CHAPTER – 5

SUMMARY AND CONCLUSION
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Although the propensity for allergy can manifest at any age, allergic diseases frequently develop in the first years of life, suggesting that very early events play an important role in initiating these disease processes. Decades of research have provided a detailed knowledge of the immunological processes that underlie the acute allergic immune response. Surprisingly, little is known about how or why these inflammatory processes are initiated and why an increasing number of individuals are affected. A better knowledge of early initiating events is critical in both understanding disease pathogenesis and planning prevention strategies. The objectives of the present study were to estimate the prevalence of asthma in children aged between 3-12 years and to investigate the associated risk factors, to assess the systemic inflammation by estimating serum CRP levels, to estimate the levels of total serum IgE in asthmatic children, to determine the serum levels of Th1 (IFN-γ) and Th2 (IL-4) cytokines in order to investigate the alteration in Th1/Th2 balance in asthma, if any and to determine the frequency of some of the selected HLA class I and class II allelic groups in asthmatic and control groups.

Assessment of prevalence and associated risk factors of asthma

Epidemiological study provides an assessment of disease frequency and burden of pediatric asthma. In addition, it allows researchers to explore associations of risk factors for childhood asthma and the study of disease progression as well as the effect of therapeutic interventions. Asthma is a major global health problem, characterized as a chronic disease affecting a major proportion of pediatric population. The prevalence of asthma has been reported to increase in many places around the world during the last decades. Many factors have been reported that contribute to this increase. Increased prevalence of asthma is multifactorial in etiology.

In this hospital based study, the prevalence of asthma and the association of risk factors among the pediatric population in the age group of 3-12 years were investigated. 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.

The prevalence of asthma among children in the age group between 3-12 years was 3.06%. The assessment of risk factors showed that the family history of asthma was significantly associated with asthma in children (33% vs. 15.45% in asthmatic and control subjects respectively). The present finding of the prevalence of childhood asthma in and around Siliguri seems to be similar to the prevalence rates 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.

**Serum level of CRP**

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. C-reactive protein (CRP) is an acute-phase reactant secreted by hepatocytes in response to circulating inflammatory cytokines. It has long been used clinically to evaluate the presence and degree of inflammation because CRP blood levels increase as much as 1,000-fold within 24 hours after the onset of inflammation. Therefore, serum CRP concentration was determined in inhaled corticosteroid (ICS)-naïve and ICS-inhaling asthmatic children to understand the inflammatory process(es) in asthma.

Serum level of CRP was studied among 87 asthmatic children (15 ICS-naïve and 72 ICS-inhaling). Freshly separated serum samples were used for the test. Commercially available CRP kit ‘IMMUNOSTAT’ (Ranbaxy Fine Chemicals Ltd., HP, India) was used for the detection of CRP level in the serum sample. 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).
The result of the study revealed that 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 asthmatic subjects (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 to normal level in the ICS-inhaling children.

**Total serum immunoglobulin E**

IgE has been shown to be a major contributing factor for the development of bronchial hyperresponsiveness in asthma. An elevation in serum IgE level 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 control subjects and to investigate the relationship of various demographic and clinical characteristics with the total serum IgE level in asthmatics.

The levels of total serum IgE were measured in asthmatic and control subjects using the ELISA kits (AccuBind, Monobind Inc., USA). The relevant demographic and clinical data were obtained using the questionnaire. The results showed significantly elevated level of total serum IgE in asthmatic children compared to the controls subjects (269.21 ± 150.97 and 146.89 ± 77.32 IU/ml, respectively, p<0.001***). The levels of total IgE and IL-4 in sera of 44 asthmatic children showed a significant positive correlation (r=0.56, p<0.001***). 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. The present findings suggest 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.
Serum Levels of IL-4 and IFN-γ

Asthma is a chronic disease of the lung characterized by shortness of breath, wheeze, cough, reduced airflow on expiration, and airway hyperreactivity to non-specific bronchoconstrictors. Recent evidence suggests that asthma is not a single disease, but consists of several subtypes, including allergic and steroid-resistant asthma. Allergic asthma is mediated by the Th-2 cytokines. 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 TNF-β), is predicted to drive the asthma phenotype. Elevated levels of IL-4, an essential cofactor for IgE production, and IL-5, responsible for the final differentiation, activation and recruitment of eosinophils, have been found in serum of patients with asthma. 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 also been reported.

