RELATIONSHIP BETWEEN HIGH SERUM PERIOSTIN AND ASTHMA CONTROL IN CHILDREN

THESIS SUBMITTED TO

ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR

In partial fulfillment of the requirement for the degree of

DOCTOR OF MEDICINE (M.D.)

(PAEDIATRICS)

JUNE, 2022 **DR. SARITA CHOUDHARY**

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(DECLARATION BY THE CANDIDATE)

DECLARATION

I hereby declare that the thesis titled **"Relationship between high serum periostin and asthma control in children"** embodies the original work carried by the undersigned in All India Institute of Medical Sciences Jodhpur.

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This is to certify that the thesis titled "**Relationship between high serum periostin and asthma control in children**" is the bonafide work of **Dr. Sarita Choudhary** carried out under our guidance and supervision in the Department of Paediatrics, All India Institute of Medical Sciences, Jodhpur.

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CERTIFICATE

This is to certify that the thesis titled **"**Relationship between high serum periostin and asthma control in children" is carried out by **Dr. Sarita Choudhary** a postgraduate student in the Department of Paediatrics, AIIMS Jodhpur, in partial fulfillment of the requirement for the degree of **M.D. PAEDIATRICS** is a bonafide work done by her under our direct supervision and guidance.

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Dr. Sarita Choudhary

ABBREVIATIONS

ACT	Asthma Control test
FVC	Forced vital capacity
FEV1	Forced Expiratory Volume in 1 second
GINA	Global Initiative for Asthma
ICS	Inhaled Corticosteroids
ISAAC	The International Study of Asthma and Allergies in Childhood
SPT	Skin Prick Test
AEC	Absolute eosinophils count
IL	Interleukin
ECM	Extracellular matrix
MMP	Matrixmatelloprotein
FeNO	Fraction excretion of nitric oxide

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INTRODUCTION

1. INTRODUCTION

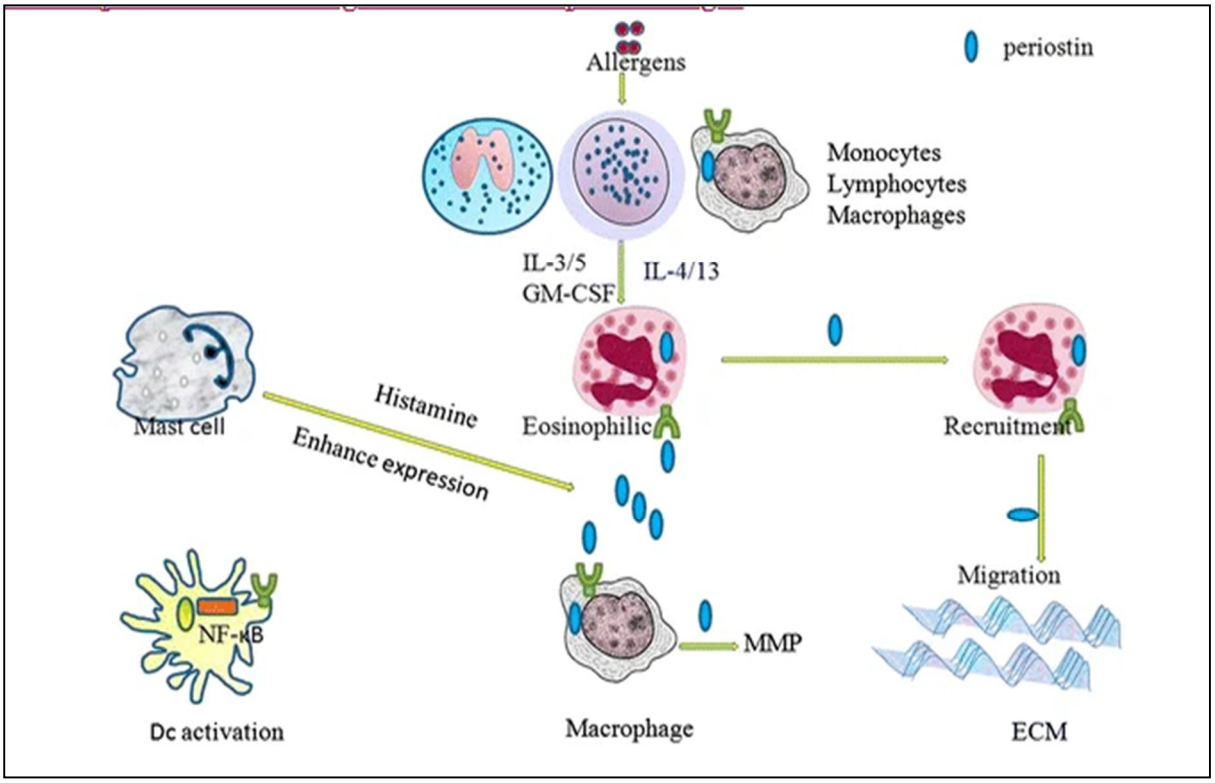
Asthma is a chronic inflammatory condition of the lung airway resulting in episodic airflow obstruction; this chronic inflammation heightens the twitchiness of the airway**.**

It affects up to 15% of children and pieces of evidence reflect that asthma is becoming more frequent and more severe with a marked increase in emergency visits. Asthma prevalence study was conducted by The International Study of Asthma and Allergies in Childhood (ISAAC), which suggested that the global prevalence of asthma is increasing. The phase 3 ISAAC found an estimated prevalence of wheeze is 7%, and more than 50% of this study had severe uncontrolled asthma. The true burden of asthma is not known in India but earlier studies showed a median prevalence of 3% [(1)], although there are strong sheds of evidence suggesting that, this is just the tip of an iceberg. The frequent disease exacerbations suffered by these patients, and multiple emergency department visits and hospitalizations represent a heavy social and economic burden. Among the leading cause of years lived with disability, asthma ranked 16th [(2)].

It is characterized by chronic airway hyper-responsiveness leading to wheezing, shortness of breath, chest tightness, and cough that vary over time in their occurrence, frequency, and intensity. These signs and symptoms are associated with variable expiratory airflow limitation i.e. difficulty in breathing air out of the lungs due to bronchoconstriction, airway wall thickening, and increased mucus. Both symptoms and airflow limitation have a waxing and waning course.

Asthma is a disease with many phenotypes, usually characterized by chronic airway inflammation. The inflammatory response in asthma is complex including a variety of cells and cellular elements. It is important to recognize the different inflammatory phenotypes of asthma to understand the underlying diseases processes and because of these diverse characteristics of asthma, it is difficult to control the symptoms by single, suitable phenotyping and tailored treatment are essential. The major feature of the disease is eosinophilic type 2 airway inflammation. Type 2 immune responses involve the accumulation of eosinophils, mast cells, basophils, Th2 cells, IgE producing B cells, and cytokines like IL-4, IL-5, and IL-13 [(3)].

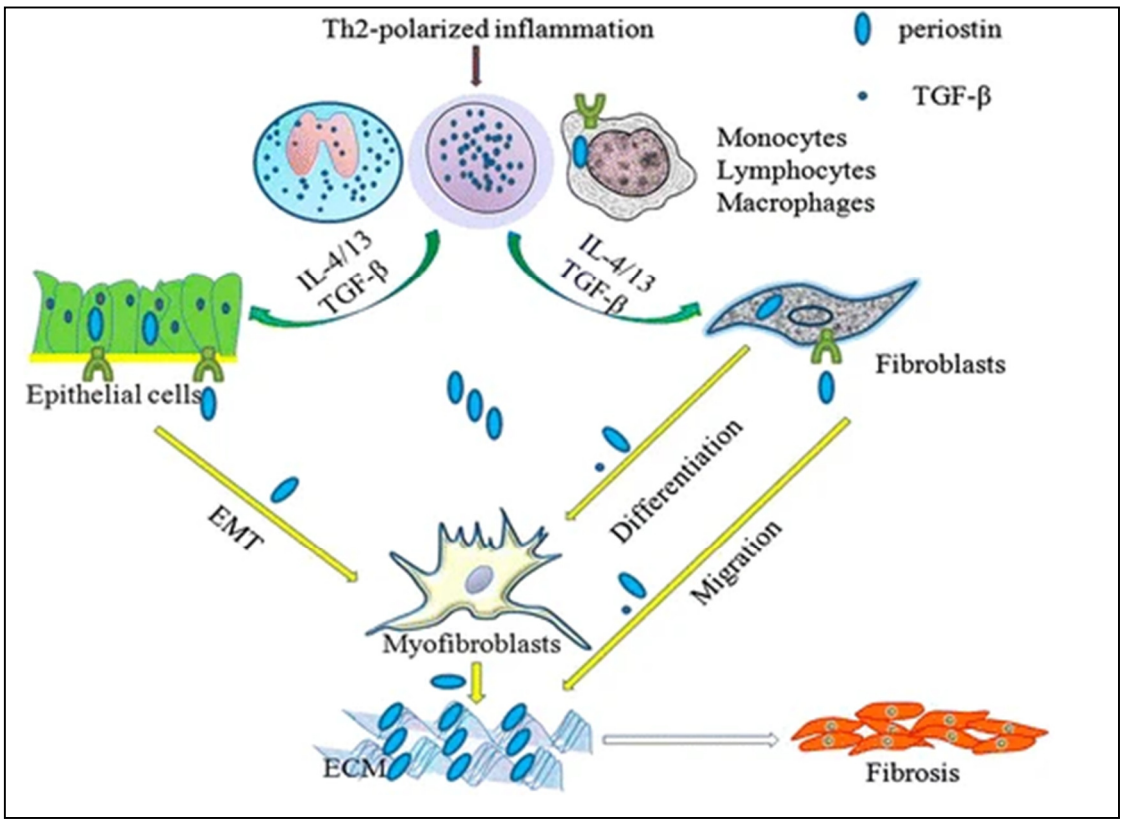
.In search of a novel molecule for asthma phenotype, it was found that Periostin is a downstream molecule of interleukin-4/13, signature cytokines of type 2 inflammation and signaling[4]. Periostin is an extracellular matrix protein that belongs to the fasciculin family. It also acts as a matricellular protein that functions in the activation of the cell by binding to its receptor, on the cell surface.Type-2 airway inflammation and remodeling are linked by this critical molecule and in the bronchial epithelial cells, are upregulated by type 2 cytokines IL-4 and IL-13.



1. Allergen induces the secretion of IL-4 and IL-13 from the immune cells, which in turn induces the eosinophils cells to produce periostin.
2. Periostin, in turn, acts on eosinophils via an autocrine mechanism to increase their adherence and recruitment to asthmatic airways.
3. Periostin augments eosinophilic inflammation in asthmatic airways through the aMb2 integrin and increases the generation of a superoxide anion (O2_) from eosinophils; it also stimulates the production of several cytokines and mediators from eosinophils, including IL-6, IL-8, transforming growth factor (TGF)-b1, TGFb2, cysteinyl leukotrienes, and prostaglandin E 2 [5,6].

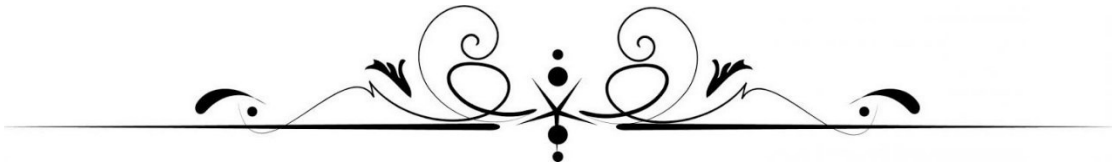
4. Macrophages produce periostin and this can enhance the secretion of matrix metalloproteinase. Periostin expression by other cells was likewise increased in the presence of histamine. This creates a vicious cycle of periostin release and inflammation.

