ABSTRACT

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. It is well known that the prevalence of asthma has been reported to increase in many places around the world during the last decades. The increased prevalence of asthma is multifactorial in etiology. 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 present in asthma, as shown by increased levels of plasma fibrinogen and serum amyloid A. Serum levels of the well-known inflammatory marker C-reactive protein (CRP) can be simply and inexpensively measured in order to assess systemic inflammation. Asthma typically begins in early childhood, with an earlier onset in males than females. Atopy is present in the majority of children with asthma over the age of 3, and allergen-specific sensitization is one of the most important risk factors for the development of asthma. 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. Immune and inflammatory responses, mediated by cytokines, play important roles in the pathophysiology of asthma. These responses are associated with over expression of T helper (Th)-2 cytokines, particularly interleukin (IL)-4, IL-5, and IL-13 and decreased expression of Th-1 cytokines, IL-2 and IFN-γ. 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 overall objectives of the study were to estimate the prevalence of asthma in children aged between 3-12 years and to investigate the associated risk factors, to determine the serum CRP concentration in asthmatic children to understand the inflammatory process in asthma and to study the effect of corticosteroid on serum CRP level, 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 level of total serum IgE in asthmatics, 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.

For the prevalence study, we considered children who visited the Out-Patient Department of Pediatrics, North Bengal Medical College and Hospital, from May 2009 to April 2010. Asthma was diagnosed by the physician. The relevant data were collected using the questionnaire. In this hospital-based study, the mean prevalence of asthma among children in the age group between 3 to 12 years was found to be 3.06%. Further analysis of associated risk factors revealed that family history of asthma was significantly associated with asthma (33% versus 15.45% in asthmatics and controls respectively, p<0.05). The prevalence rate of childhood asthma in and around Siliguri seems to be comparable to the prevalence rates prevailing in other rural areas of the country as reported by various studies. Results of our study also indicated the association of family history of asthma/atopy with asthma suggesting that genetic predisposition may be an important etiology for the development of asthma.

The latex agglutination test was performed for determining serum CRP concentration among 87 asthmatic children. The limitation of detection of the test was <6mg/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 asthmatic children compared to ICS-inhaling 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 to the normal level.

The level of total serum IgE was measured using ELISA kits (AccuBind, Monobind Inc., USA). The absorbance was measured at 450nm 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. The results showed that asthmatic children had significantly elevated level of total serum IgE compared to the control subjects. The levels of total IgE and IL-4 in sera of 44 asthmatic children showed a significant positive correlation. Total serum IgE>150IU/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. The higher age group, exposure to cigarette smoke and raised eosinophil count were associated with the elevated level of total serum IgE.

Serum levels of IL-4 and IFN-γ were determined among eighty children (18 steroid-naive, 30 steroid-treated children with asthma and 32 healthy controls) using commercially available ELISA kits (Endogen Human IL kit, Pierce Biotechnology, Inc., Rockford). Absorbance was measured at 450nm in a microtitre plate reader (Opsys MR, Dynex Tehnologies). Serum level of IL-4 was significantly higher in steroid-naive group of asthmatic children compared to the control subjects and was lower in steroid-treated group though the level was statistically not significant. In contrast, serum levels of IFN-γ were significantly lower in both steroid-naive and steroid-treated groups of asthmatic children compared to 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.

Molecular typing of the selected HLA class I and class II allelic groups was performed by polymerase chain reaction using sequence-specific primers (PCR-SSP). The PCR products were electrophoresed in 2% pre-stained agarose gel and the result was interpreted for the presence of a specific band of the HLA allelic group. The results of the present study showed a significantly higher frequency of HLA-DRB1*03 in asthmatics than in controls (11.43% versus 3.64%, OR=3.78, 95% CI=1.61 – 8.85, p=0.0025, p_{corr}<0.05). Analysis of HLA allelic groups in two groups of asthmatic children with high and low total serum IgE levels revealed no significant association. HLA-DRB1*03 may be implicated in the susceptibility to asthma in the pediatric population.