Questionnaire

PULMONOLOGY CLINIC
DEPT. OF PAEDIATRIC MEDICINE
NORTH BENGAL MEDICAL COLLEGE, SILIGURI

GENERAL INFORMATION:

❖ Name ____________________________
❖ Father’s/Mother’s name ________________________
❖ Address ____________________________
❖ Phone ____________________________
❖ Date of Birth ________________________
❖ Sex ________
❖ Caste/Ethnic group ____________________

Registration No. - ________________________
Date: ________________________
### DATE:

<table>
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<tr>
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**PRE:**

**POST:**
NAME: ______________________________________________________

AGE: ___________ SEX: ___________ HEIGHT: ___________ WT: ___________

Immunization History

Vaccines not taken:

DPT | OPV | MEASLES | TYPHOID | VARICELLA | HIB | INFLUENZA | HEP-A | MMR

Exclusive Breastfeeding: ________________________________

When symptoms first started?

☐ < 1yr ☐ 1-3 yrs

☐ 4-6 yrs ☐ 7-12 yrs

How it started and progressed?

____________________________________________________

Family History: Parents / Siblings

ASTHMA | ECZEMA | ALLERGIC RHINITIS | URTICARIA | TB | OTHERS

Significant Past History

_____________________________________________________

-2-
KNOWING COUGH

1. Is it associated with fever? Yes / No
2. Is it associated with running nose initially? Yes / No
3. When the cough is worst? Day / Night
4. Any early morning cough? Yes / No
5. Does sleep get disturbed with cough / breathlessness? Yes / No
6. What increases cough?
7. Is cough associated with vomiting? Yes / No
8. Any relation with exercise? Yes / No
9. Any relation with crying, laughing, depression or other emotions? Yes / No
10. What relieves symptoms?
11. Can you hear wheeze? Yes / No
12. History of hospitalization for cough / breathlessness. Yes / No
13. History of any inhalers used / prescribed. Yes / No
14. Seasonality. Yes / No

ASSOCIATED CO-MORBIDITIES

- ALLERGIC RHINITIS
- EAR DISCHARGE
- FEATURES OF SINUSITIS
- FEATURES OF GERD
- ALLERGIC CONJUNCTIVITIS
HABITAT:

SANITATION:

BATH + TOILET ........................................... (in the house / Community)

WASHING ...................................................(Laundry / Local arrangement)

HYGIENIC CONDITION AROUND HOUSE (Good / Fair / Poor)

NUTRITION .................................................

GENERAL:

OVERCROWDING ...........................................(Yes / No)

COOKING MODE ...........................................(Smokeless / With smoke)

TRIGGERS:

<table>
<thead>
<tr>
<th>PET</th>
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<tbody>
<tr>
<td>PLANTS</td>
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<tr>
<td>SMOKE</td>
<td></td>
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<tr>
<td>CIG. SMOKE</td>
<td></td>
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<tr>
<td>MOSQUITO REPELLENT</td>
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<tr>
<td>INSECTICIDE</td>
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<td>BODY SPRAY</td>
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<td>PERFUMES</td>
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<td>ROOM FRESHNER</td>
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<td>TOYS</td>
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<td>CARPET</td>
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<td>FOOD</td>
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<td>CHANGE IN CLIMATE</td>
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<tr>
<td>EXERCISE</td>
<td></td>
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<td>OTHERS</td>
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ANY COMMENT:
PEAK FLOW METER READING:

<table>
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<tr>
<th>VISIT</th>
<th>1(^{ST})</th>
<th>2(^{ND})</th>
<th>3(^{RD})</th>
<th>4(^{TH})</th>
<th>5(^{TH})</th>
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TC, DC: ..........................................................

Mx TEST: ..........................................................

IgA + IgE LEVEL: ..................................................

CD4 COUNT: ..........................................................
## Gradation & Treatment

<table>
<thead>
<tr>
<th>Steps</th>
<th>Symptoms</th>
<th>FEV1 / PEF Variable</th>
<th>Specific Note</th>
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</thead>
<tbody>
<tr>
<td>Mild Intermittent</td>
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<td></td>
<td></td>
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<tr>
<td>Mild Persistent</td>
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<tr>
<td>Moderate Persistent</td>
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<tr>
<td>Severe Persistent</td>
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### Drug History

<table>
<thead>
<tr>
<th>Name</th>
<th>Duration</th>
<th>Name</th>
<th>Duration</th>
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</tbody>
</table>

### Results After Starting Medication If Any

- [ ] Improved
- [ ] No Change
- [ ] Deteriorated

### Any Specific Response to Medication

<table>
<thead>
<tr>
<th>PEFR</th>
<th>WT</th>
<th>Height</th>
<th>Date</th>
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<tbody>
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<tr>
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### Advice (Medication)

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<th>Name</th>
<th>Duration</th>
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</tbody>
</table>

### Devices

- Technique: [ ]
- Duration: [ ]
- Maintenance: [ ]

### Other Advice:

- [ ]
CHEMICALS, REAGENTS & KITS

1. 20 X SSC (pH 8.0)
   NaCl - 175.3g
   Na-Citrate - 88.2g
   Dissolve in 1000ml distilled water and adjust pH to 8.0 with NaOH.

2. HSB (High Salt Buffer) pH 7.6
   10mM Tris-HCl - 605.7mg
   10mM KCl - 372.8mg
   10mM MgCl₂ - 1.02g
   0.4 M NaCl - 11.69 g
   2mM EDTA - 404.7g
   Dissolve in 1000 ml distilled water and adjust pH to 7.6.

3. 10% SDS
   Dissolve 1g SDS in 10ml distilled water.

4. 50mM KCl
   3.728g of KCl in 1000ml distilled water.

5. 4M NaCl
   116.9g NaCl in 500ml distilled water.

6. 4M NaOH
   Dissolve 50g of NaOH in 500ml distilled water. Filter the solution through Whatman no. 3 filter paper.

7. Tris hydrochloride (Tris-HCl), Mol. Wt. 157.6 (Himedia)

8. Tris NH₄Cl
   TRIS - 20.6g/L dH₂O
   NH₄Cl - 0.83g/100ml dH₂O
9. **RCLB (Red Cell Lysis Buffer)**

NH₄Cl - 4.15 g  
0.1M Tris HCL - 50mL  
Make up to 500mL with distilled water and adjust pH to 7.5 ±0.2.

10. **Proteinase-K Solution (8mg/ml)**

Dissolve 8mg Proteinase-K to 1ml distilled water.

11. **Phenol Chloroform (4:1)**

4 parts Phenol + 1 part Chloroform. The pH of phenol should be adjusted to 8.5-9.0 adding Tris-HCl.

12. **Deoxyribonucleotide Triphosphate (dNTPs) set: Bangalore Genei, India**

The deoxyribonucleotide triphosphates are the monomers of DNA polymer consisting of dATP, dCTP, dGTP, dCTP. The dNTPs are used at saturating concentration in PCR amplification of DNA.

13. **PCR Buffer with MgCl₂ (Bangalore Genei, India)**

The PCR buffer is optimized for use in PCR experiments. Generally, the PCR buffer is supplied along with Taq Polymerase by the commercial companies.

14. **Ethidium Bromide (Gibco BRL, USA): 0.5µg/ml TBE Buffer**

15. **Gel Loading Dye/ Solution**

0.05% Bromophenol blue - 50 mg  
4.0% Sucrose - 20 g  
0.1 M EDTA - 1.46 g  
0.5% SDS - 250 mg  
Dissolve EDTA in 25ml distilled water by adjusting the pH to 8.0 with 5N NaOH and add bromophenol blue. Once dissolved add sucrose and finally SDS. Adjust the final volume to 50 ml and stir at 80°C to make the solution viscous. 1 volume of gel loading solution is optimal to 1-4 volumes of sample. Bromophenol blue serves as the tracking dye while
sucrose adds density and facilitates sample loading. EDTA is included to terminate the action of intrinsic DNAase activity. SDS helps to dissociate DNA-protein complexes which can otherwise interfere the electrophoresis.

16. Taq DNA Polymerase (Bangalore Genei, India)

17. Genomic DNA (100ng -50µg)

18. 10 X TBE Buffer

0.9 M TRIS       -       109.06 g
0.02 M EDTA      -       7.44 g
0.9 M Boric Acid -       55.647 g

Dissolve in 1000ml distilled water and store at 4°C. Prepare 1X as working buffer.

19. TE Buffer/Solution

1mM TRIS            -       121.16 mg
0.1 mM EDTA         -       37.224 mg

Dissolve in 950ml distilled water and adjust pH to 7.5. Adjust the final volume to 1000ml adding distilled water.