In addition to type 2 inflammation, periostin is also involved in airway remodeling, which is an additional feature of eosinophilic asthma.

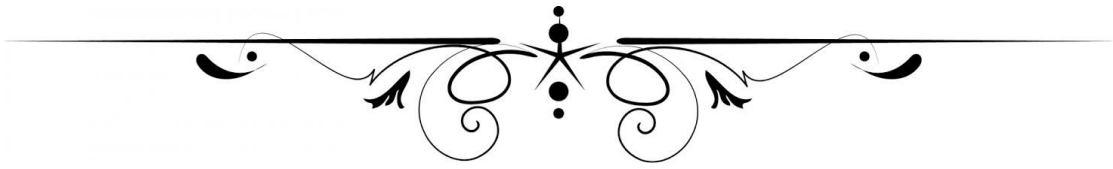


1. Asthma is characterized by Th2 inflammation, which causes recruitment of various immune cells like macrophages, lymphocytes, etc, and these cells release IL-4 and IL-13 along with TGF-β to stimulate fibroblast and epithelial cells to produce periostin.
2. Periostin via the autocrine pathway acts on the fibroblast and epithelial cells causing a vicious cycle.
3. Periostin also acts on fibroblasts cause their migration and differentiation into myofibroblasts.
4. Periostin as a cofactor of TGF-β promotes extracellular matrix production
5. By attaching to ECM proteins (collagen, fibronectin) and promoting collagen fibrillogenesis, it also promotes fibrosis.

Despite the well-known interaction of periostin in asthma pathophysiology, there is a lack of evidence regarding the potential role of periostin level in asthmatic children for better control. Therefore, with the rising prevalence of asthma in children & many tools aiding to diagnosis, with the potential role of periostin in the pathophysiology of asthma, research has been carried out to investigate the significance of serum periostin in asthma. Few studies have quoted a strong correlation between higher serum periostin and asthma symptom control. Few studies have shown no significance. In this study, we aimed to explore the relationship between periostin level and asthma control in Paediatrics patients. Our study's other objective was to determine the effect of IgE levels, eosinophils percentage, absolute eosinophils count, and FEV1 in asthma control.



REVIEW OF LITERATURE



2. REVIEW OF LITERATURE

Evidence suggests that periostin is associated with increased airway hyper-responsiveness, lower pulmonary functions, eosinophilia, poor asthma control, and possibly steroid resistance**.** Therefore, there are many trials have been done to assess the role of periostin in asthma control. We reviewed the literature for the same and decided to assess the effect of Periostin and IgE in addition to other factors in asthma control.

Published literature about the topic:

In a study done at the University of Bonn, Germany, a total of 158 children was enrolled, and among them, 125 were physician-diagnosed asthma cases and 33 were age and sex-matched control, with a median age of 10.2yrs. Asthma severity was assessed retrospectively, from the level of treatment required to control the symptoms according to GINA guidelines. It was found that children with asthma had higher serum periostin levels as compared to control. Periostin levels were also varied according to the severity of asthma, i.e. children with severe asthma had higher periostin levels as compared to mild/moderate asthma. [(7)]

A study was done in Hallym University Kandong Sacred Heart Hospital, Seoul, Korea, a total of 87 subjects were recruited. Divided into two groups, the patient group comprised 58 asthmatics and the control group 29 healthy subjects. Asthma definition and severity were according to the GINA guidelines. The outcome suggested that Periostin levels were significantly higher in the asthmatics than in the healthy controls. There was a significant correlation between serum periostin and peripheral blood eosinophils count. There were no significant correlations were found between serum periostin levels, gender, and ages in both groups. They also found that the periostin levels were significantly correlated with the bronchial provocation test results measured using both methacholine and mannitol-induced AHR. Therefore periostin level in peripheral blood might be useful as a clinical diagnostic tool in asthmatic patients. [(8)]

Using microarray and polymerase chain reaction analyses of bronchial epithelial brushings from a total of 70 patients including 42 patients with mild-to-moderate asthma and 28 healthy control subject. They identified three highly induced genes in airway epithelial cells from the asthmatic patients: POSTN, CLCA1, and SERPINB2. They also showed that in vitro IL-13 directly induces the expression of these three genes in epithelial cells. POSTN and CLCA1 can also be induced by IL-4. [(9,10)]

Another study was done in Jikei University Daisan Hospital, Izumihoncho, Komae city, Tokyo, Japan. 28 children with asthma and 27 children without asthma aged 6-16 years were enrolled. Asthma was diagnosed according to the ISAAC questionnaire. The severity and level of asthma control were classified as per GINA guidelines. FeNO, lung function test, blood eosinophils counts, total IgE, and serum periostin level were measured and then the comparison of results was done between the asthma group and control group. This study had two main findings. One was, that periostin levels were significantly higher in the asthmatics group, allowing it to differentiate from the control group. The second was patients with periostin levels above 50% had high FeNO levels as compared to patients with levels below 50%, which suggested that this finding was specific to airway pathology in asthma. ROC AUC value of periostin was 0.70 and that of FeNO was 0.72, both were comparable. So, after this study, researchers concluded that periostin can be a useful biomarker for the diagnosis of asthma in children who either have difficulty in undergoing a lung function test or FeNO measurement is excluded. [(11)]

A study was done by Takayama et al. to look for the role of periostin in sub-epithelial fibrosis in bronchial asthma analyzed the induction of periostin in lung fibroblasts by IL-4 or IL-13 and the expression of periostin in patients with asthma and in ovalbumin-sensitized and saline-inhaled mice. They also evaluated the binding capacity of periostin to other extracellular matrix proteins. They observed sub-epithelial portions by light microscopy and found that was thickened in patients with bronchial asthma, with specifically localized periostin, whereas periostin deposition was not detected in bronchial tissues of non-asthmatics. Periostin was also deposited in sub-epithelial portions in ovalbumin inhaled wild-type mice, but not in saline-inhaled mice, and was significantly decreased in IL-4 or IL-13 knockout mice. Final results suggested that periostin was involved in sub-epithelial fibrosis of bronchial asthma, and it is a downstream molecule of IL-4 and/or IL-13 signals. [(4)]

Hisako Matsumoto et al. demonstrated that activated epithelial cells of the airway secret a large amount of periostin into the underlying matrix, where it has autocrine effects on epithelial cell function, including MMP-2- and MMP-9–mediated activation of TGF-β and up-regulation of collagen. They also demonstrated that periostin secreted by epithelial cells causes activation of airway fibroblast via TGF-β mediated activation. This study concluded periostin is an unexpected regulator of TGF-β airway inflammatory cells, which can change the biochemical property of the underlying matrix and cause fibroblast activation. [(12)]

At Keio University Hospital, Japan, patients who were k/c/o difficult to treat asthma of age > 20yrs were enrolled. Asthma was diagnosed as per the Japanese society of Allergology guidelines and the severity of asthma was diagnosed according to GINA guidelines. Asthma requiring Step 4 or 5 to achieve symptomatic control was considered severe asthma. Healthy subjects without a history of allergic diseases and patients with mild to moderate asthma, who are on step 1 to 3 treatment actions of GINA were taken as control. This study enrolled 11 healthy subjects and 190 asthmatics including 42 with mild-moderate asthma and rest severe. The median serum periostin levels in asthmatic patients were significantly higher than in the control. However, there was no significant difference in periostin concentration among patients in GINA step 1-3 and step 4-5. For further characterization of high periostin phenotype, patients with asthma were again divided into tertiles based on the serum periostin concentration. It was found that the high serum periostin group had asthma onset at an older age; they were having poor lung functions, high peripheral eosinophils counts, and had a high prevalence of associated nasal disorders and aspirin intolerance. There was a weak to moderate correlation between periostin levels and blood eosinophils count. Among the patient with severe asthma i.e. who are on the GINA step 4 and 5 groups, the high periostin group had high serum TH2 cytokine concentration. [13]

One study from Cairo Egypt enrolled 60 -non-smoker asthmatic patients of age >20years, along with 20, age and gender-matched healthy control. Asthma patients were classified twice; first according to asthma severity into mild, moderate, and severe and then according to the level of asthma control. Various tests including serum periostin level, pulmonary function test, and multi-detector computed tomography were performed for all. And they found that there was a statistically significant direct relationship between increased serum periostin and bronchial wall thickening with the severity of asthma. They also showed that increased serum periostin was more among patent with uncontrolled asthma than those with controlled asthma. They also evaluated the relationship between steroid use and serum periostin level. The study revealed that there was a highly statistically significant decreased serum periostin level and bronchial wall thickness among the steroid-treated patient as compared to a non-steroid-treated group of asthmatics. Among the uncontrolled patients and steroid-naive patients, serum periostin levels and bronchial wall thickness were significantly decreased after being adequately treated or after the daily use of ICS for 6 months from their baseline value. [14]

Makoto et al. Studied the association between airway wall thickness with serum periostin and steroid-naive asthma. They had enrolled forty-six healthy control and 56 patients with asthma, among asthmatics; 30 patients were using inhalational corticosteroids and 26 patients were steroid naive and treated with only short-acting β agonist as required. Asthma was diagnosed as per American Thoracic Society criteria. The study subjects had mild to moderate asthma and healthy subjects without any history of allergic diseases were taken as control. Serum periostin, IgE levels, pulmonary function test, CT to look for airway dimensions, and induced sputum eosinophils counts were measured. Serum periostin values were significantly higher in a patient with steroid naive asthma than those who were treated with steroids. The percentage of predicted FEV1 and FEV1/FVC were significantly lower in asthmatics than in healthy control. Among the asthmatics, those who were steroid naive had significantly lower FEV1%predicted than in patients treated with steroids. Bronchial wall thickness was significantly higher in a patient with asthma as compared to the control group. Furthermore, bronchial wall thickness was significantly increased in patients with steroid-naive asthma compared with patients treated with steroids. Sputum eosinophils were significantly higher in steroid naive asthma patients than in those who were treated with steroids and the control group. They also demonstrated that serum periostin values were inversely correlated with percentage predicted FEV1 and positively correlated with sputum eosinophils count and bronchial wall thickness in steroid naive asthmatics. No significant correlation was found between serum periostin levels and other clinical variables in asthma patients treated with steroids and control subjects. [15]

Simpson et al. Conducted a cross-sectional study to determine the utility of serum periostin to predict the presence of eosinophilic asthma and to examine the relationship between airway and serum periostin levels, inflammatory subtype, and asthma control in a group of adults with poorly-controlled asthma. They enrolled 83 patients and asthma diagnosis according to the American Thoracic Society guidelines. Enrolled patients were prescribed maintenance ICS treatment and remained poorly controlled according to an Asthma Control Questionnaire. Subjects underwent clinical examination, skin prick test, and pulmonary function test with bronchodilator reversibility, sputum induction, and blood sampling. In poorly-controlled asthma patients who are treated with inhaled corticosteroids, periostin levels are low in airway secretions, but detectable. Serum periostin values were significantly higher as compared to sputum values. They also found that periostin values were higher in a patient with eosinophilic asthma as compared to non- eosinophilic asthma. Even though serum and

