trend in the serial serum ECP measurement.

An elevated serum ECP can be used as supportive evidence in the diagnosis of bronchial asthma in Malaysian children. As the cost of serum ECP studies is prohibitive, routine serum ECP measurement in childhood asthma cannot be advocated and should be reserved for the poorly defined cases with persistent but less specific respiratory symptoms. Its serial use to monitor airway inflammation and guide childhood asthma management will require further evaluation.

Acknowledgement
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References