20. Phosphate Buffered Saline (PBS), pH 7.2 (Himedia, India)
HUMAN IL-4 ELISA KIT (Pierce Biotechnology, Inc., Rockford)

INSTRUCTIONS

Human IL-4 ELISA Kit

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
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<tbody>
<tr>
<td>EH2IL4</td>
<td>Human Interleukin-4 (IL-4) ELISA Kit, sufficient reagents for 96 determinations</td>
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<tr>
<td></td>
<td>Kit contents:</td>
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<tr>
<td></td>
<td>Anti-Human IL-4 Precoated 96-well Strip Plate, 1 each</td>
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<tr>
<td></td>
<td>Lyophilized Recombinant Human IL-4 Standard, 2 vials</td>
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<tr>
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<td>Standard Diluent, 14 ml</td>
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<td></td>
<td>Biotinylated Antibody Reagent, 8 ml</td>
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<td>30X Wash Buffer, 50 ml</td>
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<td></td>
<td>Streptavidin-HRP Concentrate, 75 µl</td>
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<tr>
<td></td>
<td>Streptavidin-HRP Dilution Buffer, 13 ml</td>
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<tr>
<td></td>
<td>TMB Substrate, 12 ml</td>
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<tr>
<td></td>
<td>Stop Solution, 13 ml, contains 0.16 M sulfuric acid</td>
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<td>Adhesive Plate Covers, 4 each</td>
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<table>
<thead>
<tr>
<th>EH2IL45</th>
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<tbody>
<tr>
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<td>Kit contents:</td>
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<tr>
<td></td>
<td>Anti-Human IL-4 Precoated 96-well Strip Plate, 5 each</td>
</tr>
<tr>
<td></td>
<td>Lyophilized Recombinant Human IL-4 Standards, 5 vials</td>
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<td>Standard Diluent, 75 ml</td>
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<td>TMB Substrate, 5 × 13 ml</td>
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<td>30X Wash Buffer, 200 ml</td>
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<td>Stop Solution, 55 ml, contains 0.16 M sulfuric acid</td>
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<td>Adhesive Plate Covers, 30 each</td>
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</table>

For Research Use Only. Not for use in diagnostic procedures.

Storage: For maximum stability, store in a non-defrosting -20°C freezer and refer to the expiration date for frozen storage on the label. Alternatively, store at 2-8°C and refer to the expiration date for refrigerated storage. Once thawed, store at 4°C until the expiration date for refrigerated storage. Kit is shipped on dry ice.

Introduction

This Thermo Scientific ELISA Kit is for measuring human IL-4 in serum, plasma, urine and culture supernatants.
**HUMAN IFN-γ ELISA KIT** (Pierce Biotechnology, Inc., Rockford)

**INSTRUCTIONS**

**Human IFNγ ELISA Kit**

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<th>Number</th>
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<tbody>
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<tr>
<td>EHIFNG2</td>
<td>Human Interferon gamma (IFNγ) ELISA, sufficient reagents for 2 × 96 determinations</td>
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<tr>
<td>EHIFNG5</td>
<td>Human Interferon gamma ELISA, sufficient reagents for 5 × 96 determinations</td>
</tr>
</tbody>
</table>

**Kit Contents**

- Anti-human IFNγ Precoated 96-well Strip Plate: 1 each
- Iyophilized Recombinant Human IFNγ Standard: 2 vials
- Standard Diluent, contains 0.1% sodium azide: 12mL
- 30X Wash Buffer: 50mL
- Biotinylated Antibody Reagent, contains 0.1% sodium azide: 8mL
- Streptavidin-HRP Concentrate: 7.5μL
- Streptavidin-HRP Dilution Buffer: 14mL
- TMB Substrate: 2×13mL
- Stop Solution, contains 0.16M sulfuric acid: 13mL
- Adhesive plate covers: 6 each

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**Storage:** For maximum stability, store in a non-defrosting -20°C freezer and refer to the expiration date for frozen storage on the label. Alternatively, store at 2-8°C and refer to the expiration date for refrigerated storage. Once thawed, store at 4°C until the expiration date for refrigerated storage. Kit is shipped on dry ice.

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**Introduction**

The Thermo Scientific Human Interferon gamma (IFNγ) ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of IFNγ in serum, plasma, urine and culture supernatant.
IgE ELISA KIT (Accu-Bind, Monobind Inc., USA)

CRP KIT (IMMUNOSTAT®, RANBAXY FINE CHEMICALS LTD.)


A study of the association of childhood asthma with HLA alleles in the population of Siliguri, West Bengal, India

M. Lama1,2, M. Chatterjee3 & T. K. Chaudhuri1

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2 Department of Zoology, The University of Burdwan, Golapbag, West Bengal, India
3 Department of Pediatrics, North Bengal Medical College & Hospital, Siliguri, India

Key words
allelic group; asthma; children; DRB1*03; human leukocyte antigen

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Received 5 March 2014; revised 2 May 2014; accepted 27 May 2014
doi: 10.1111/tan.12403

Abstract
Asthma is a heterogeneous disease for which a strong genetic basis is firmly established. It is a complex disorder influenced by gene–environment interaction. Human leukocyte antigen (HLA) genes have been shown to be consistently associated with asthma and its related phenotypes in various populations. The aim of this study was to determine the frequency of the selected HLA classes I and II allelic groups in asthmatic and control groups. HLA typing was performed using polymerase chain reaction-sequence-specific typing (PCR-SSP) method. The allele frequency was estimated by direct counting. Frequency of each HLA allelic group was compared between asthmatic group and control group using $\chi^2$ test. $P$-value was corrected by multiplying with the number of the allelic groups studied. Odds ratio (OR) and its corresponding 95% confidence interval (CI) for each allelic group were calculated using GRAPHPAD INSTAT. The results of this study showed a significantly higher frequency of HLA-DRB1*03 in asthmatics than in controls (11.43% vs 3.64%, OR = 3.78, 95% CI = 1.61–8.83, $P = 0.0025$, $P_{corr} < 0.05$). Analysis of HLA alleles in low and high total serum immunoglobulin E (IgE) level in asthmatics revealed no significant association. HLA-DRB1*03 may be implicated in the susceptibility to asthma in the pediatric population.

Asthma is a common chronic inflammatory disease of the conducting airways which undergo distinct structural and functional changes, leading to non-specific bronchial hyper-responsiveness (BHR) and airflow obstruction that fluctuates over time (1). It is a complex disease determined by many genes and molecular mechanisms. Indeed, the genetic factors contribute to disease susceptibility but the manifestation of the disease is modulated by environmental exposures and the interactions between both of these components. Candidate-gene and linkage studies followed by positional cloning have already provided a large number of genes accountable for the susceptibility to asthma (2). In addition, numerous genome-wide association studies (GWASs) published in recent decade have identified several genetic loci to be associated with asthma and/or its related phenotypes in different populations (3–5).

The major histocompatibility complex (MHC), designated as human leukocyte antigen (HLA) in human, genes map on chromosome 6p21 play an important role in the regulation of the immune system (6). Many studies have documented that 6p21 region is strongly linked to atopic phenotype and asthma and it is considered a major locus influencing allergic diseases (7–9). Further, numerous studies have investigated the association of HLA alleles and/or antigens with asthma. Some of the earlier studies have reported the association of various HLA class I alleles/antigens (10, 11), while large number of studies have investigated the association of HLA class II alleles/haplotypes with asthma in different populations. Choi et al. reported a strong association between HLA-DRBI*0301 and aspirin-intolerant asthma in Korean population (12). In another study, Movahedi et al. reported the association of HLA class II alleles with asthma and high total IgE levels in Iranian children (13). Lara Marquez et al. have reported the association of HLA class II alleles with asthma and high total IgE levels in Venezuelan population (14). Similarly, Guo et al. also reported the association of HLA-DQ genes with asthma and positive specific IgE response to Dermatophagoides species in Chinese population (15). In a study on Korean population, Kim et al. have reported the association of HLA haplotype with isocyanate-induced occupational asthma (16). Another study on Greek children with allergic asthma revealed the association of HLA-DRBI*04...
and DQAI*0501 with susceptibility to mites sensitive asthma (17).

Siliguri is located in the foothills of the Himalayas. It is a city in the Indian state of West Bengal and known as the gateway to North East India, Bhutan, Nepal and Bangladesh. The adjoining areas of Siliguri are comprised of tea gardens and villages. People of diverse communities are the inhabitants of this region. The majority of population belongs to Bengali community while other minority communities include Nepali, Marwari, Bihari and native tribal community (http://en.wikipedia.org/wiki/Siliguri. Accessed on 16.1.2014).

There is a paucity of data particularly on HLA association with asthma in pediatric population of this region. Therefore, the purpose of this study was to determine the frequencies of some of the selected HLA classes I and II allelic groups in asthmatic and control subjects.

A total of 105 unrelated asthmatic children, aged 3–12 years, were recruited in this study from the Out Patient Department of Pediatrics, North Bengal Medical College & Hospital, Siliguri, West Bengal, India. Asthma was diagnosed by a physician based on the physical examination, clinical symptoms, response to bronchodilators, etc. Age and sex matched 110 unrelated children of the same ethnic background were included as controls. The criteria for the selection of control subjects included: no history of allergy, airway hyper-responsiveness, upper respiratory tract infections and lung disease. The study was approved by the Institutional Ethics Committee, University of North Bengal. The written informed consent was obtained from the parents/guardians for their children to participate in the study. The blood samples were collected under appropriate conditions by vein puncture method and the demographic and clinical characteristics of the participating subjects were collected using the questionnaire.

Genomic DNA was extracted from the venous blood using Phenol-Chloroform method. Molecular typing of the selected HLA classes I and II allelic groups was performed by polymerase chain reaction using sequence-specific primers (PCR-SSP). We studied the allelic groups which have been reported previously to be associated with childhood asthma in various populations (10, 11, 13, 17–25). The primer sequences of HLA class I allelic groups were taken from Bunce et al. (26) and of class II allelic groups were obtained from Zhu et al. (27) and Sacchetti et al. (28). The primers were synthesized and supplied by Integrated DNA Technologies (IDT).