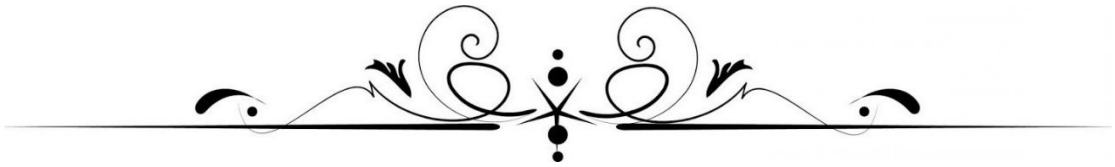
sputum periostin levels are significantly related to sputum eosinophils proportions, their ability to predict eosinophilic asthma in poorly-controlled asthma is relatively unpretentious. (16)

Anderson et al. Enrolled two hundred eighty-nine newborns, with either parental history of asthma or one of the parents having one or more positive aeroallergen skin tests. Peripheral blood samples were taken at annual visits, at ages 2, 4, 6, and 11years.Peripheral blood eosinophils number and IgE antibodies specific for common inhalant allergens were also measured. Diagnosis of asthma was made at ages 6 and 11years according to GINA guidelines. Periostin levels at ages 2, 4, 6, and11years and blood eosinophils count at ages 2, 6, and 11 were compared with the diagnosis of asthma by the ages6 and 11years. It was found that periostin value is higher at ages2, 4, and 6years in the subjects who developed asthma by the age of 6. Periostin levels were also higher at ages 2, 4, and 6years in the individual who were diagnosed with asthma at age of 11years, although it was not statistically significant. Peripheral blood eosinophils counts were significantly higher at age 2 and 6 for both the categories who developed asthma at 6years or 11years of age. Periostin and peripheral eosinophils counts were higher in the children who developed asthma by the age of 6 and 11years as compared to non-asthmatics. They also demonstrated early life aeroallergen sensitization whether at the age of 2 years or 6 years, increases the asthma risk. They concluded that the use of combinations, of two or more positive biomarkers, predict a higher risk of development of asthma at age 6 and 11 years. In the end, the closing statement was that the children with evidence of activation of multiple pathways of type 2 inflammation in early life are at greater risk for the development of asthma in their subsequent life. (17)

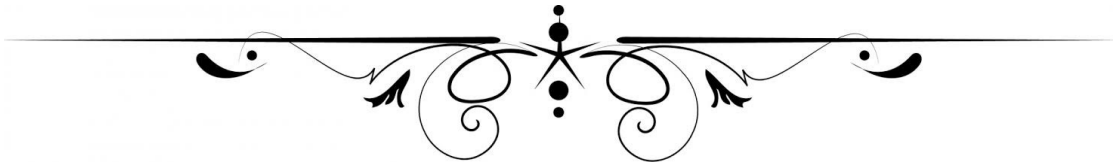
Jia et al did a study to identify the systemic biomarkers of eosinophilic airway inflammation in asthmatic patients. They collected sputum, performed a bronchoscopy, and matched peripheral blood samples from the 67 asthmatic patients who remained symptomatic despite receiving treatment with maximal inhaled corticosteroids or had poor asthma control according to Asthma Control Questionnaire. Serum periostin levels were measured and it was found that levels were significantly increased in asthmatic patients in whom bronchoscopy and sputum examination report was suggestive of eosinophilic airway inflammation relative to those with minimal eosinophilic airway inflammation. By using a logistic regression model including sex, age, body mass index, IgE levels, blood eosinophils counts, FeNO levels, and serum periostin levels in 59 patients with severe asthma, they showed that among all these indices, serum periostin was the most valuable predictor of airway eosinophilia.(18)

Kanemitsu et al did an observational study to find out whether increased periostin associated with greater airflow limitation in patients receiving inhaled corticosteroids; they enrolled a total of 224 patients with the mean age at enrolment age was 62.3± 13.7years and who were diagnosed with asthma at 40years of age or older, from 9 different institutions belonging to the Kinki Hokuriku Airway disease Conference. Asthma was diagnosed using the American Thoracic Society criteria. Inclusion was based on the following criteria, the total duration of ICs treatment should be more than 4 years, they should have undergone 3 or more lung function tests, or exacerbation free for more than 1 month. A pulmonary function test was performed twice; first at 1 year after the commencement of ICS treatment and the other at 25years of age or older. Patients with h/o smoking in the past 1year or who had other pulmonary diseases were excluded. Adherence to ICS therapy or other medications, frequency of exacerbation, or requirement of systemic corticosteroids during the past 6 months were recorded. Control of symptoms was assessed by using Asthma Control Test. The step of treatment required for controlling symptoms was determined according to GINA guidelines. At enrolment, patients underwent spirometry and blood tests. Spirometry was repeated at 6 months and then at 12months, after the enrolment. Blood eosinophils and neutrophil counts, serum total IgE levels, IgE specific against common inhaled allergens, high-sensitivity C reactive protein, and periostin levels were measured. According to the questionnaire which was filled out by the patients, medication adherence was satisfactory; 49% of the participants never and 38% seldom forgot to take ICS or other medications. Based on ACT scores, 50% of the patients were controlled, and 38% of patients scored from 20 to 24, indicating that they were well controlled. Serum periostin levels of asthmatic patients were significantly higher than those of healthy subjects. In the analysis of the decline in FEV1 values, it was found that a greater decline in FEV1 was associated with higher serum periostin levels at enrolment, patients who had poor asthma control (lower ACT score), who were on step 5 treatment, or had other co-morbidities. They also observed that serum periostin levels were weakly associated with blood eosinophils counts, serum IgE, ICS-untreated period, which is the period between onset of asthma and the initiation of ICS therapy, daily maintenance doses of ICS at enrolment, and frequency of asthma exacerbation. The value of serum periostin levels was significantly higher in patients on high-dose ICS (≥1000 mg daily) than in the remaining patients. Finally, serum periostin levels were also higher in patients with sinusitis than in patients without sinusitis. Serum periostin levels were not associated with any seasonal variability or with age at the onset of asthma. [19]

HibernateMena et al. Conducted an observational, cross-sectional prospective, single-center study. They had enrolled children aged 5 to 14 years with uncontrolled asthma according to the level of asthma symptom control based on the GINA guidelines and FEV1. Diagnosis of severe asthma was made after the second visit and after proper treatment in mild, moderate, and severe asthma according to GINA guidelines. A questionnaire was filled out by parents regarding demographic and psychological factors, personal and family medical history. FeNO was measured, SPT was done; weal greater than 3mm was considered positive and levels of serum periostin were measured. Asthma control was determined using Asthma Control Test in children over the age of 12years and the Childhood Asthma Control Test for children under 12 years. Finally, 2 groups were formed: one with uncontrolled severe asthma and another made of the remaining children. IgE levels were elevated in the uncontrolled severe asthma group. Periostin values were generally very high. An increase in mean levels of periostin was in a trend, it was observed with increasing patient age (838 ng/mL for children aged 5-7 years, 961ng/mL for children aged 8-12, and 1242 ng/mL in those aged >12 years). Gender distribution was also noted in periostin value; higher in boys than in girls. However, no correlation was found between serum periostin and total IgE levels, eosinophils levels, FEV1, or FeNO. [20]



LACUNAE IN EXISTING LITERATURE



3. LACUNAE IN THE EXISTING LITERATURE

1. Almost all of the studies are done in the western world. There is a scarcity of studies done on the Indian population.
2. The study population age group in most of the studies is above 20years.

RESEARCH QUESTIONS

4. RESEARCH QUESTION

- Is high serum periostin associated with poor asthma control in children?

AIM & OBJECTIVES

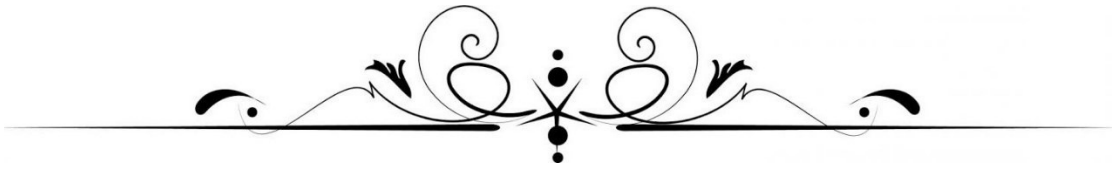
5. AIM AND OBJECTIVES

5.1 Primary Objective

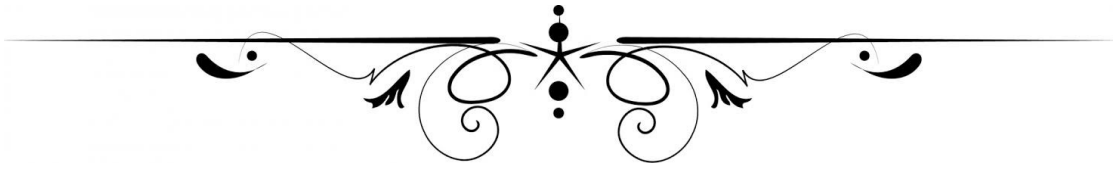
- To determine the association between serum periostin level and asthma control in children.

5.2 Secondary Objective

- To determine the relationship between the blood eosinophils counts, serum IgE levels, and pulmonary function test with the Asthma control
- Comparison of serum periostin level in asthma patients and non-respiratory patients.



MATERIALS & METHODS



6. MATERIALS AND METHODS

6.1 Study design: Cross-sectional study

6.2 Study settings: Department of Paediatrics AIIMS, Jodhpur.

6.3 Eligibility criteria:

Inclusion criteria:

Children 6-17 years of age with a physician-diagnosed case of asthma (by GINA 2019 guideline).

Children who have completed at least 12 weeks of inhaled corticosteroid (ICS) therapy.

Exclusion criteria

Patients with a history of prematurity, bronchopulmonary dysplasia, coexisting primary parenchymal pulmonary disease (e.g. cystic fibrosis), or coexisting congenital/acquired heart diseases and patients of epilepsy on anticonvulsants.

Patients with acute exacerbation of asthma or requiring steroids within the last 3months.

6.4 Sample size: For sample size calculation we used recent data by Yavuz et al [7] which indicates that serum periostin in children with asthma is 53.1 ± 13.1 and 43.0 ± 11.2 ng/mL in the control group. Assuming a power of 90% with a 5% level of significance and case to control the ratio of 2:1, we will require a minimum of 44 children with asthma and 22 controls.

6.5 Data collection:

- Children aged 6-17 years with physician-diagnosed asthma were enrolled in the study along with age and sex-matched control, who visited our outpatient department with non-respiratory complaints. The flow of the study is depicted in figure 1.
- Asthma was diagnosed as GINA guidelines i.e. current symptoms (wheeze and cough) and positive bronchodilator responsiveness. [20]
- Asthma severity was assessed retrospectively, from the level of treatment required to control the symptoms, according to GINA guidelines.

- Asthma control was assessed by using ACT/ C-ACT scoring. Children with C-ACT/ACT ≤19 were considered as uncontrolled asthma while scores of > 19 were considered well-controlled asthma.
- All the patients underwent skin prick tests using common allergens. Histamine and normal saline were used as positive and negative controls respectively. Wheal circumference greater than 3mm than the negative control was considered positive.
- Anthropometry measurements were taken in all the patients, and later on Z- scores were calculated.
- Pulmonary function tests were performed using a spirometer with RMS Helios software
- The blood sample was for blood eosinophil counts, IgE levels, and serum periostin.
- The serum sample was stored at -80ºC, in the Department of Biochemistry AIIMS Jodhpur.
- Measurement of serum periostin and IgE levels was made with ELISA kit i.e. Human POSTN/OSF2 ELISA Kit 96T Fine Test and Total IgE ELISA Kit 96T Make Xema. The kits were standardized as per manufacturer instructions. The standard curve for serum IgE and serum periostin are shown in Figures 2 and 3.

