MATERIAL & METHODS

The present study was conducted at the Allergy Clinic, Immunology and Biochemistry laboratory, Department of Paediatrics, M.L.B. Medical College and Hospital, Jhansi. A total number of 21 children between 4 and 13 years of age attending the Paediatrics Out Patient department and Allergy Clinic were selected for this study. Cases were grouped as follows:

A - Study group:

Sixteen children of bronchial asthma were selected from the out patient department and Allergy clinic of Paediatrics department, M.L.B. Medical College, Jhansi. Those children having liver disorder, renal disorder known immuno-deficiency disease and receiving corticosteroid were excluded from the study. Diagnosis were based on detailed history, clinical examination and relevant investigations.

B - Normal healthy controls:

Five children those of the staff members of the hospital with no history of allergic disorder, no history of atopy in family, no intestinal parasitic infestation were taken as control cases for this study.
Present, Past and Family History:

From parents or other family members detailed history was obtained regarding present illness, in a chronological order. Beside name, age, sex, address and socio-economic status, following facts were recorded in each case: a detailed history with emphasis on the age at the onset of the first attack; duration of disease; symptom free period; frequency per year; precipitating factors for illness.

The other detail recorded was that of history regarding environmental exposure to allergens. An attempt was made to elicit the role of food, drugs and physical agents in initiating or aggravating the symptoms of asthma.

Past History:

History of worm infestation, bronchiolitis, pertussis and measles was elicited.

Family History:

History of atopy especially that of bronchial asthma in other family members, tuberculosis in any family member was elicited.
Immunization history:

History of immunization was asked from the parents. For BCG vaccination, confirmation was done from the scar mark.

Social history:

A detailed account of living conditions of the patients was noted, with special reference to the type of house (kaccha/Tacca), flooring, water supply and toilet facilities. The status of hygiene was noted, as well.

Physical examination:

Besides routine physical examination of the whole body, a record of the detailed examination of the system(s) involved was made. Thorough clinical examination was done especially to note wheezing, nasal/congestion and rhinorrhoea.

Central nervous system, cardiovascular system and respiratory system were examined in each cases. Main emphasis was given to respiratory system. Lung capacity was measured by spirometry in each cases. Abdomen was specifically examined for liver, spleen enlargement and tenderness of colon.
Children with bronchial asthma were assessed on the basis of their personal history of allergy, family history of allergy, degree of eosinophilia, decrease in lung capacity and age of onset of illness.

**Weight:**

Weight was recorded nearest to 0.05 kg by using infant weighing scale, if the weight was less than 10 kg. Adult type weighing machine was used in children weighing more than 10 kg and weight was recorded nearest to 0.1 kg.

**Investigations:**

The following investigation were done in each case:

1). Total and differential leucocyte count.
2). Haemoglobin.
3). Erythrocyte sedimentation rate (ESR).
4). Stool examination for ova and cysts.
5). Chest skiagram.
6). Estimation of IgE level in serum.
8). Absolute eosinophil count (AEC)
    \[ AEC = TLC \times \text{Percentage of eosinophil}/100. \]
9). Peak expiratory flow (litre/minutes) by Peak flow meter.
Collection of blood sample:

From each patient blood sample of 6 ml was collected by a sterile dry syringe from the anticubital vein. Total leucocyte count was done by neubaur chamber and differential count was done after making a smear and staining with Leishman stain. Erythrocyte sedimentation rate was done. Absolute eosinophil count was calculated with the help of following formula.

\[
\text{Absolute eosinophil count} = \left( \frac{\text{Total leucocyte count} \times \% \text{ of eosinophils}}{100} \right)
\]

The serum was separated within 30 minutes after collection of sample and was kept in a freezer at -20°C.

IgE estimation:

Serum level of IgE was measured by single radial immuno-diffusion technique (Mancini 1965) using low concentration commercially prepared IgE plates and IgE standard serum. Automatic micro pipettes were used to make different dilutions of the standard serum and for filling the wells.

20μl of the serum (standard or patient serum) was placed into the wells, followed 30 minutes later, by another 20 μl: thus, making total volume of 40 μl per well for the quantitative determination of IgE.
After loading, the plates were allowed to stand open for 10 minutes and then close with the plastic lid, the plates were subsequently left standing at room temperature.

The diameter of precipitin rings was read off with an accuracy of 0.1 mm after allowing diffusion to occur for five days.

Reference curve was drawn after plotting the square of diameter on x-axis against the known concentration (IU/ml) of reference serum plotted on Y-axis.

The concentration of the Ig- in the sample was then read off directly on this straight line.

This method of Ig- estimation was sensitive to detect serum Ig- level between 920 IU/ml and 11,000 IU/ml.

Complement C3 and C4 estimation:

Serum level of complement C3, C4 were measured by single radial immuno-diffusion technique (Mancini 1965) using commercially prepared low concentration C3, C4 plates and C3, C4 standard serum 5 μl capillary tubes were used for filling the wells. Automatic micro pipettes were used to make different dilutions of the standard serum.
5 µl of serum (standard or patient serum) was placed into wells, for quantitative determination of complement C3 & C4.

After loading, the plates were allowed to stand open for 10 minute, then closed with the plastic lid and left for the development of the precipitin rings in inverted position at room temperature.

The diameter of precipitin rings was read off at an accuracy of 0.1 mm after diffusion was allowed to take place for three days.

Reference curve was drawn after plotting the square of diameter on x-axis against the known concentration (mg/ml) of reference serum plotted on Y-axis.

The concentration of the complement C3 and C4 in the sample was then read off directly on this straight line.

**Measurement of lung capacity by peak flow meter:**

Lung capacity was measured by peak flow meter in each case on each visit and on every day in admitted cases.
Forced expiratory volume by peak flow meter:
(Litre/Minute)

The patient was asked to take in deep breath as far as possible and then expel the same as hard and as fast as possible into the peak flow meter. Reading was noted from the indicator of peak flow meter.