A 25 μl reaction mix was prepared and the reaction was carried out in a thermal cycler (MJ Mini™ Gradient Thermal Cycler, PTC-1148, Bio-Rad, Singapore). Touchdown PCR was adopted for the amplification of the DNA with the following reaction conditions: Initial denaturation at 94°C for 3 min followed by five cycles of 94°C denaturation for 30 s, 2°C higher annealing temperature (varying for different alleles) for 35 s and 72°C extension for 40 s, and 25 cycles of 94°C denaturation for 30 s, 2°C lower annealing temperature for 50 s and 72°C extension for 1 min. A final extension at 72°C for 7 min was performed. In order to avoid technical error in amplification, two different internal controls were used. An internal control (hemoglobin gene) of 256 bp was used for typing of class I allelic groups and another internal control (a fragment of human growth hormone gene) of 439 bp was used for class II allelic groups. The PCR products were electrophoresed in 2% prestained agarose gel. We also analyzed the association of HLA allelic groups with the elevated level of total serum Immunoglobulin E (IgE) in asthmatic subjects. Available data of total serum IgE of 70 asthmatic subjects of our earlier study were used for this purpose (29).

The frequencies of the HLA allelic groups were determined by direct counting. The frequency of each allelic group observed in the asthmatic group was compared to control group using χ² test. P-values were corrected (Pcorr) by multiplying with the number of allelic groups studied. A P-value <0.05 was considered to be statistically significant. The odds ratio (OR) with its corresponding 95% CI for each HLA allelic group was calculated using the GRAPH PAD IN STAT version 3.10.

The characteristics of asthmatic and control subjects are summarized in Table 1. The result showed the higher frequencies of A*01 (15.71% vs 10.45%), A*03 (17.62% vs 14.09%), A*24 (16.67% vs 13.64%), A*26 (12.86% vs 8.64%), B*08 (21.90% vs 18.64%), B*37 (19.05% vs 15.45%), DRB1*01 (6.67% vs 3.18%), DRB1*03 (11.43% vs 3.64%), DQBI*0201 (18.57% vs 14.09%), DQBI*0302 (14.29% vs 10.00%) and DQAI*0501 (14.76% vs 10.91%) and the lower frequencies of A*11 (8.57% vs 10.00%), B*51 (6.19% vs 8.18%), B*52 (4.29% vs 5.91%), DRB1*04 (15.24% vs 18.18%) and DQBI*0603/8 (2.86% vs 4.55%) in asthmatic group than in controls, respectively (Table 2). Among these, the frequency of HLA-DRB1*03 was significantly higher in asthmatics than in controls (11.43% vs 7.64%, OR = 3.78, 95% CI = 1.61–8.85, P = 0.0025, Pcorr < 0.05) while the frequencies of rest of the allelic groups did not show significant difference between the two groups. When the frequencies of HLA-DRB1*03 were compared between asthmatic

| Table 1 Characteristics of asthmatic and control subjects |
|-------------------------------------------|----------------|----------------|
| **Study groups**                         | **Asthmatics** | **Controls**  |
| Total no. of subjects                    | 105            | 110           |
| Males                                    | 57             | 58            |
| Females                                  | 48             | 52            |
| Age (years): mean ± SD                   | 7.33 ± 2.62    | 7.71 ± 2.74   |
| Age of onset Before 5 years              | 85             |               |
| After 5 years                            | 20             |               |
| Height (cm): mean ± SD                   | 116.35 ± 13.28 | 117.63 ± 13.03|
| Weight (kg): mean ± SD                   | 19.19 ± 5.94   | 20.95 ± 6.28  |
| **HLA class I Allelic Groups**           |                |               |
| Bengali                                   | 78             | 69            |
| Bihari                                    | 12             | 15            |
| Nepali                                    | 7              | 15            |
| Others                                    | 8              | 11            |

SD, standard deviation.
In this study, it was observed that the frequency of HLA-DRB1*03 was significantly higher in asthmatic subjects than in controls (OR = 3.78, 95% CI = 1.61–8.85, P = 0.0025, P<0.05). The association of HLA-DRB1*03 with childhood asthma, in this study, is consistent with the findings of various earlier studies. A study conducted by Ivković-Jureković et al. in Croatian asthmatic children, showed a significantly higher frequency of HLA-DRB1*03 specificity among the asthmatic patients with total serum IgE ≥ 400 kU/l (30). In another study, Juhn et al. reported that HLA-DRB1*03 allele was the most significantly associated with an increased risk of asthma (HR: 1.5, 95% CI: 1.0–2.4, P = 0.006) (23). Similarly, Hanchard et al. suggested the role of HLA-DRB1*03 in asthma susceptibility independent of ancestral-haplotype-mediated linkage disequilibrium (31). In addition, Rajagopalan et al. have reported that HLA-DRB1*03 allele plays an important role in determining the eosinophilic airway inflammation, a Th2 mediated inflammation (32).

The frequency of HLA-DRB1*01 was higher in asthmatic subjects than in controls, although the difference was not significant (6.67% vs. 3.18%, respectively; OR = 2.26, 95% CI = 0.88–5.85, P = 0.136). Analysis of HLA alleles in association with the elevated level of total serum IgE showed that none of the HLA alleles was found to be associated with the elevated level of total serum IgE in asthmatic subjects. Many studies have reported the positive significant association of HLA-DRB1*01 with the elevated level of total serum IgE in asthmatics and/or allergic patients. Torio et al. showed a significant association of DRB1*01 with the elevated level of total serum IgE in Spanish Artemisia sensitive asthmatics (24). Woszczek et al. reported the significantly higher total serum IgE levels in allergic patients with HLA-DRB1*01 compared to patients without these allele (25). Similarly,

### Table 2 Frequencies of HLA class I and class II allelic groups in asthmatic and control subjects

<table>
<thead>
<tr>
<th>HLA allelic group</th>
<th>Asthmatics (N=105) Freq. % (n)</th>
<th>Controls (N=110) Freq. % (n)</th>
<th>χ²</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*01</td>
<td>15.71 (33)</td>
<td>14.09 (22)</td>
<td>2.564</td>
<td>0.109</td>
<td>1.73 [0.94–3.21]</td>
</tr>
<tr>
<td>A*03</td>
<td>17.62 (37)</td>
<td>10.00 (22)</td>
<td>3.62</td>
<td>0.056</td>
<td>1.90 [1.08–3.38]</td>
</tr>
<tr>
<td>A*11</td>
<td>14.09 (23)</td>
<td>9.09 (20)</td>
<td>0.116</td>
<td>0.734</td>
<td>1.19 [0.61–2.24]</td>
</tr>
<tr>
<td>A*24</td>
<td>16.67 (35)</td>
<td>13.64 (30)</td>
<td>0.670</td>
<td>0.413</td>
<td>1.33 [0.34–2.39]</td>
</tr>
<tr>
<td>A*26</td>
<td>10.48 (22)</td>
<td>9.09 (20)</td>
<td>0.734</td>
<td>0.374</td>
<td>1.19 [0.61–2.34]</td>
</tr>
<tr>
<td>B*08</td>
<td>21.90 (46)</td>
<td>18.64 (41)</td>
<td>0.701</td>
<td>0.403</td>
<td>1.31 [0.76–2.26]</td>
</tr>
<tr>
<td>B*44</td>
<td>5.71 (12)</td>
<td>3.64 (8)</td>
<td>0.662</td>
<td>0.416</td>
<td>1.65 [0.64–4.20]</td>
</tr>
<tr>
<td>B*45</td>
<td>7.14 (15)</td>
<td>5.00 (11)</td>
<td>0.569</td>
<td>0.451</td>
<td>1.50 [0.65–3.44]</td>
</tr>
<tr>
<td>B*51</td>
<td>6.19 (13)</td>
<td>5.91 (13)</td>
<td>0.314</td>
<td>0.575</td>
<td>0.70 [0.29–1.71]</td>
</tr>
<tr>
<td>B*52</td>
<td>4.29 (9)</td>
<td>5.91 (13)</td>
<td>0.314</td>
<td>0.575</td>
<td>0.70 [0.29–1.71]</td>
</tr>
<tr>
<td>B*37</td>
<td>19.05 (40)</td>
<td>15.45 (34)</td>
<td>0.931</td>
<td>0.335</td>
<td>1.38 [0.78–2.42]</td>
</tr>
<tr>
<td>DRB1*01</td>
<td>11.43 (24)</td>
<td>3.64 (8)</td>
<td>9.106</td>
<td>0.0022</td>
<td>3.78 [1.61–8.85]</td>
</tr>
<tr>
<td>DRB1*02</td>
<td>15.24 (32)</td>
<td>18.18 (40)</td>
<td>0.593</td>
<td>0.441</td>
<td>0.77 [0.43–1.35]</td>
</tr>
<tr>
<td>DRB1*12</td>
<td>8.57 (18)</td>
<td>5.45 (12)</td>
<td>1.258</td>
<td>0.216</td>
<td>1.69 [0.77–3.71]</td>
</tr>
<tr>
<td>DQB1*02</td>
<td>14.76 (31)</td>
<td>10.91 (24)</td>
<td>1.295</td>
<td>0.255</td>
<td>1.50 [0.81–2.78]</td>
</tr>
<tr>
<td>DQA1*02</td>
<td>14.76 (31)</td>
<td>10.91 (24)</td>
<td>1.295</td>
<td>0.255</td>
<td>1.50 [0.81–2.78]</td>
</tr>
</tbody>
</table>

CI, confidence interval; HLA, human leukocyte antigen; OR, odds ratio.

a% = (n/N) × 100

bP corrected <0.05.