Figure 1: Flow chart describing the enrollment of cases and control

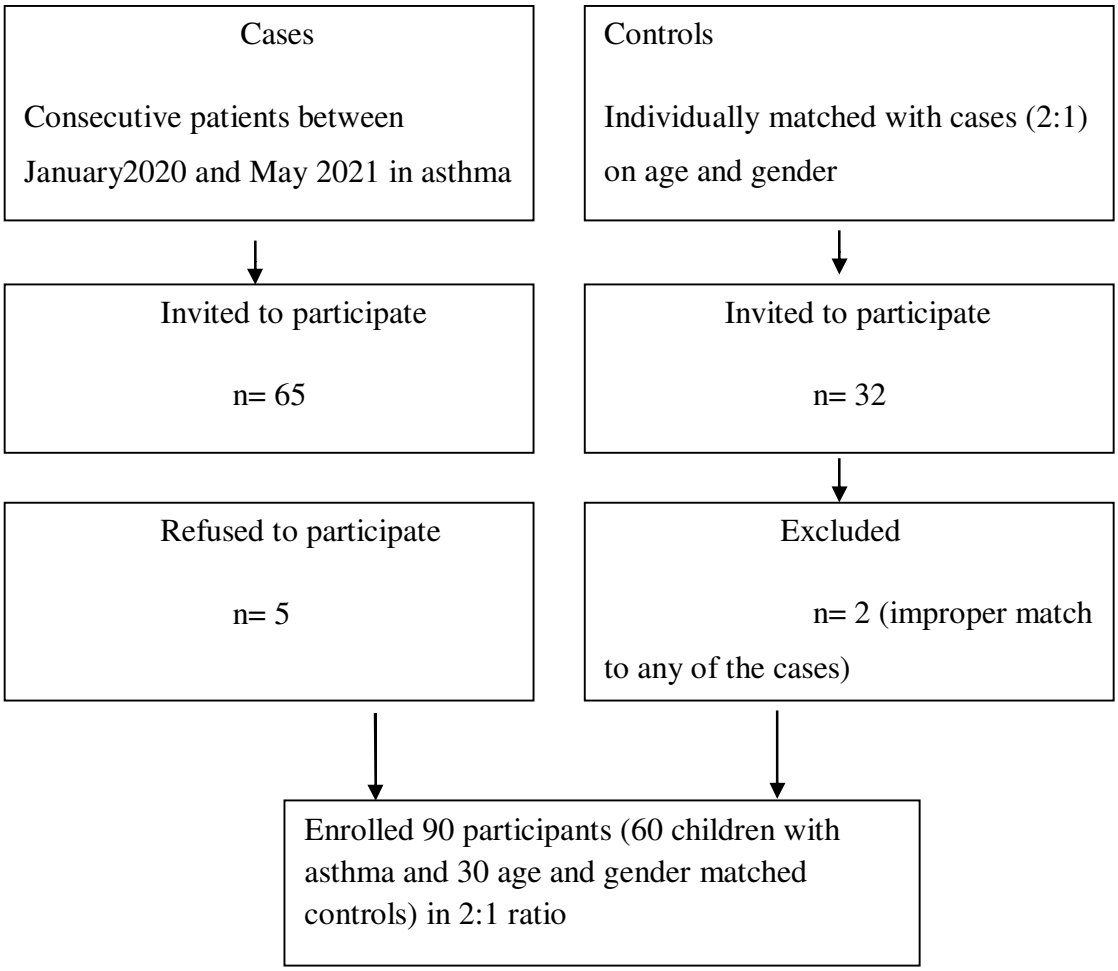


Figure 2: Standard curve of IgE measurements

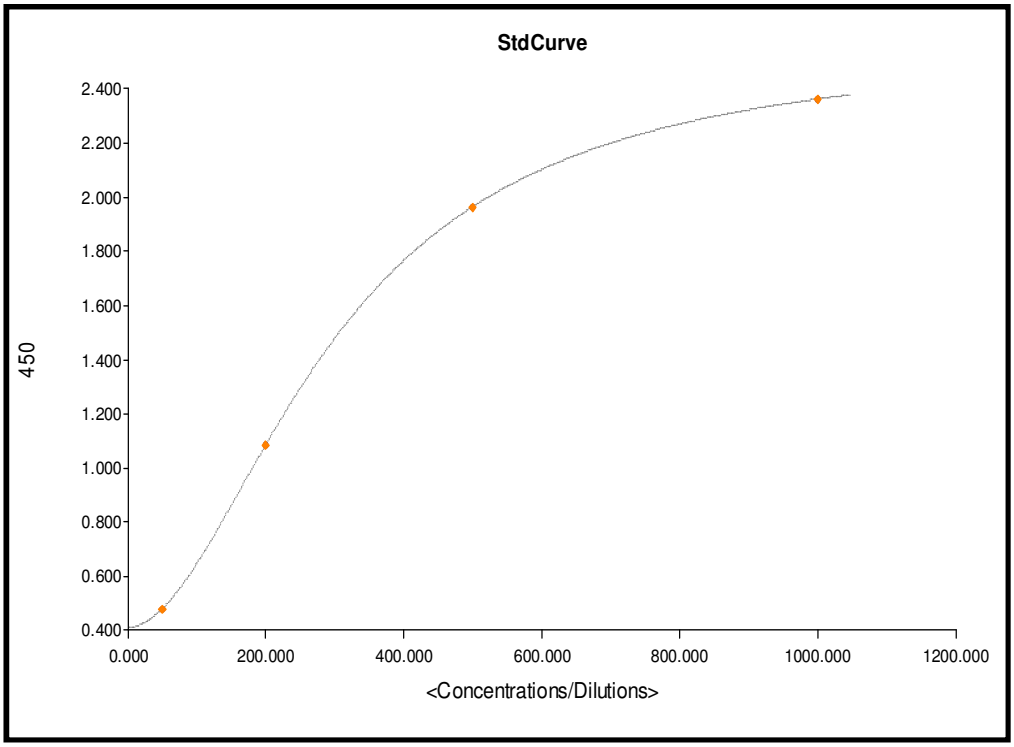
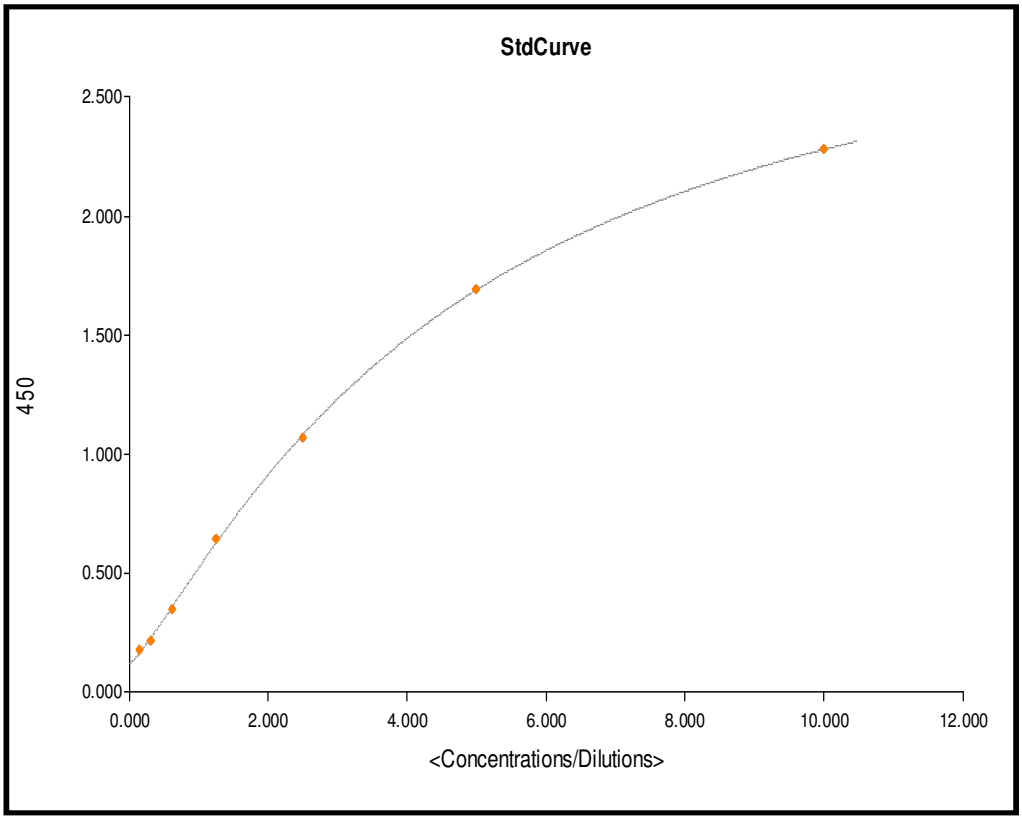


Figure 3: Standard curve of serum periostin levels



6.6 Data Analysis:

Data were entered into case record form and then manually transferred into a Microsoft Excel worksheet. All statistical analyses were performed by SPSS (Version 22) (IBM SPSS Statistics for Windows, IBM Corporation). Results were calculated either as mean ± standard deviation if normally distributed or median (IQR) for skewed data. Comparison among the groups was performed using the Chi-square test to identify differences in categorical variables. Spearman's coefficient correlation was applied for the associations between two quantitative variables. A p-value < 0.05 was considered statistically significant.

6.7. Ethical Justification:

Research ethics approval: The study was undertaken after the ethical clearance from the institute`s ethical committee.

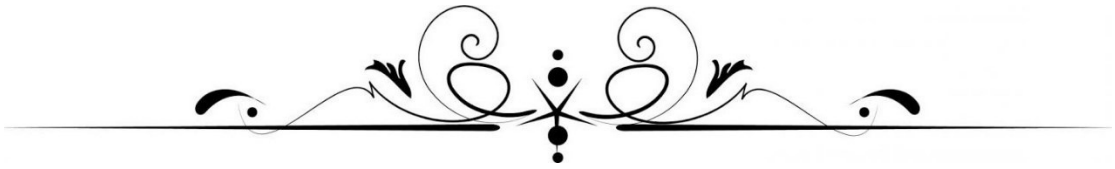
6.8 Consent:

Informed written consent was obtained by the participant's parents/or guardian.

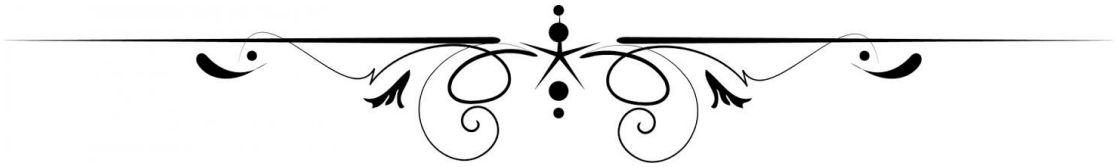
The purpose and design of the study were explained to the participant's parents in their mother language.

The parents or consenting family members were informed that they can withdraw from the study at any time.

6.9 Confidentiality: The confidentiality of information obtained was maintained and revealed only to the doctor/auditor/ involved in the study and if required to regulatory authorities.



OBSERVATIONS & RESULTS



7. OBSERVATIONS AND RESULTS

A total of 90 patients including 60 physician-diagnosed asthma according to GINA guidelines and 30 age and gender-matched control, presented to the OPD with non-respiratory complaints. Asthma control was assessed by using ACT/C-ACT scoring or GINA guidelines. Relation of asthma control with the other variables like blood eosinophils, lung function test, IgE level was also seen in this study. We have also compared serum periostin levels between asthma patients and non-asthmatic patients.