Serum levels of IL-4 and IFN-γ were determined among 48 asthmatic children (18 steroid-naïve and 30 steroid-treated) and 32 control subjects using Enzyme Linked Immunosorbent Assay (ELISA) kits with the objectives of comparing the serum levels of IL-4 and IFN-γ between the asthmatic and control subjects, investigating any alteration in Th-1/Th-2 balance, and analyzing whether there is any deviation in the levels of cytokines with corticosteroid treatment in asthmatic subjects.

Serum level of IL-4 was significantly higher in steroid-naïve group of asthmatic children as compared to control group (52.25 ± 21.91 versus 32.81 ±16.28 pg/ml; p< 0.001***) while it was lower in steroid-treated group of asthmatic children 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 children compared to control group (21.62 ± 9.91 versus 30.79 ± 14.28; p = 0.02* and 23.03 ± 10.54 versus 30.79 ± 14.28 pg/ml; p = 0.019*), respectively. 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.
Association of HLA with asthma

Asthma is a heterogeneous disease for which a strong genetic basis is firmly established. It is a complex disorder influenced by gene-environment interaction. HLA genes have been shown to be consistently associated with asthma and its related phenotypes in various populations. The aim of the present study was to determine the frequency of the selected HLA class I and class II allelic groups in asthmatic and control subjects.

Frequencies of HLA alleles were determined among 105 asthmatic and 110 control subjects using PCR-SSP method. The allele and two loci haplotype frequencies were estimated by direct counting. Frequency of each HLA allele and/or haplotype 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 95% CI for each allele were calculated using GRAPHPAD INSTAT version 3.10.

The result of our study did not show the significant association of HLA class I allelic groups with asthma. Among class II allelic groups, the frequency of $HLA-DRB1^*03$ was significantly higher in asthmatic children than in controls (11.43% vs. 3.64%, OR=3.78, 95% CI=1.61 – 8.85, p=0.0025, $p_{corr}<0.05$). Further analysis of HLA allelic groups in two groups of asthmatic subjects with high and low total serum IgE levels did not show the significant association. Therefore, $HLA-DRB1^*03$ may be implicated in the susceptibility to asthma in the pediatric population.

On the basis of our observation, the following concluding remarks may be drawn:

1. The prevalence of childhood asthma observed in our hospital based study is comparable to the prevalence rates reported from other rural areas of India, although we need to estimate the actual prevalence among the general pediatric population taking into consideration the school going children of different age groups.

2. The family history of asthma/allergy was found to be associated with asthma suggesting that genetic predisposition may be an important etiology for the development of asthma in children.
3. Serum CRP level was found to be elevated in ICS-naïve asthmatics reflecting the ongoing systemic inflammation. While serum CRP level in ICS-treated asthmatics was normal and this could be due to the anti-inflammatory action of the ICS. Therefore, CRP may be considered as a marker of inflammation in asthma.

4. The increased level of IL-4 and decreased level of IFN-γ in serum of asthmatic children suggest the Th-2 mediated pathogenesis supporting the hypothesis of Th1/Th2 imbalance in asthma.

5. Significantly higher level of total serum IgE in asthmatic children may indicate the allergic etiology of asthma. Further, the study showed the association of higher age group, exposure to cigarette smoke, and raised eosinophil count with the high titer of total serum IgE in asthmatic children.

6. The present preliminary finding suggests the possible association of \textit{HLA-DRB1}*03 with asthma in children. Further study in a large cohort of asthmatic subjects needs to be done to strengthen the present finding.