 Fisher exact = 0.0018.
Ulbrecht et al. reported a weak association of HLA-DRB1*01 with specific IgE-positive cases compared to negative controls (33).

Asthma and its associated trait ‘atopy’ were some of the first complex diseases for which a strong genetic basis was established (34). HLA class II antigens play a key role in antigen presentation to CD4+ T-lymphocytes and therefore influence the specificity of the immune response. HLA genes have been implicated in triggering an allergen-specific IgE response. The amino acid constituents of the specific epitopes of allergens have been identified and specific HLA-DR and DQ gene products have been shown to present these epitopes (35). HLA-DR alleles are found to be associated with the development of specific IgE response to seasonal as well as perennial allergens (36, 37). Murray suggested the potential role of HLA genes in determining the Th1 vs Th2 immune response through the interaction between T-cell receptor, peptide and MHC molecules (38). Blumenthal et al. have suggested a different role of HLA gene polymorphism. They showed that in pollen allergy, the possible association of HLA-DQA1*0103 allele with high level of total serum IgE in asthmatic sub-populations has been documented (40, 41). Therefore, the regulation of the total IgE presents a complicated process involving several genetic loci.

In this study, we failed to establish the association of HLA alleles with high level of total serum IgE in asthmatic subjects. It could be because of the limitations of our study which include: relatively small number of subjects in total IgE < 150 IU/ml group of asthmatics. Further, we studied only limited number of allelic groups which have been reported previously to be associated with pediatric asthma in different populations. Therefore, further study in large cohort of asthmatic subjects of this region taking into account as many HLA allelic groups as possible is needed to support the present finding.

In conclusion, the present preliminary finding suggests the possible association of HLA-DRB1*03 allelic group with asthma in the pediatric population of Siliguri region of West Bengal, India. Therefore, HLA-DRB1*03 allelic group may be implicated in the susceptibility to childhood asthma.

Conflicts of interest

The authors have declared no conflicting interests.

References

Frequencies of HLA alleles in asthmatic and control subjects


Total Serum Immunoglobulin E in Children with Asthma

M. Lama, M. Chatterjee & T. K. Chaudhuri

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Total Serum Immunoglobulin E in Children with Asthma

M. Lama · M. Chatterjee · T. K. Chaudhuri

Abstract Immunoglobulin (Ig) E has been shown to be a major contributing factor for the development of bronchial hyperresponsiveness in asthma. An elevation in serum IgE levels contributes to asthma and is considered a potent predictor of the development of asthma. The objectives of the present study were to estimate the levels of total serum IgE in asthmatic and healthy control subjects and to investigate the relationship of various demographic and clinical characteristics with the total serum IgE level in asthmatics. We measured the levels of total serum IgE using the ELISA kits (AccuBind, Monobind Inc., USA). The relevant demographic and clinical data were obtained using the questionnaire. The results showed that asthmatic children had significantly elevated level of total serum IgE compared to that of the healthy controls. The levels of total IgE and IL-4 in sera of 44 asthmatic children showed a significant positive correlation. Total serum IgE >150 IU/mL was found to be significantly associated with the age, exposure to cigarette smoke, and raised eosinophil count in asthmatic children. In conclusion, the elevated level of total serum IgE may demonstrate the allergic etiology of asthma in the subjects studied.

Keywords Immunoglobulin E · Asthma · Children · Eosinophil · Interleukin-4

Introduction

Asthma is the most common chronic disorder in childhood, characterized by reversible airway obstruction, bronchial hyperresponsiveness (BHR) and atopy [1]. Total IgE level estimation provides evidence in support of atopy. Atopy is a nearly universal finding in children with asthma which is described as a tendency to produce excess amount of immunoglobulin (Ig) E antibodies when exposed to allergens [2]. Patients with asthma tend to have increase airway reactivity to a variety of stimuli such as allergens, irritants, exercise, cold air, and viruses [3]. The concentration of IgE in serum is age dependent and normally remains at levels less than 10 IU/mL in most infants during the first year of life [4].

Various population studies have shown an association between the prevalence of asthma/BHR and the total serum IgE levels, independent of specific reactivity to common allergens or symptoms of allergy [5, 6]. Burrows and his colleagues found a close correlation between serum IgE levels and the self-reported asthma [2].

The objectives of the present study were to estimate the levels of total serum IgE in asthmatic and healthy control subjects and to investigate the relationship of various demographic and clinical characteristics with the total serum IgE level in asthmatics.
Materials and Methods

Subjects and Collection of Blood Samples

In the present study, a total of 140 (70 asthmatic and 70 control) subjects were included. Children of the age group 3–12 years with asthma but free of other ailments such as parasitic infection, etc., diagnosed by the physicians were registered for the study. Each patient was thoroughly examined by the physician and the proforma was filled accordingly. Age and sex matched healthy subjects with no history of respiratory disorder, other atopic signs and symptoms, helminths or parasitic infection, were considered as control subjects. The registration of participants, data collection, and blood sample collection were done in the Out-Patient Department of Pediatrics, North Bengal Medical College & Hospital, Siliguri, West Bengal, India. All the laboratory investigations were performed in the Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, Siliguri, West Bengal, India. The blood samples were collected by vein puncture method at appropriate conditions. Sera were separated and stored in aliquots at \(-70^\circ\text{C}\) until analysis.

Measurement of Total Serum IgE

Total serum IgE level was measured using Immunoenzymometric sequential assay (Type 4), ELISA kits (AccuBind, Monobind Inc., USA). The principle of the method involves the immobilization of the biotinylated monoclonal anti-IgE antibody on the surface of a microplate well on interaction with the streptavidin coated on the well. On addition of serum containing the native antigen, antibody–antigen complex is formed. Another antibody (directed to a different epitope) labeled with an enzyme is added which results in the formation of an enzyme labeled antibody–antigen–biotinylated–antibody complex on the surface of the wells. On addition of the substrate color is formed which is measured using a microplate spectrophotometer. The concentration of the unknown samples is determined from the standard curve created using reference samples with known antigen concentration.

The assay procedure was followed as per the manufacturer’s instruction. The absorbance was measured at 450 nm in the ELISA plate reader (Bio rad). The sensitivity of the IgE AccuBind™ ELISA test system was 1.0 IU/mL with the intra- and inter-assay precisions of 1.95–5.87 % and 3.52–8.42 %, respectively.

Determination of Serum Level of Interleukin-4

In our previous study, we investigated serum levels of IL-4 and IFN-γ in 48 asthmatic and 32 control subjects [7].

A total of 44 asthmatics whose sera were used for both IL-4 and IgE estimation were considered for determining the correlation between IL-4 and total serum IgE.

Ethics

This study was approved by the “Institutional Human Ethics Committee”, University of North Bengal, Siliguri, West Bengal, India. The written informed consent was obtained from the guardians/parents for their children to participate in this study.

Statistical Analysis

The data were compiled and tabulated in MS Excel 2007. Statistical analyses were done by the statistical computer software SPSS 16.0. First, means and SDs were calculated for the variables and \(t\) tests were applied for the comparison of means. For attributes, the percentages were calculated and \(\chi^2\) test was used for the comparison. Pearson’s Chi-square test was used for analyzing the correlation between the total serum IgE and IL-4 in asthmatic subjects. A \(p\) value of \(<0.05\) was considered to be statistically significant. The scatter diagram was plotted using the OriginLab v8.5.

Results and Discussion

The demographic and biochemical profile of asthmatics and controls are presented in Table 1. Table 2 shows the relationship of demographic and clinical characteristics of asthmatic subjects with the elevated level of total serum IgE. The results showed no significant associations of gender, family history of asthma/atopy, exclusive breastfeeding up to 6 months and residential set up with the elevated level of total serum IgE. The higher age group, exposure to cigarette smoke and the raised eosinophil count showed the significant associations with the elevated levels of total serum IgE in asthmatics. Further, there was a significant positive correlation \((r = 0.36, \ p < 0.001***\) between the total serum IgE and IL-4 in 44 asthmatic children (Fig. 1).

It was observed that out of 70 asthmatics, 50 (71.43 %) subjects had total serum IgE > 150 IU/mL. The mean total serum IgE level was significantly higher in asthmatic subjects compared to that of the control subjects, 269.21 ± 150.97 IU/mL versus 146.89 ± 77.32 IU/mL; \(p < 0.001***\) (Fig. 2).

In the present study, the higher age group, exposure to cigarette smoke, and the raised eosinophil count showed the significant association with the elevated level of total serum IgE in asthmatic children. These findings are
consistent with the findings of several earlier studies. Cline et al. [8] reported the higher total serum IgE levels in the age group of 8–14 years. Similarly, Strachan and Cook [9] showed the potential role of passive smoking on IgE in a study conducted in children. Satwani et al. [10] showed eosinophilia along with raised serum IgE levels to be a significant allergic marker. Peripheral blood eosinophil counting has tremendously important clinical implication in order to demonstrate the allergic etiology of the disease, to monitor its clinical course and to address the choice of therapy [11].