7.1 Demographic details of the subjects: A total of 90 children (60 with physician-diagnosed asthma and 30 age and sex-matched control subjects) with a mean age of 12.1 years (12.1±2.77SD) were enrolled. Among the 90 patients, 77.7% were male and the rest were female patients. In terms of age, sex, anthropometric parameters; there was no significant difference between the children with asthma and the control group. Characteristics of the study groups are shown in Table 1.

Table 1: Baseline characteristics of study participants (n=90)

Variables	**Asthma (n=60)**	**Control (n=30)**	**p-value**
Age	11.8±3.04	12.6±2.1	0.23
Male sex (%)	76.67	80	0.12
Height (cm)	-0.074±1.17	-0.16±1.15	0.79
Weight (kg)	-0.53±1.10	-0.23±1.12	0.20
BMI- Z score	-0.63±1.09	-0.17±1.14	0.07
Data are presented as percentage and mean ± standard deviation			

7.2 Clinical and laboratory characteristics of study population: Serum periostin, eosinophil %, absolute eosinophil counts, and total IgE levels were statistically significantly high in asthma as compared to the control group ($p<0.005$) [Figure 4, 5, 6]. Aero-allergen sensitization was also more in asthma as compared to the control ($p<0.005$) [Figure 7].

Table 2: Laboratory parameter of study participants (n=90)

Variables	**Asthma (n=60)**	**Control (n=30)**	**p-value**
Serum Periostin (ng/mL)	23.5 (22,26)	22 (19.40,22.96)	0.04*
Eosinophils, %	6.75 (0.95,12.1)	1.25 (0.9,3.2)	<0.01*
Eosinophils (/mL)	511.5 (75.9,765)	96.5 (35,179)	<0.01*
Total IgE (IU/mL)	524 (292,1047.5)	131.35 (23.8,315)	<0.01*
Aeroallergen sensitization (%)	71.67	26.67	<0.01*

* Statistically significant

Data are presented as a percentage, median (inter-quartile range).