The major finding of the present study confirmed that 71.43% of the asthmatic subjects had total serum IgE levels >150 IU/mL. The mean total serum IgE level in asthmatic group was 269.21 ± 150.97 and 146.89 ± 77.32 IU/mL in control group. The difference was

<p>| Table 1 Demographic and biochemical profile of asthmatic and control subjects |
|-----------------------------|-----------------------------|-----------------------------|----------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Asthmatic subjects (n = 70)</th>
<th>Control subjects (n = 70)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>6.93 ± 2.63</td>
<td>7.02 ± 2.29</td>
<td>-0.211</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/Female (%)</td>
<td>37/33 (52.86/47.14)</td>
<td>39/31 (55.71/44.29)</td>
<td>0.115</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>116.03 ± 17.16</td>
<td>119.59 ± 13.28</td>
<td>-1.372</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>18.74 ± 6.01</td>
<td>19.80 ± 5.57</td>
<td>-1.079</td>
</tr>
<tr>
<td>Study community</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bengali/non-Bengali</td>
<td>54/16 (77.14/22.86)</td>
<td>46/24 (65.71/34.29)</td>
<td>2.24</td>
</tr>
<tr>
<td>Level of total serum IgE (IU/mL)</td>
<td>269.21 ± 150.97</td>
<td>146.89 ± 77.32</td>
<td>-6.03</td>
</tr>
</tbody>
</table>

*** Significant at p < 0.001

<table>
<thead>
<tr>
<th>Table 2 Relationship of demographic and clinical characteristics of asthmatic children with elevated level of total serum IgE (&gt;150 IU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Age group</td>
</tr>
<tr>
<td>3–7 years</td>
</tr>
<tr>
<td>8–12 years</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Eosinophil count</td>
</tr>
<tr>
<td>Raised</td>
</tr>
<tr>
<td>Normal</td>
</tr>
<tr>
<td>FHA</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>EBF up to 6 months</td>
</tr>
<tr>
<td>Given</td>
</tr>
<tr>
<td>Not given</td>
</tr>
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<td>Exposure to cigarette smoke</td>
</tr>
<tr>
<td>Exposed</td>
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<tr>
<td>Not exposed</td>
</tr>
<tr>
<td>Residential set up</td>
</tr>
<tr>
<td>Rural</td>
</tr>
<tr>
<td>Urban</td>
</tr>
</tbody>
</table>

FHA family history of asthma/atopy; EBF exclusive breastfeeding
* Significant at p < 0.05, ** Significant at p < 0.01

Fig. 1 Correlation between serum levels of IL-4 and total IgE in 44 asthmatic subjects. The correlation coefficient was 0.56 and was statistically significant (p < 0.001***)

Fig. 2 Comparison of total serum IgE levels (IU/mL) between asthmatic and control subjects

in order to demonstrate the allergic etiology of the disease, to monitor its clinical course and to address the choice of therapy [11].
statistically significant ($p < 0.001^{***}$). Several studies have reported the elevated levels of total serum IgE in asthmatics [12, 13]. Therefore, it is in accordance with the well known fact that IgE plays a central role in the pathophysiology of allergic disorder such as asthma.

In the present study, it was also observed that there was a significant correlation between total IgE and IL-4 in sera of 44 asthmatic subjects. This finding is consistent with the finding of Afshari et al. [14] who reported considerably higher levels of serum IgE and IL-4 in asthmatics than non-asthmatic controls. IL-4 is one of the two cytokines known to cause switching in B-cells, a prerequisite for elevated IgE synthesis [15].

This study is a preliminary investigation and it has of course certain limitations. Further study investigating the prevalent allergens and the specific IgE estimation is warranted to strengthen the present study.

In conclusion, the elevated level of total serum IgE may demonstrate the allergic etiology of asthma in the subjects studied. Further, it also reveals the significant association of higher age, exposure to cigarette smoke and raised eosinophil count with the elevated level of total serum IgE in asthmatics.

Acknowledgments Authors are thankful to the University Grants Commission (UGC), New Delhi, for the financial support provided to carry out this study. The authors are indebted to all the participants and their parents for their participation and cooperation. Further, authors would like to extend sincere thanks to medical officers of Pediatric Department, North Bengal Medical College and Hospital, Siliguri, for their kind help throughout the study.

References
Short Communication

Increased interleukin-4 and decreased interferon-γ levels in serum of children with asthma

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Steroid-treated

A B S T R A C T

Background and purpose: Immune and inflammatory responses, mediated by cytokines, play important roles in the pathophysiology of asthma. These responses are associated with overexpression of T helper (Th)-2 cytokine, particularly interleukin (IL)-4 and IL-5, and decreased expression of Th-1 cytokine, IL-2 and IFN-γ. We hypothesized that there would be an imbalance in the levels of circulating IL-4 and IFN-γ in the asthmatic subjects.

Method: We investigated serum levels of IL-4 and IFN-γ among eighty children (18 steroid-naïve, 30 steroid-treated children with asthma and 32 healthy controls) using commercially available ELISA kits.

Results: Serum level of IL-4 was significantly higher in steroid-naïve group of asthmatic children compared to healthy control subjects. The results of our study suggest that serum level of IL-4 may be elevated in concert with decreased level of IFN-γ in asthma. Determination of serum levels of IL-4 and IFN-γ may be a useful tool for understanding the disease processes in asthma.

1. Introduction

Asthma is the most common chronic disease of childhood and the leading cause of childhood morbidity as measured by school absences, emergency department visits, and hospitalizations [1]. The asthma phenotype is characterized by a T helper (Th)-2 mediated inflammatory response involving alteration in the fine balance between Th-1 and Th-2 responses towards Th-2 bias and a complex interaction between a wide network of inflammatory and structural cells and the inflammatory mediators which they release [2].

In asthma, the development of immune response depends on a repertoire of cytokines produced by numerous cells, including CD4+ helper T cells. These lymphocytes can be divided into two subsets, T helper type 1 (Th-1) and T helper type 2 (Th-2), based on their cytokine profiles [3]. Effector Th1 cells are involved in delayed-type hypersensitivity through their production of IFN-γ and IL-2, whereas Th2 cells secrete IL-4, IL-9, IL-10 and IL-13, and promote antibody-mediated humoral immune responses [4]. It has been suggested that an alteration in cytokine milieu, with excess Th-2 products (IL-4, IL-5, and IL-13) in concert with decreased Th-1 products (IFN-γ and IL-2), is predicted to drive the asthma phenotype [5]. Elevated levels of IL-4, an essential cofactor for immunoglobulin E (IgE) production, and IL-5, responsible for the final differentiation, activation and recruitment of eosinophils [6], have been found in serum of patients with asthma [7–10]. On the other hand, IFN-γ is thought to protect against the development of asthma by regulating Th-2 cytokine production, although a mixed Th-1/Th-2 pattern has been reported [11].

One of the first studies measuring cytokine concentrations in children with allergic disease, revealed a significant increase in the level of IL-4 in serum from atopic asthmatics compared to controls, which correlated with IgE [7]. Other subsequent studies in serum and blood supported the importance of IL-4 in childhood asthma [12–14]. The differences in IL-4 levels are likely to be dependent on disease severity, since analysis of children with mild/moderate asthma revealed no differences in IL-4 concentrations compared to normal controls [15]. Several other invasive studies involving bronchoalveolar lavage (BAL) fluid and lung biopsies have confirmed that a Th-2-like mediated immune response is seen in asthma [16–18]. Gemou-Engesaeth et al. and Krouwels et al. [19,20] have reported the imbalance in the production of...
IL-4 and IFN-γ in children with atopic asthma, and corticosteroids appear to correct it.

Although there are various studies showing cytokine profile of asthmatic subjects, mostly employing either bronchoalveolar lavage (BAL) fluid or the exhaled breath condensate, the data on the circulating levels of cytokines in the asthmatic subjects are limited. Moreover, it has been suggested that asthma being the most heterogeneous airway disease, it may also demonstrate the systemic pattern of the disease outside the respiratory tract. Therefore, the aims of the present study were: (1) to characterize Th-1 cytokine (IFN-γ) and Th-2 cytokine (IL-4) profiles in children with asthma and healthy control, (2) to investigate any alteration in Th-1/Th-2 balance, and (3) to analyze whether there is any deviation in the levels of cytokines with corticosteroid treatment in asthmatic subjects.

2. Materials and methods

2.1. Subjects

This study included asthmatic as well as healthy children in the age group of 3–12 years. Children with asthma were recruited from the pediatric Outpatient Department of North Bengal Medical College and Hospital, Siliguri, West Bengal, India. Healthy age-matched control subjects were selected on the basis of having no history of lung disease or allergy, no airways hyperresponsiveness and no upper respiratory infections. We obtained approval for the study from the ‘Human Ethics Committee’, University of North Bengal, Siliguri, West Bengal, India. The study was explained in detail to the parents/guardians of the children who participated in this study and an informed consent was obtained from each parent/guardian.

2.2. Study design

Physical examination of each child was performed by the physician. Demographic data and other clinical characteristics of the study subjects were collected using the questionnaires. Weight and height were recorded for every child. Asthma was diagnosed by the physician on the basis of medical history, physical examination, chest X-ray, and peak expiratory flow rate (PEFR). The entire asthmatic subjects were divided into two groups: asthmatic children who were not under steroid treatment were included in steroid-naive group and those under steroid treatment were included in the steroid-treated group.

2.3. Sample size and blood collection

A total of 48 asthmatic subjects (18 under steroid-naive group and 30 under steroid-treated group) and 32 healthy control subjects were included in this study. All the participating subjects were from Indian origin residing in and around Siliguri, West Bengal, India. The majority of the children were Bengali. The blood samples from the healthy controls and asthmatic subjects were collected between 11 am and 2 pm by venipuncture method at appropriate conditions. Serum was acquired after coagulation of the blood for 1–2 h at room temperature. The supernatant was centrifuged for 10 min at 2000 g. The serum thus acquired was then aliquoted and stored at −20°C until analysis.

2.4. Determination of serum levels of cytokines

Serum levels of IL-4 and IFN-γ were determined by Enzyme Linked Immunosorbent Assay (ELISA) method. Commercially available 96 well ELISA kits (Endogen Human IL kit, Pierce Biotechnology, Inc., Rockford) were used to measure serum levels of IL-4 and IFN-γ. The sensitivity for both IL-4 and IFN-γ was <2 pg/ml with inter and intra-assay coefficient of variation of <10% in each case. Absorbance was measured by a microtitre plate reader (Opsys MR, Dynex Technologies) at 450 nm. Each assay was carried out by the same investigator in the Cellular Immunology Laboratory, Department of Zoology, University of North Bengal, Siliguri, India.

2.5. Data analysis

The data collected were statistically analyzed by the statistical computer software SPSS, version 15.0. A p value of less than 0.05 was considered to be statistically significant. Mean and standard deviation were calculated for the variables and t-test was employed for the comparisons. For the attributes, percentages were calculated first and then χ² test was used for comparisons. The figures were drawn with the help of OriginLab 6.1.

3. Results

Forty-eight asthmatic children, 18 steroid-naïve and 30 steroid-treated, and 32 healthy children participated in this study. The demographic and clinical characteristics of the study groups are presented in Table 1. Eosinophil count in the asthmatic subjects was significantly higher as compared to control subjects (p < 0.05). The age, gender and other clinical characteristics did not differ significantly between the two groups. The demographic and clinical characteristics such as family history of asthma, total lymphocyte count, eosinophil count, etc. did not show significant correlation with serum levels of IL-4 and IFN-γ.

Serum levels of IL-4 and IFN-γ in three groups, viz. steroid-naïve, steroid-treated children with asthma and healthy control subjects, are summarized in Figs. 1 and 2, respectively. Serum IL-4 level was significantly higher in steroid-naive group of asthmatic children as compared to healthy control group (52.25 ± 21.91 versus 32.81 ± 16.28 pg/ml; p < 0.001) and it was lower in steroid-treated group but not statistically significant when compared with steroid-naïve group (40.80 ± 17.77 versus 52.25 ± 21.91 pg/ml; p = 0.054, NS). In contrast, serum level of IFN-γ was significantly lower in both steroid-naïve and steroid-treated groups of asthmatic subjects as compared to healthy control subjects (848 ± 741.20 versus 9891.67 ± 1756.25 pg/ml; p < 0.001). The sensitivity for both IL-4 and IFN-γ was <2 pg/ml with inter and intra-assay coefficient of variation of <10% in each case.

Table 1

<table>
<thead>
<tr>
<th>Demographic and clinical characteristics of asthmatic and healthy control subjects.</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthmatic subjects</td>
<td>Control subjects</td>
</tr>
<tr>
<td>Age (years)</td>
<td>6.74 ± 2.70</td>
</tr>
<tr>
<td>Age of onset</td>
<td></td>
</tr>
<tr>
<td>Up to 4 years</td>
<td>9448 ± 1831.16</td>
</tr>
<tr>
<td>5–8 years</td>
<td>848 ± 741.20</td>
</tr>
<tr>
<td>9–12 years</td>
<td>18 [37.5%]</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male/female</td>
<td>25/23</td>
</tr>
<tr>
<td></td>
<td>[52.08%/47.92%]</td>
</tr>
<tr>
<td>Treatment status</td>
<td></td>
</tr>
<tr>
<td>Steroid-naive</td>
<td>22/26</td>
</tr>
<tr>
<td>Yes/no</td>
<td>[45.83%/54.17%]</td>
</tr>
</tbody>
</table>

* p < 0.05.
and the receptor for TNF-α have reported increased concentrations of exhaled IL-4 in steroid-naive group of asthmatic children and a decreased concentration of exhaled IFN-γ in both steroid-naive and steroid-treated groups compared to control subjects. Further, they have also reported that the exhaled IL-4 level was significantly lower in asthmatic children who were on steroid treatment. In a study, it was revealed that the expression of T-bet mRNA and the level of IFN-γ were lower, but the level of serum IL-4 was higher in asthma patients compared to healthy subjects. With the Astragalus membranaceus intervention, the level of IFN-γ and the expression of T-bet mRNA were increased and the level of IL-4 was decreased in the peripheral blood mononuclear cells (PBMCs) supernatant [26]. Several other studies have also reported that allergic and asthmatic subjects are more likely to have elevated levels of Th-2 cytokines and reduced levels of Th-1 cytokines [13,27–32].

In fact, IL-4 demonstrates a broad range of biological activities. It is a main cytokine involved in the pathogenesis of allergic responses and at the same time it can also down-regulate acute inflammatory changes [33]. IL-4 has also got additional effects on asthma pathogenesis which include stimulation of mucus producing cells and fibroblasts leading to airway remodeling [34–36]. It has also been confirmed that the crucial role of IL-4 lies in its effect on Th-2 development, rather than on the induction of IgE synthesis and subsequent mast cell degranulation [37]. On the other hand IFN-γ is a potent inhibitor of IgE synthesis [38]. Thus, this imbalance in the serum levels of IL-4 and IFN-γ is predicted to drive the asthma pathogenesis.

The findings of our study also revealed the association of steroid treatment with the reduction of IL-4 level in serum of asthmatic subjects. Serum level of IL-4 was lower in steroid-treated asthmatic subjects, although not significant as compared to steroid-naive asthmatic subjects (40.80 ± 17.77 versus 52.25 ± 21.91 pg/ml; p = 0.054, NS). The previous findings suggest that steroids inhibit both IL-4 and IFN-γ synthesis but the inhibitory action on IFN-γ is less marked [36]. Therefore, our finding of lower level of IL-4 in steroid-treated asthmatic subjects supports the earlier findings and suggests that steroid treatment down-regulates the level of IL-4 in asthma.

Although Th-1/Th-2 paradigm provided a simplistic model for initially describing involvement of T cells in asthma but still it does not fully support the complexities of this disease. Moreover, IFN-γ possesses a number of proinflammatory activities including the up-regulation of ICAM-1 [40] and the receptor for TNF-α [41], it is likely, that under certain circumstances IFN-γ may exert its pro-inflammatory activities and potentiate the inflammatory response in children with asthma. Therefore, it appears that some Th-1 and Th-2 cytokines are indeed elevated in asthma phenotypes of children. However, their effects in childhood asthma are largely unknown. In fact, there is an urgent need for complete understanding of T cell cytokine responses in childhood asthma. Moreover, it is crucial to understand the disease process for unraveling such complexities.

To conclude, the findings of our study support the hypothesis of Th1/Th2 cytokine imbalance and suggest that serum level of IL-4 may be elevated in concert with decreased level of IFN-γ, in asthma. Determination of serum levels of IL-4 and IFN-γ may be useful for understanding and monitoring the inflammatory response in asthma.

**Acknowledgments**

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No. 48-106(D-2008, Dated: 15.09.2008). Authors would like to thank all the participants and their families, without whom this study would simply be impossible. Authors are also thankful to the medical officers of the Department of Pediatrics, North Bengal Medical College and Hospital, Siliguri, for their kind help particularly during the recruitment of the participants and blood sample collection.

References


Epidemiology of Childhood Asthma: A Hospital Based Study

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** Dept. of Pediatrics, North Bengal Medical College & Hospital, Sushrutnagar, Siliguri

ABSTRACT
a) Introduction: Asthma is a major global health problem, characterized as a chronic disease affecting a major proportion of pediatric population. Increased prevalence of asthma is multifactorial in etiology.
b) Aims and Objectives: The prevalence of asthma among the pediatric population in the age group 3-12 years and its associated risk factors have been investigated in a hospital based study carried out from May 2009-April 2010 in the Outpatient Department of Pediatrics (OPD), North Bengal Medical College & Hospital, Siliguri. c) Methodology: Children who visited the Out-Patient Department of Pediatrics, North Bengal Medical College and Hospital, from May 2009 to April 2010, were registered for the study. Asthma was diagnosed by the physician. The relevant data were collected using the questionnaire. d) Result and Analysis: In this hospital-based study, the mean prevalence of asthma among children in the age group between 3 and 12 years was found to be 3.06%. It was noted that 33 (33%) asthmatic children and 17 (15.45%) control subjects had the family history of asthma. This difference was statistically significant. e) Conclusion: The prevalence rate of childhood asthma in and around Siliguri seems to be roughly equal to the prevalence rates prevailing in other rural areas of the country as reported by various studies. Results of our study also indicated that asthma is associated with the family history of asthma/atopy suggesting that genetic predisposition may be an important etiology for the development of asthma.

KEY WORDS: Asthma, Children, Prevalence

INTRODUCTION
Pediatric asthma is a major global health concern. It is now one of the most common chronic diseases affecting an estimated 300 million people worldwide[1]. Asthma has also increased the number of preventable hospital visits and admissions. Apart from being the leading cause of hospitalization for children, it is one of the most important chronic conditions causing elementary school absenteeism[2,3]. The increased prevalence of asthma is multifactorial in etiology. The pathogenesis has not been clearly elucidated, but various factors such as economic development, exposure to tobacco smoke, exposure to air pollution, infection, climate, diet, obesity, antibiotic use, and exposure to allergens are known to be associated with childhood asthma[4].