Figure 4: Comparison of serum periostin level in asthma and control group

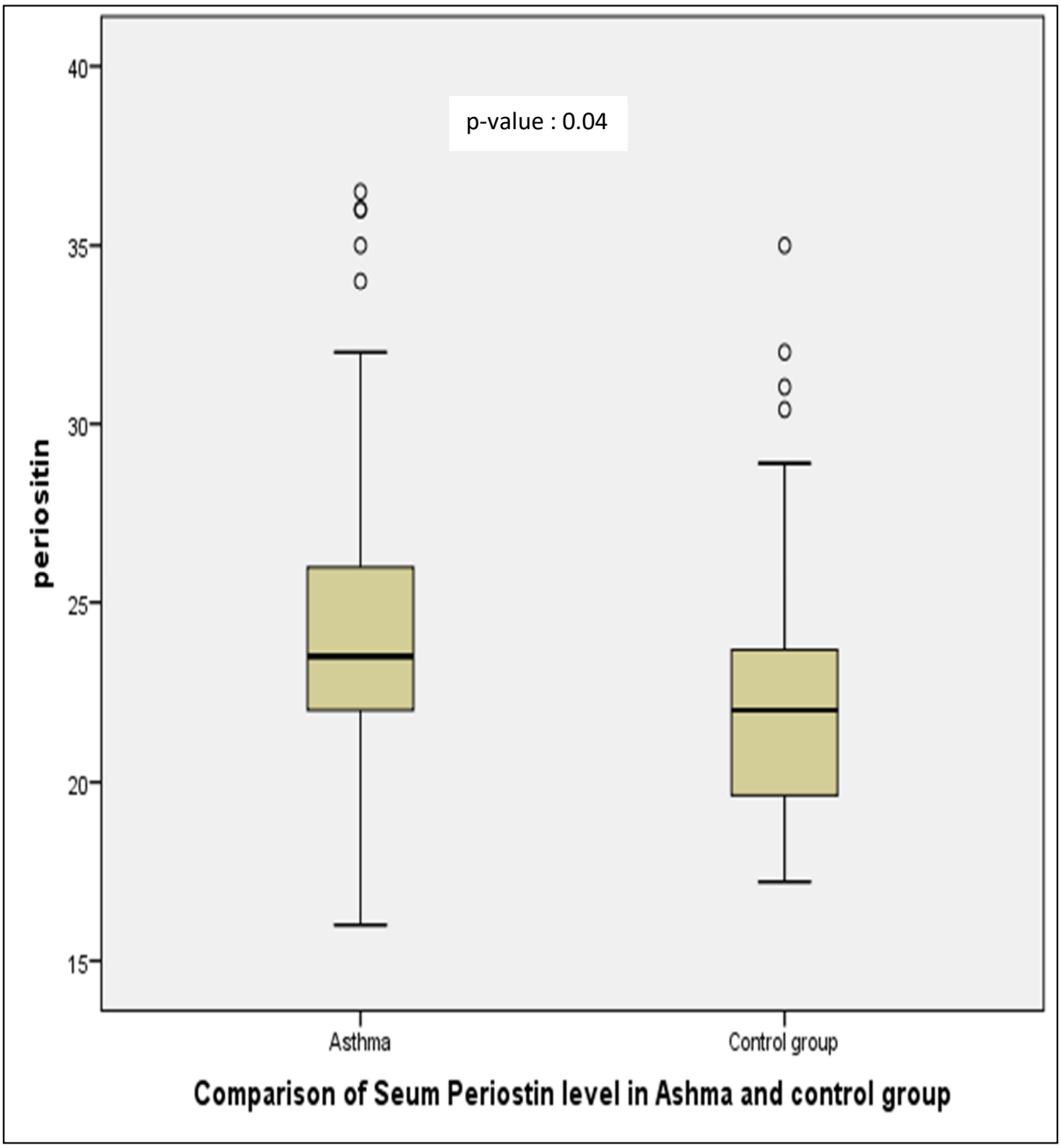


Figure5: Comparison of serum IgE level in asthma and control group

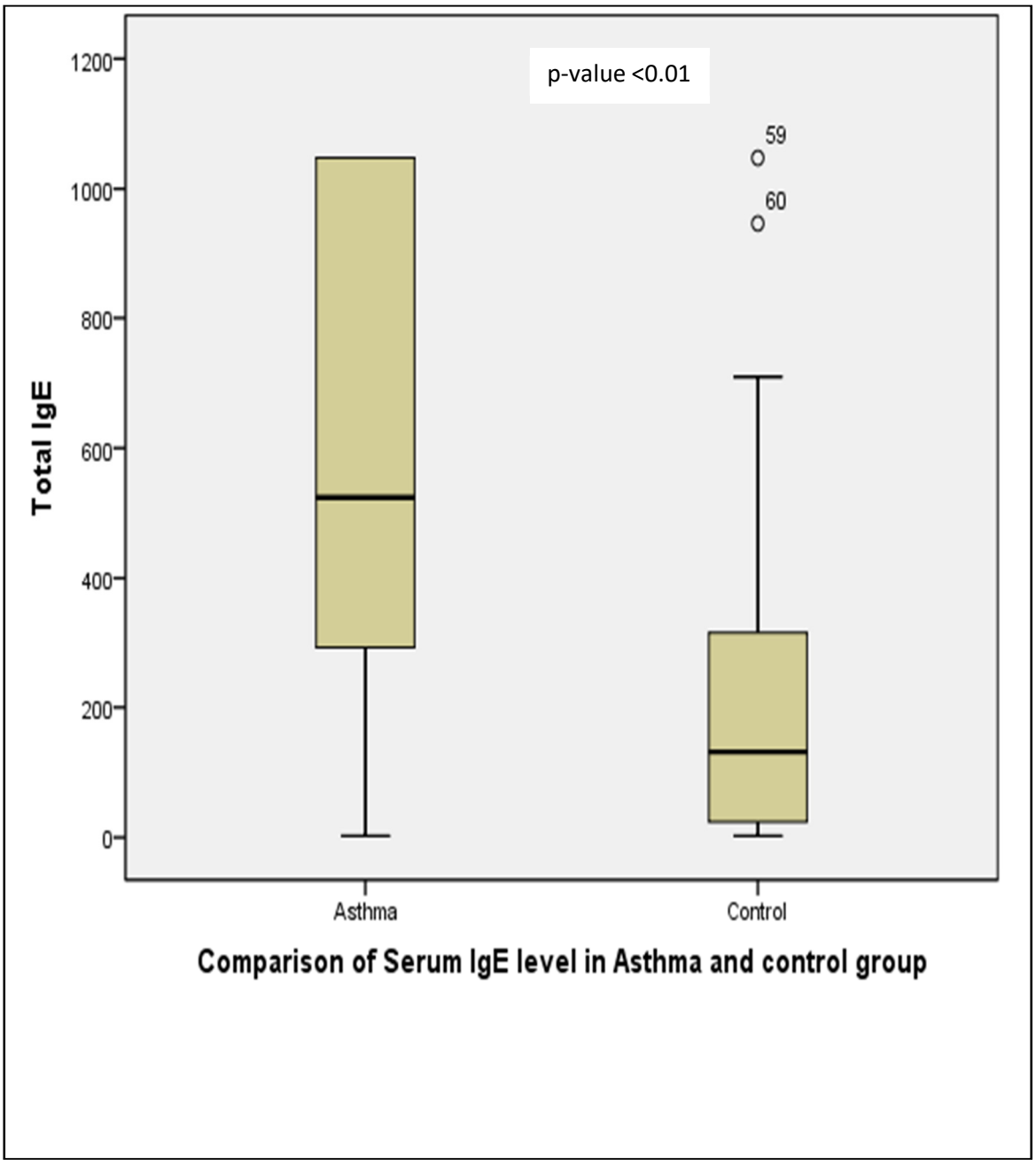


Figure 6: Comparison of eosinophils percentage in asthma and control group

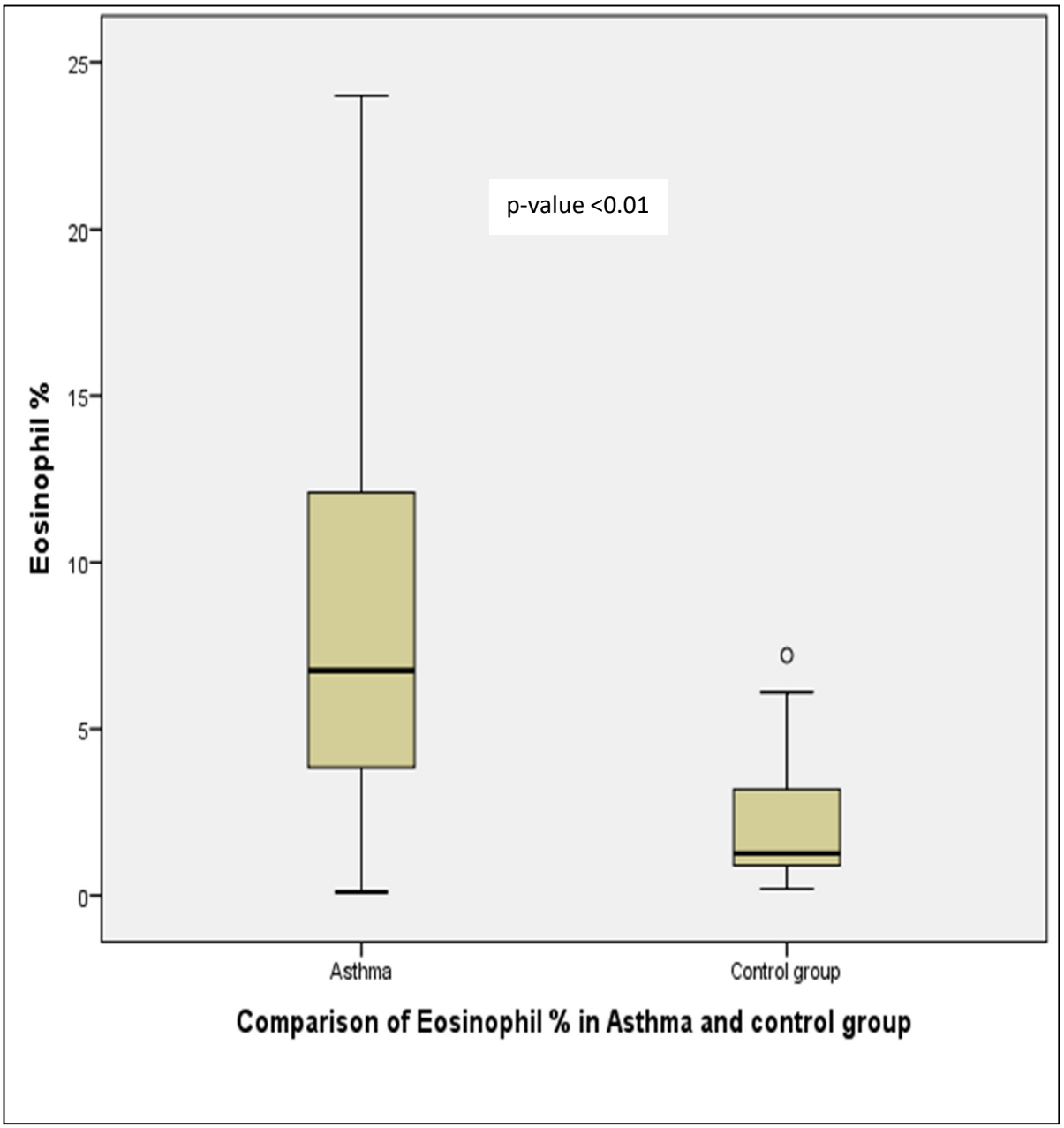
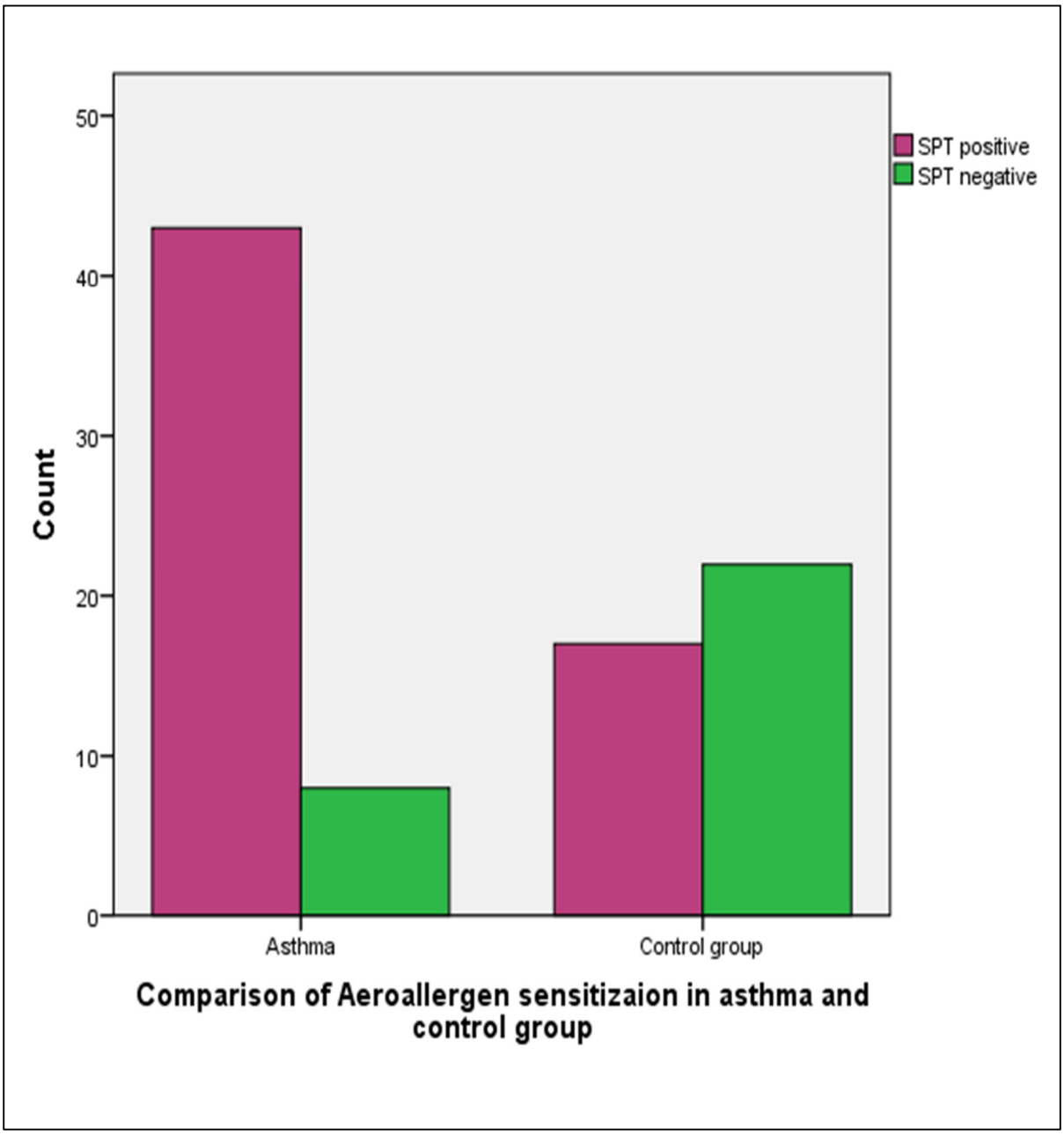


Figure 7: Comparison of aeroallergen sensitization in asthma and control group



7.3 Association of other variables with asthma control: Among the 60 asthma patients, 43.33% of patients had poor asthma control based on the C-ACT/ACT scores. Serum periostin was significantly higher in the uncontrolled asthma patients as compared to control asthma patients, shown in figure 8. No significant difference was found in age, gender, allergic rhinitis, BMI, aeroallergen sensitization, eosinophils counts/percentage, and IgE levels between well-controlled and uncontrolled asthma. Patients with well-controlled asthma were found to have significantly good pulmonary test results, depicted

in figure 9. We also found that patients, who were from rural residential, had poor asthma control. All these findings are depicted in table 3.

Table 3: Clinical and laboratory parameters of children according to asthma control (n=60)

Variables	**Uncontrolled (n=26)**	**Controlled (n=34)**	**p-value**
Age (median IQR)	13 (11,14)	11 (9,14)	0.26
Male (n, %)	19 (73)	27(79.4)	0.75
Place of residence Rural (n, %)	23 (88.5)	22 (64.7)	0.04*
Allergic rhinitis (n, %)	23(88.5)	28 (82.4)	0.71
BMI Z-score (median IQR)	-0.53(-1.23,0.01)	-0.42(-1.5, 0.11)	0.72
Aeroallergen sensitisation	17 (65.4)	26 (76.5)	0.39
Serum periostin (ng/mL)	25.8 (23.2,33.5)	23 (21,24)	0.01*
Eosinophils, %	9.1 (4.4, 14.3)	6.7 (3.8,10.6)	0.54
Eosinophils (/mL)	739 (332, 1210)	511 (298,693)	0.28
Total IgE (IU/mL)	560 (237,1047)	515 (333,996)	0.96
FEV1	80.5(60.5,94)	93 (83,103)	0.01*

* Statistically significant

Data are presented as percentage, median (inter-quartile range), or mean ± standard deviation