In Indian scenario, large numbers of studies have reported the varying rates of asthma prevalence in pediatric population. A study has reported a wide variation (4-19%) in the prevalence of asthma in school-going children from different geographic areas in India[5]. A recent investigation by Jain et al.[6] in a cross sectional community based study on rural Indian children showed the prevalence of bronchial asthma to be 10.3%. Another study by Awasthi et al.[7] on school-going children in Lucknow...
showed the prevalence of asthma to be 2.3% in age group of 6-7 years and 3.3% in age group of 13-14 years. The prevalence of childhood asthma in this region is still not known.

AIMS AND OBJECTIVE OF THE STUDY

To determine the prevalence of childhood asthma and to study the risk factors associated with the development of asthma.

METHODOLOGY

This study was carried out for one year from May 2009 to April 2010 on the pediatric population in the age group of 3- to 12-years. The North Bengal Medical College & Hospital is a main hospital in this region. The children residing in rural as well as urban areas in and around Siliguri who visited the Out-Patient Dept. of Pediatrics, North Bengal Medical College & Hospital, were registered in this study. Most of the children were from the rural areas (villages and tea gardens).

Asthma was diagnosed by a physician based on the medical history, physical examination, clinical history, family history of atopy and asthma, etc. For the study of prevalence of asthma, total number of children in the age group 3-12 years who were registered in the OPD was recorded. For the study of associated risk factors of asthma, data were collected using the questionnaires from 100 asthmatic and 110 control subjects. Age, sex, height and weight were also recorded. For each case to participate in the study we obtained informed consent from the parent and/or guardian of the patient.

Data Analysis

Data obtained from the study were statistically analyzed with the statistical software SPSS (version 15.0). For variables, first the means were calculated and t-test was employed for comparing the equality of means. For attributes, the percentages were calculated and $\chi^2$ test was used for the comparisons. A p value of less than 0.05 was considered to be statistically significant.

RESULTS

A total of 6280 children were registered in one year in the age group of 3-12 years out of which 192 were diagnosed with asthma. The monthly prevalence of pediatric asthma for one year duration from May 2009-April 2010 is shown in Fig. 1. The mean prevalence was observed to be 3.06%

The age and sex distributions of asthmatic and control subjects are presented in Table I and associated risk factors of asthma are presented in Table II. In this study, it was noted that 25 (25%) asthmatic children and 18 (16.36%) non-asthmatic children were not breast fed up to 6 months of age.

Table I. Age and sex distribution of asthmatic and control subjects

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Mean</th>
<th>SD</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthmatic subjects</td>
<td>7.26</td>
<td>2.64</td>
<td>55 [55%]</td>
<td>45 [45%]</td>
<td></td>
</tr>
<tr>
<td>Control subjects</td>
<td>7.15</td>
<td>2.52</td>
<td>59 [53.64%]</td>
<td>51 [46.36%]</td>
<td></td>
</tr>
</tbody>
</table>

0.752 NS | 0.843 NS

Fig. 1. Prevalence of pediatric asthma for one year duration (from May 2009 - April 2010)
months. This difference was not statistically significant. The family history of asthma/atopy was recorded in 33 (33%) asthmatic children and in 17 (15.45%) control subjects. This difference was found to be statistically significant (p<0.01**). The rest of the factors, hygienic condition around house, overcrowding and cooking mode did not differ significantly between asthmatic and control subjects.

**DISCUSSION**

In this hospital based study, the prevalence of pediatric asthma was recorded to be the highest during the month of November then followed by March, August and April. The lowest prevalence rate was observed during the months of February and October. This variation in prevalence rate may be due to seasonal variation, climatic factors, exposure to certain environmental factors, cold allergy, etc. The annual mean prevalence was found to be 3.06%. Various school survey, community based studies and hospital based study have shown diverse rates of asthma prevalence [5-7]. Singh et al. 8 in their study on the pediatric population in the age group of 1-15 years residing in five villages of Dehlon block of Ludhiana showed the prevalence of asthma to be 2.6%.

Although some recent reports suggest the prevalence of asthma to be declining but no overall global declining trend in the prevalence of asthma was shown in a recent review of epidemiological studies to examine international trends in asthma prevalence in children and adults for the period 1990-20089.

The genetic predisposition (family history of asthma) is considered to be an important risk factor for the development of asthma. The finding of our study is consistent with various other studies which have well documented strong association of family history of asthma and asthma development. Vishwanathan et al. 10 have observed the family history of asthma in 42% of asthmatic subjects but in only 10% non-asthmatics. Similarly, Ninan et al. 11 observed parental history of asthma in 42% patients with polysymptomatic asthma as compared to 13% in asymptomatic children (p<0.001). The present study showed no significant association of asthma with exclusive breastfeeding, hygiene condition around house, overcrowding and cooking mode (with smoke/smokeless). These observations are consistent with previous studies. The findings of Gergen et al.12 showed non-significant association between overcrowding and asthma. Schenker et al.13 and Chhabra et al. 14 have reported no significant association between the prevalence of wheeze and asthma with the type of fuel used in kitchen.

The importance of breast feeding to childhood

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**Table II. Associated risk factors of asthma as noted in asthmatic and control subjects**

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Type of risk Factors</th>
<th>Asthmatic subjects (n=100)</th>
<th>Control subjects (n=110)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBF up to 6 months</td>
<td>Done</td>
<td>75 [75%]</td>
<td>92 [83.64%]</td>
<td>0.1214 NS</td>
</tr>
<tr>
<td></td>
<td>Not done</td>
<td>25 [25%]</td>
<td>18 [16.36%]</td>
<td></td>
</tr>
<tr>
<td>Family history of</td>
<td>Yes</td>
<td>33 [33%]</td>
<td>17 [15.45%]</td>
<td>0.0029 S**</td>
</tr>
<tr>
<td>asthma/atopy</td>
<td>No</td>
<td>67 [67%]</td>
<td>93 [84.55%]</td>
<td></td>
</tr>
<tr>
<td>Overcrowding</td>
<td>Yes</td>
<td>39 [39%]</td>
<td>31 [28.18%]</td>
<td>0.0967 NS</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>61 [61%]</td>
<td>79 [71.82%]</td>
<td></td>
</tr>
<tr>
<td>Cooking mode</td>
<td>With smoke</td>
<td>78 [78%]</td>
<td>91 [82.73%]</td>
<td>0.388 NS</td>
</tr>
<tr>
<td></td>
<td>Smokeless</td>
<td>22 [22%]</td>
<td>19 [17.27%]</td>
<td></td>
</tr>
</tbody>
</table>

S=Significant, NS=Non significant, Abbreviation: EBF, Exclusive breastfeeding, **Significant at p < 0.01
asthma is a controversial issue. However, in a study by Oddy et al. it was reported that the exclusive breast feeding >4 months was a significant protective factor for wheezing LRI, current asthma and atopy, following multivariate adjustment.

This is a preliminary epidemiological study carried out in the North Bengal region. There are indeed certain limitations of this study. We could not involve the general pediatric population of specific age group in this study as it was restricted only to the hospital visiting children. Future study based on school survey using the standard questionnaire (ISSAC questionnaire) designed particularly for the epidemiological study may be warranted to further support this study.

CONCLUSION
The present hospital-based study shows the mean annual prevalence of childhood asthma to be 3.06% in and around Siliguri, West Bengal. Results of our study also indicate that the family history of asthma/atopy is associated with asthma in children suggesting the genetic predisposition to be an important etiology for the development of asthma.

ACKNOWLEDGEMENTS
This study was supported financially by the University Grants Commissions (UGC), New Delhi (Vide Award letter, Ref. No. 48-106/D-2008, Dated: 15.09.2008). The authors are indebted to all the participants and their parents for their cooperation during the study procedure. Further, authors would like to extend sincere thanks to the medical officers of Pediatric Department, North Bengal Medical College and Hospital, for their kind help.

REFERENCES
ELEVATED SERUM C-REACTIVE PROTEIN CONCENTRATION IN INHALED CORTICOSTEROID-NAÏVE CHILDREN WITH ASTHMA

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ABSTRACT

Objective-Serum C-reactive protein (CRP) concentration was determined in inhaled corticosteroid (ICS)-naïve and ICS-inhaling asthmatic children to understand the inflammatory process (es) in asthma. The latex agglutination test was performed for determining the serum CRP concentration among 87 asthmatic children. The limitation of detection of the test was less than 6 mg/L. Further, CRP was treated as a categorical variable: elevated (≥ 6 mg/L) and normal (< 6 mg/L). Among 87 asthmatic children, 15 children were ICS-naïve and 72 were ICS-inhaling. The elevated serum CRP concentration was detected in 13 (86.7%) ICS naïve-children and in only 3 (4.2%) ICS-inhaling children. The CRP concentration was significantly elevated in the serum of ICS-naïve sub-group of asthmatic children (p < 0.001). This study suggests that the asthmatic inflammation is associated with the elevation of serum CRP concentration and the ICS, which has the anti-inflammatory properties, might have played a role in reducing the CRP concentration in the ICS-inhaling children.

Key words: Asthma, C-reactive protein, Inflammation, Inhaled corticosteroid.

INTRODUCTION

Asthma is the most common chronic disease in childhood. It is responsible for significant social, economic and psychological impact on the family. Acute asthma leads to disturbed sleep, restriction in day to day activities and school absenteeism. Risk factors associated with development of asthma include: family history of asthma and atopic diseases,
bronchiolitis during infancy, sensitization to allergens during childhood and passive smoking\textsuperscript{1}.

Asthma is characterized by airway hyperresponsiveness and inflammation, in which various cells (such as eosinophils, neutrophils, macrophages and T-lymphocytes) cytokines and mediators play a role. Beside local inflammation, systemic inflammation is also present in asthma, as shown by the plasma fibrinogen and serum amyloid A\textsuperscript{2}.

C-Reactive protein (CRP), the best-studied major acute phase protein in humans, was initially described in 1930 by Tillet and Francis Jr.\textsuperscript{3} as the serum factor responsible for the precipitation of acute phase sera with the C-substance (C-polysaccharide,CPS) of pneumococcal cell walls. It is produced by the liver at a higher concentration, when the organism is challenged by a significant inflammatory stimulus, such as endotoxins from the membranes of Gram-negative bacteria inhaled into the bronchial tree of asthmatic patients\textsuperscript{4}. A population based study has shown associations of increased levels of serum CRP with a high frequency of airway hyperresponsiveness and low forced expiratory volume in one second (FEV\textsubscript{1}) among subjects without heart disease\textsuperscript{5}, suggesting that systemic inflammation may be associated with respiratory impairment. Another epidemiological study showed that elevated levels of hs-CRP correlate significantly with respiratory symptoms and with prevalence of non-allergic asthma\textsuperscript{6}. Recent publications suggest that CRP could be taken into consideration as a simple, cheap and reliable marker for monitoring asthmatic inflammation\textsuperscript{5-7}.

In the present study, we therefore, determined the serum CRP concentration in asthmatic children with and without ICS treatment in order to understand if serum CRP concentration could be taken as a marker for asthmatic inflammation. The relationships of the elevated serum CRP concentration to the other demographic variables were then investigated.

**EXPERIMENTAL**

**Study subjects**

The study included children (3-12 years) with asthma, who attended the out-patient department of North Bengal Medical College & Hospital, Siliguri, West Bengal. Children were diagnosed to have asthma according to the clinical criteria (history, physical examination, chest X-rays and peak flow expiratory rate (PEFR). A total of 87 asthmatic children (49 males and 38 females, age = 7.09 ± 2.55 years) were registered in the study from February 2009 – March 2010. After obtaining the informed consents from the
parents/guardians of the patients, blood samples of 2-3 mL were obtained in appropriate conditions by vein-puncture method.

**Ethics**

This study was approved by the Institutional Human Ethics Committee, University of North Bengal.

**CRP test**

The blood sample was allowed to coagulate at room temperature for 3-4 hours. Blood clot was cut and centrifuged at 2000Xg for 10 minutes to separate the serum. Freshly separated serum samples were used for the C-reactive protein test. Commercially available CRP kit ‘IMMUNOSTAT’ (Ranbaxy Fine Chemicals Ltd., HP, India) was used for the detection of CRP concentration in the serum. The limitation of detection of the test was < 6 mg/L. We treated CRP as a categorical variable, elevated (≥ 6 mg/L) and normal (≤ 6 mg/L). A positive result indicating the elevated serum CRP concentration was interpreted by the development of a clearly visible agglutination. It indicated CRP content of 6 mg/L and above in the test samples. Serum samples that showed no visible agglutination were considered to have normal CRP concentration.

**Statistical analysis**

Data were statistically analyzed with statistical software version 15.0 (SPSS package). Firstly, we calculated the mean, standard deviation and the frequency tables. To compare the two sub-groups (ICS-naïve and ICS-inhaling), the Student’s t-test and the chi-square test were used. Chi-square test was employed for the homogeneity testing, t-test for equality of means of independent populations and paired t-test for the equality of means for the dependent variables. The p-values of less than 0.05 were considered as significant.

**RESULTS AND DISCUSSIONS**

Among 87 asthmatic children, 15 children were ICS-naïve and 72 were ICS-inhaling. The elevated serum CRP concentration was detected in 13 (86.7%) ICS-naïve children and in only 3 (4.2%) ICS-inhaling children.

The demographic data and the ICS status of patients (ICS-naïve and ICS-inhaling) of the entire group and the two sub-groups of children with asthma are shown in Table 1. Age, sex distribution, exclusive breast feeding up to 6 months and the family history of asthma/atopy did not differ between the two sub-groups. The result showed that the CRP
concentration was significantly elevated in the serum of ICS-naïve sub-group of asthmatic children (p < 0.001).

Table 1: Demographic data and CRP concentration (elevated and normal) in the entire group and two sub-groups of patients (ICS-naïve and ICS-inhaling)

<table>
<thead>
<tr>
<th>Demographic data and serum CRP concentration</th>
<th>Age (Years) Mean ± SD</th>
<th>Sex M/F</th>
<th>Exclusive breast feeding (up to 6 months) Yes/No</th>
<th>Family history of asthma (atopy) Yes/No</th>
<th>Elevated CRP/Normal CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire group (N = 87)</td>
<td>7.09 ± 2.55</td>
<td>49/38  (56.3% /43.7%)</td>
<td>68/19 (78.2% /21.8%)</td>
<td>31/56 (35.6% /64.4%)</td>
<td>16/71 (18.4% /81.6%)</td>
</tr>
<tr>
<td>ICS-naïve sub-group (N = 15)</td>
<td>7.11 ± 2.14</td>
<td>9/6 (60% /40%)</td>
<td>12/3 (80% /20%)</td>
<td>6/9 (40% /60%)</td>
<td>13/2 (86.7% /13.3%)</td>
</tr>
<tr>
<td>ICS-inhaling sub-group (N = 72)</td>
<td>7.08 ± 2.64</td>
<td>40/32 (55.6% /44.4%)</td>
<td>56/16 (77.8% /22.2%)</td>
<td>25/47 (34.7% /65.3%)</td>
<td>3/69 (4.2% /95.8%)</td>
</tr>
<tr>
<td>Statistical</td>
<td><strong>χ² = 0.10 df = 1 p = 0.752 NS</strong></td>
<td><strong>χ² = 0.36 df = 1 p = 0.850 NS</strong></td>
<td><strong>χ² = 0.151 df = 1 p = 0.698 NS</strong></td>
<td><strong>χ² = 50.23 df = 1 p &lt; 0.001 S</strong>*</td>
<td></td>
</tr>
</tbody>
</table>

NS: Not Significant *significant at p < 0.05
S: Significant **significant at p < 0.01
***significant at p < 0.001

In the present study, it was observed that the serum CRP concentration was elevated in the ICS-naïve children with asthma while ICS-inhaling children showed to have normal serum CRP concentration. Our finding is similar to the finding of Takemura M et al. They reported that serum hs-CRP levels were significantly increased in steroid-naïve patients compared with controls, but not in patients on inhaled corticosteroid. Further, among steroid-naïve patients, serum hs-CRP levels negatively correlated significantly with indices of pulmonary function and positively with sputum eosinophil count. In another study by Kasayama et al., it was revealed that the plasma CRP levels were significantly reduced in corticosteroid-naïve asthmatic patients treated with inhaled corticosteroid for 3 months. In this respect, it is likely that in the present study, the ICS, which has well characterized anti-inflammatory properties, might have reduced serum CRP concentration to the normal state among the ICS-inhaling group of asthmatic children.
Bronchial hyperresponsiveness (BHR) is a crucial attribute of asthma. It is due to chronic asthmatic inflammation and may reflect the level of the inflammatory process\(^{10}\). An elegant work on a population-based study, Kony et al.,\(^5\) revealed an association between a higher frequency of BHR and and higher CRP levels in study participants, which could reflect local inflammation within the bronchi. Fujita M et al.,\(^{11}\) reported that increased hs-CRP levels may be associated with allergic inflammation, particularly eosinophilic inflammation, and the degree of airway obstruction in asthmatic patients. In another study by Szalai et al.,\(^{12}\) it has been suggested that an increase in CRP concentration may accompany the acute phase of allergic inflammation. This phenomenon may occur as a secondary reaction connected with the ability of CRP to stimulate the expression of anti-inflammatory cytokine-10, which down-regulates the activity of the Th\(_2\) lymphocyte population. Thus, these findings suggest that asthmatic inflammation results in the elevation of CRP concentration. However, the exact role of the CRP in asthmatic inflammation is not clearly understood.

Although CRP is structurally distinct from the immunoglobulins, it shares with them the ability to initiate several biological functions including precipitation\(^3\), opsonization\(^{13}\), capsular swelling\(^{14}\) and agglutination\(^{15}\). Two major biological activities of CRP have been well defined: first, it is able to bind several biological substrates that are distributed widely in nature\(^{16}\). Second, it has significant activation capabilities, in particular to activate the complement system\(^{17}\) and to bind to and modulate the function of phagocytic leukocytes\(^{18,19}\). These effects support the concept that this serum protein may have a potentially central role in the host defense mechanisms.

To our knowledge, this is the first such attempt to investigate the interrelationship between serum CRP concentration and asthma in this region. The present study has certain limitations: we did not consider the severity score of the disease in our study. Moreover, the sample size in the present study is also small; study in large sample size will be needed to strengthen the present study further.

**CONCLUSION**

In conclusion, it was observed that the ICS-naïve status of the asthmatic children was associated with the elevated serum CRP concentration, which suggests that the serum concentration of CRP can be a marker, reflecting the degree of inflammation in asthma. It was further observed that the serum CRP concentration was reduced in the ICS-inhaling asthmatic children. The ICS, which has potent anti-inflammatory properties, might have reduced the CRP concentration to the normal state.
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REFERENCES


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