Figure 8: Comparison of serum periostin level according to asthma control

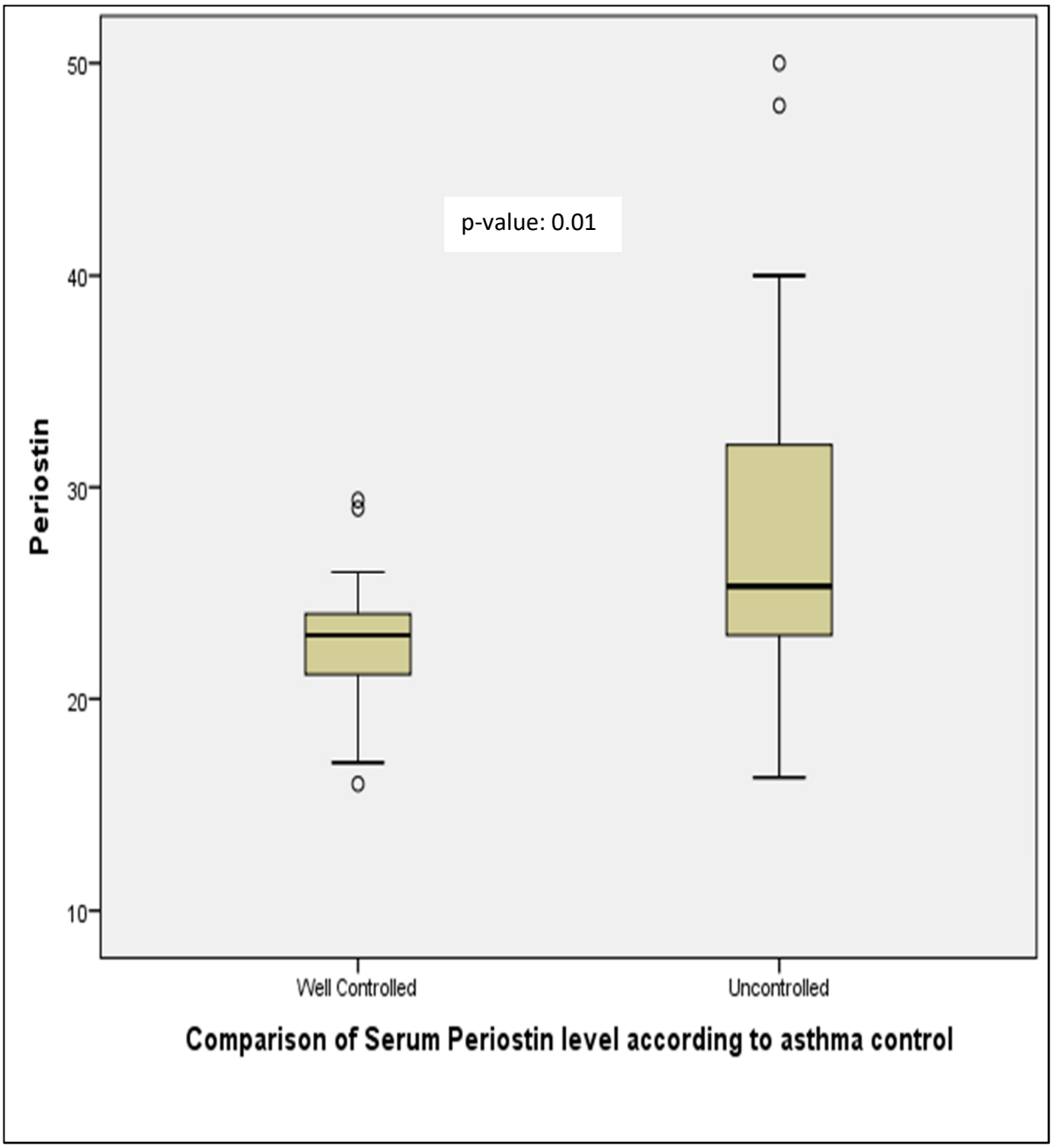
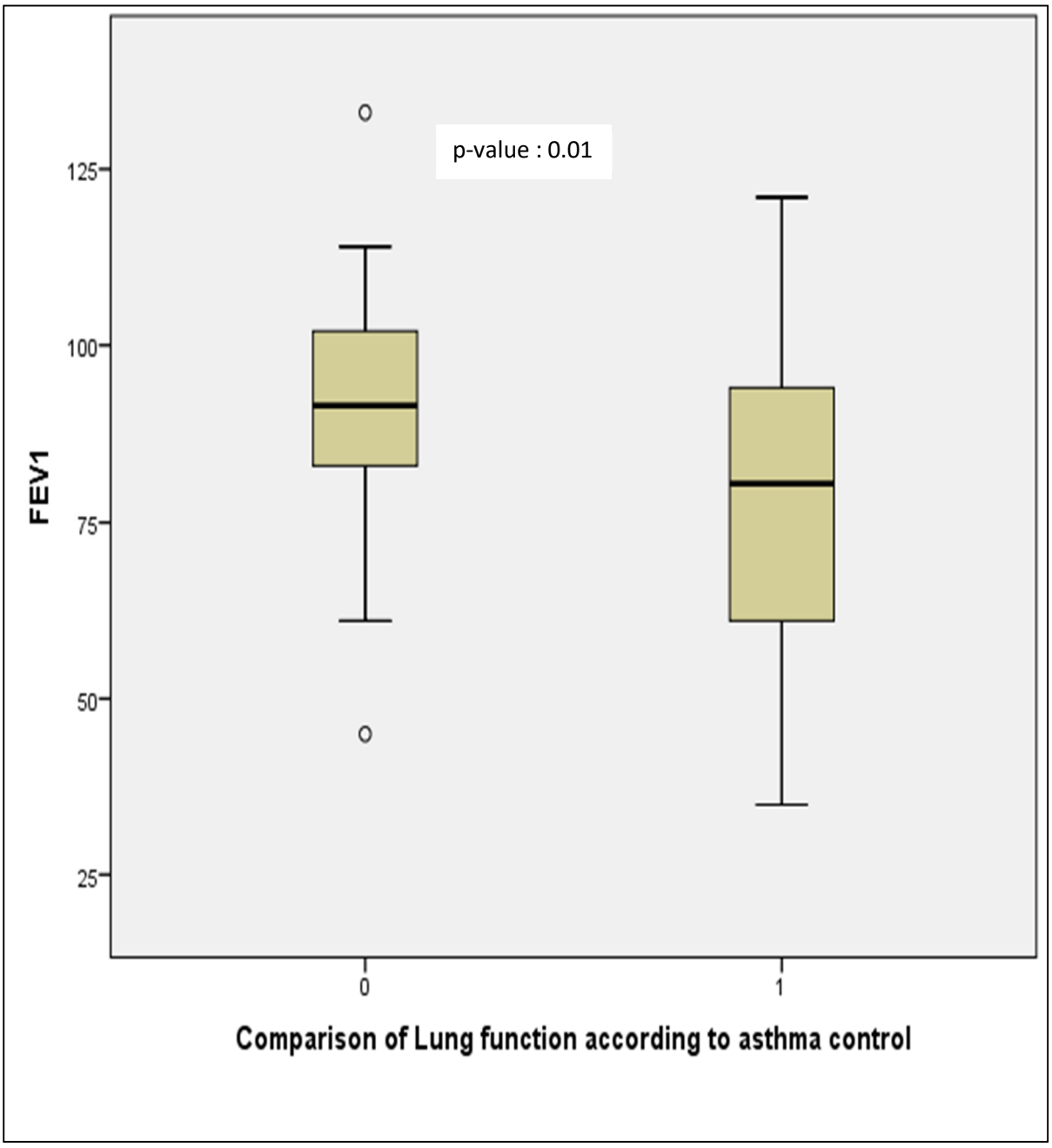


Figure 9: Comparison of lung function level according to asthma control



7.4 Association of aeroallergen sensitization with various variables: Among 90 enrolled patients, 71% of the asthmatics and 26% of the control showed aeroallergen sensitization. There was a significant association found between blood eosinophils count or percentage and aeroallergen sensitization (Figure 10). However, we did not find any association between IgE levels, serum periostin levels with aeroallergen sensitization. These findings are shown in table 4.

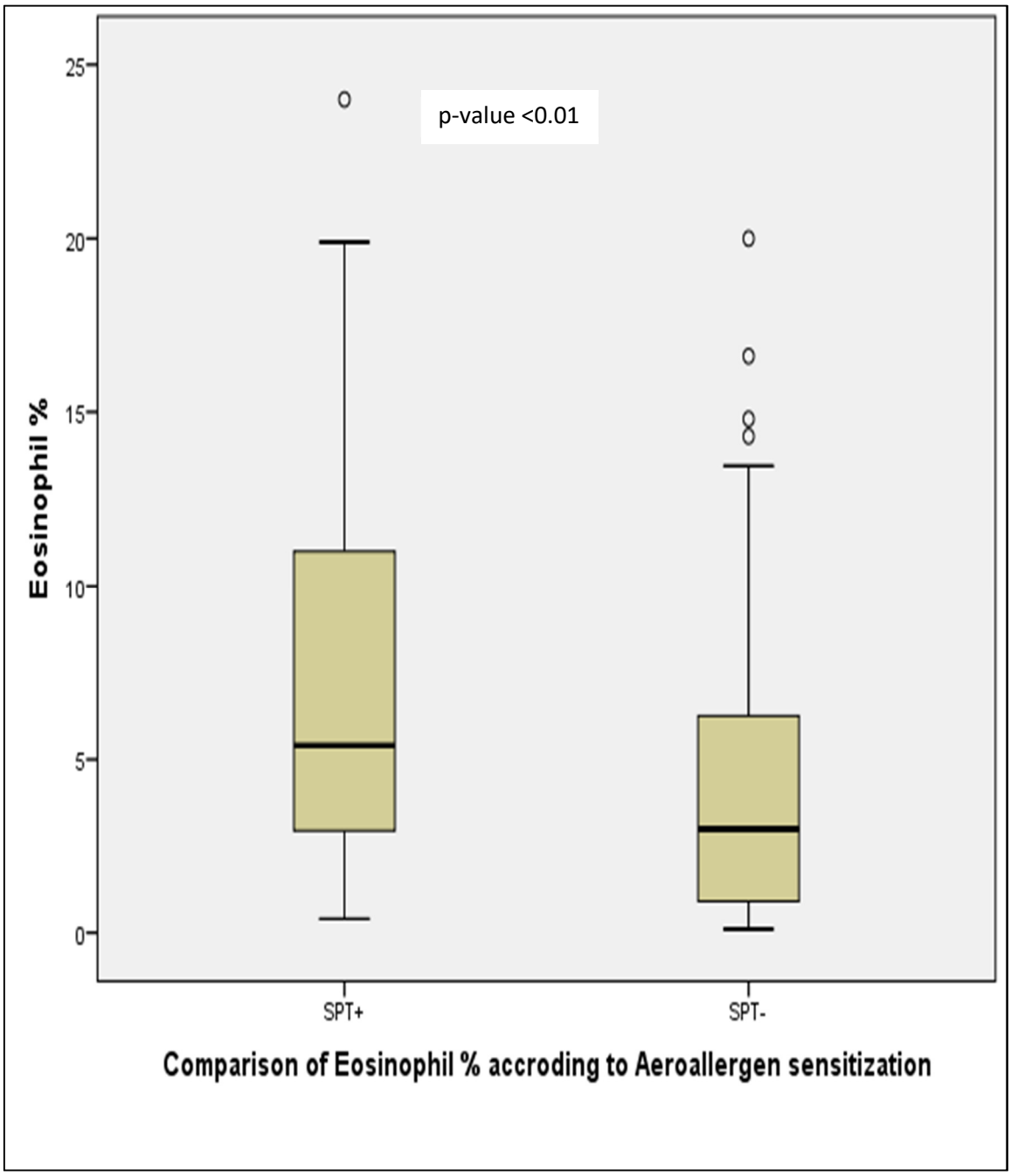
Table 4: Laboratory parameters according to aeroallergen sensitisation (n=90)

Aeroallergen sensitisation	**Present(n=51)**	**Absent (n=39)**	**p-value**
IgE level	449 (215,1048)	277 (48,709)	0.09
AEC	368 (224,717)	179 (69,481)	<0.01*
Blood eosinophils %	5.4 (2.6,11)	3 (1,6.4)	<0.01*
Serum periostin (ng/mL)	23 (22,26)	23 (21,30)	0.65

* Statistically significant

Data are presented as a percentage, median (inter-quartile range), or mean ± standard deviation

Figure 10: Comparison of eosinophils percentage according to aeroallergen sensitization



7.5 Serum periostin levels according to clinical variables in children with asthma: We found that serum periostin values were significantly higher in the patient with uncontrolled asthma compared to the ones who had good asthma control. Our study did not show any significant difference in the periostin values, with gender status, aeroallergen sensitization, or history of allergic rhinitis. All the findings are shown in table 5.

Table 5: Levels of serum periostin according to the clinical characteristics in children with asthma (n=60).

Variable	Serum periostin (ng/mL)	p-value
Gender		
Male	25.5 ± 7.5	0.53
Female	26.8 ± 5.5	
Asthma control status		
Controlled	24.1 ± 5.7	0.03*
Uncontrolled	28.0 ± 8.1	
Aeroallergen sensitization		
Yes	25.2 ± 6.4	0.32
No	27.2 ± 8.5	
Allergic rhinitis		
Yes	26.1 ± 7.5	0.37
No	23.8 ± 3.2	
* Statistically significant Data are presented as mean ± standard deviation		

7.6 Correlation between the clinical variables related to asthma and serum periostin: No correlation was found with the age, BMI z score, asthma control status, accompanying atopic diseases such as allergic rhinitis, total IgE levels, eosinophils counts or percentage, and pulmonary function parameters on spearman's correlation. (Table 6).

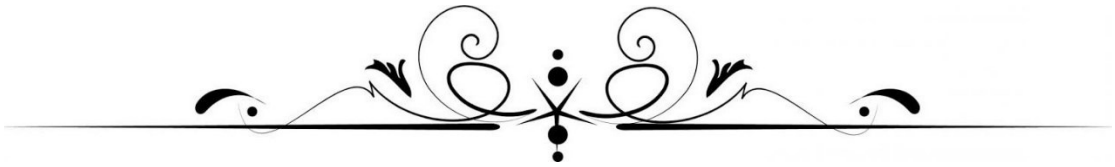
Table 6: Spearman's correlation coefficients between serum periostin level and other clinical variables in children with asthma (n=60)

	Serum periostin (ng/mL)	
Variables	R	p-value
Age	0.068	0.06
Asthma control status	-0.170	0.19
BMI, Z-score	0.170	0.19
Total IgE (IU/mL)	-0.056	0.67
Eosinophils (%)	0.092	0.48
Eosinophils (/mL)	0.055	0.67
FEV1	-0.182	0.18
Allergic rhinitis	-0.105	0.42

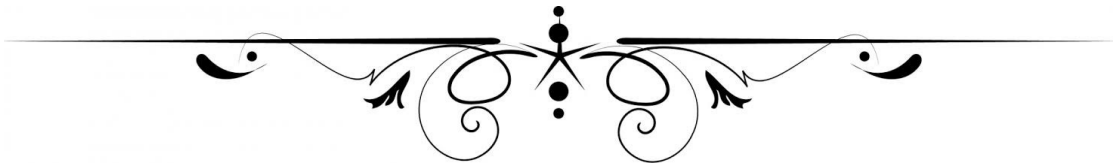
7.7 Multivariable logistic regression analysis for asthma control: Multivariable logistic regression analysis showed that serum periostin levels were associated with poor asthma control in children. However, other variables like BMI z score, aeroallergen sensitization, eosinophils percentage, IgE levels, and FEV1 weren't associated with asthma control. Results are shown in Table 7.

Table 7: Multivariable logistic regression analysis for asthma control (n=60)

Variables	**OR (95%CI)**	**p-value**
Age	1.21(0.96-1.51)	0.09
BMI, z score	1.19(0.59-2.4)	0.61
Aeroallergen sensitisation	0.99(0.23-4.09)	0.98
Serum periostin (ng/mL)	1.12(1.01-1.24)	0.02*
Eosinophils, %	1.06(0.95-1.18)	0.28
Total IgE (IU/mL)	1.00(0.99-1.00)	0.58
FEV1	0.97(0.93-1.00)	0.08
* Statistically significant		



DISCUSSION



8. DISCUSSION

In this cross-sectional study, we observed that serum periostin levels were significantly higher in children with asthma as compared to age and gender-matched control. Our finding also suggested an association between serum periostin levels and poor asthma control in children independently from the confounding factors such as age, BMI, aeroallergen sensitization, eosinophil percentage, and total IgE.

In our study mean age group of study participants was 12.1±2.77 years. The mean age in asthma and control group was 11.8±3.04 years and 12.6±2.1 years respectively without any statistically significant difference. Baseline variables like weight, height, and BMI score also did not show any significant difference in both groups. Yavuz et al, enrolled 158 children (125 with asthma and 33 age- and sex-matched control subjects) with a median age of 10.2 years (range 6.0-17.0). There were no significant differences between the children with asthma and control groups in terms of age, sex, body mass index, which is coherent with our study.[7]

We compared serum periostin levels in children with asthma and control and found a statistically significant difference in values between these two groups. Yavuz et al. also found that children with asthma had significantly higher periostin levels than controls. [7] According to Song et al. Periostin levels in asthmatics were substantially higher than in healthy controls. [8] Inoue et al. also demonstrated in their cross-sectional study that serum periostin levels in the bronchial asthma group were significantly higher than those in the control group. [11]

We found that the IgE levels in asthma patients were statistically significantly higher than in the control group. Adawy et al. also found statistically significant higher total IgE levels among asthmatics than in control groups. [14] Inoue et al. also demonstrated that the asthmatic group had a significantly higher total IgE level as compared to healthy controls.[11] The findings of Song et al.'s study were in line with our study as they also had found that the asthmatic group had a significantly higher total IgE level as compared to healthy controls.[8]

Our study showed that the percentage of eosinophils or absolute eosinophils count was significantly higher in patients with asthma than in the control group. Yavuz et al, also found that blood eosinophils count and percentage were higher in asthma patients as compared to healthy controls.[7] Adawy et al. also found statistically significant higher blood eosinophil

levels among asthmatics than in control.[14] Inoue et al. found that the asthmatic group had a significantly higher eosinophils count in comparison to controls. [11] Song et al. also found that the asthmatic group had significantly higher total blood eosinophils counts as compared to the healthy control. [8]

All of the 90 patients underwent skin prick testing to common aeroallergen for our region and found that aeroallergen sensitization was higher in the patients with asthma than in the control group. A significant number of the children (71.6%) with asthma have positive SPT to one or more of aeroallergens, in comparison to control (26.67%). This finding was coherent with the study done by Yavuz et al., who found that the children with asthma reported considerably greater rates of aeroallergen sensitivity.[7] The finding of Inoue et al.'s study was similar to our study as they found that the asthmatic group had significantly higher skin prick test positivity as compared to healthy controls.[11]

We found that median serum periostin levels of children with uncontrolled asthma were significantly higher than in children with controlled asthma. Adawy et al. also showed that there was a statistically significant increase in serum periostin levels among uncontrolled asthmatic patients than those with properly controlled asthma.[14] Jia et al. also concluded that serum periostin had a promising significant role for detecting uncontrolled asthma than its severity, thus detecting the group of patients who are at high risk of frequent life-threatening frequent exacerbation and in turn in need of more controller medications than ordinary ones.[18] Basha et al. demonstrated that children with an acute asthma exacerbation have significantly higher serum periostin levels as compared to the stable controlled asthma group. [22] Park et al. also stated that there was statistically significant decreased serum periostin level and bronchial wall thickness among patients with uncontrolled asthma after being adequately and properly controlled than their baseline uncontrolled states.[23] In contrast to the above studies, Meena et al. observed that children with uncontrolled severe asthma were associated with lower serum periostin levels.[20]

We found that percentage of predicted FEV1 was significantly higher in the controlled asthma group as compared to the uncontrolled asthma group. Kanemitsu et al. demonstrated that a low percentage predicted FEV1 is more in the patient who had poor asthma control according to the ACT scoring. [19]

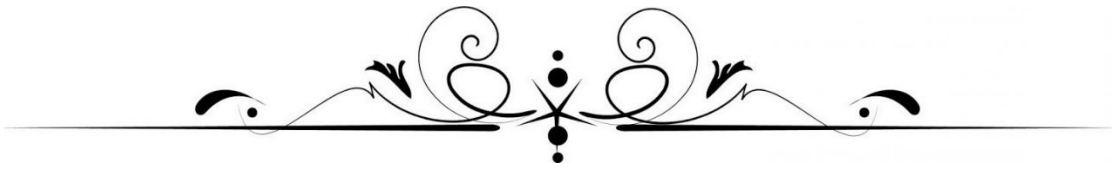
We did not find any significant difference in aeroallergen sensitization, total IgE levels, eosinophils %, and absolute eosinophils count between controlled and uncontrolled asthma groups. Yavuz et al, also found that there was no difference in the groups between IgE levels, blood eosinophils counts. [7]

We also analyzed the association of aeroallergen sensitization with various variables like IgE levels, absolute eosinophils count, and percentage of eosinophils. We found that all these variables were higher in the group with the positive skin prick test. However, a statistically significant association was found only with AEC and eosinophils. Khadadah et al. also found a statistically significant association between blood eosinophilia and aeroallergen sensitization. [25] Mahmoud et al. showed that increased aeroallergen sensitization was associated with a significant increase in the eosinophils count and percentage. [26]

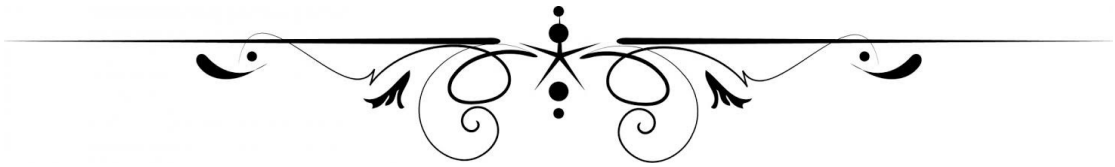
We investigated the effect of different clinical variables (gender, aeroallergen sensitization, and allergic rhinitis) on the serum periostin levels in children with asthma. No significant difference in the periostin values was present when compared with gender status, aeroallergen sensitization, or history of allergic rhinitis. Yavuz et al. also did not find any significant difference in serum periostin values when compared to gender status, aeroallergen sensitization, or history of allergic rhinitis. [7] Kim DY et al. found in their study that allergic rhinitis or allergic sensitization did not influence serum periostin levels. [27]

In our study, we did not find any significant correlation of serum periostin with age, BMI, Z-score, asthma control status, total IgE, eosinophils count and percentage, FEV1, allergic rhinitis. Yavuz et al. also didn't find any correlation with age, accompanying allergic diseases, asthma control status, total IgE levels, eosinophils counts/percentage, lung function. [7] Our study did not show any correlation of serum periostin with the BMI, which was different from the study of Yavuz et al. The possible explanation might be the small sample size and different settings in our study.

We found a significant and independent association of asthma control and serum periostin levels on multivariable logistic regression analysis. The observations of our study were consistent with the previous study [22]. In contrast, Yavuz et al. did not find a significant association between asthma control and serum periostin. [7]



STRENGTH & LIMITATIONS



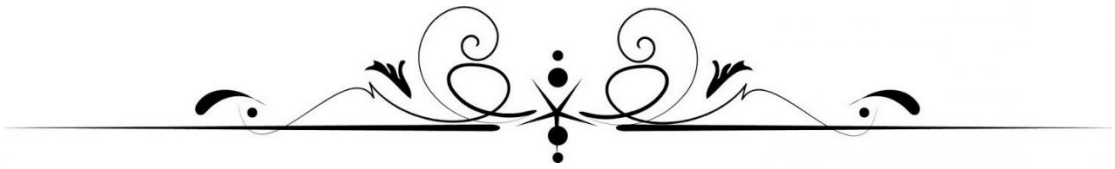
9. STRENGTH AND LIMITATIONS OF STUDY

9.1 The main strength of our study was-

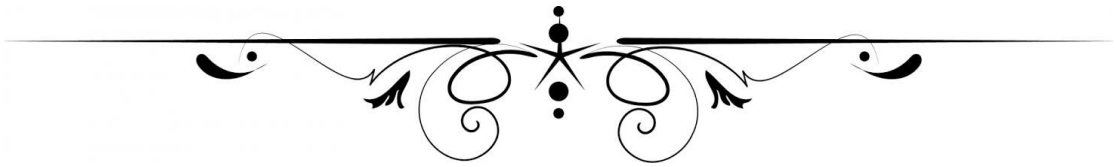
This is the first study from India to the best of our knowledge that looked into the association of serum periostin with asthma control in children. Moreover, we used a validated score i.e. C-ACT/ACT for evaluation of asthma control.

9.2 Limitations of the study:

Serum periostin is a bone-derived extra-cellular matrix protein and it is secreted by bone osteoblast. Its value is affected by the growth of children. Therefore, we were not able to define the cut-off for asthma control in children. Moreover, this study was done at a single tertiary care center, and the data collected were from one tertiary hospital. Therefore, the result of our study can not be generalized.



SUMMARY & CONCLUSION



10. SUMMARY AND CONCLUSION

10.1 Title of the study: Relationship between High Serum Periostin and Asthma Control in children

10.2 Background: Periostin has emerged as a novel biomarker in the pathogenesis of asthma. However, there are limited data on the association of periostin and asthma control in children.

10.3 Objectives

Primary Objective

- To determine the association between serum periostin level and asthma control in children.

Secondary Objective

- To determine the relationship between the blood eosinophils counts, serum IgE levels, and pulmonary function test with the Asthma control**.**
- Comparison of serum periostin level in asthma patients and non-respiratory patients.

10.4 Methods: Children aged 6-17 years with physician-diagnosed asthma were enrolled in the study along with age and sex-matched control subjects who visited our outpatient department with non-respiratory complaints. Asthma control was assessed by using ACT/ C-ACT scoring. All the patients underwent skin prick tests using common allergens. Anthropometry measurements and pulmonary function tests were performed on all the patients. The blood sample was taken for blood eosinophils counts, IgE levels, and serum periostin.

10.5 Results: A total of 90 children (60 with asthma and 30 age and sex-matched control subjects) with mean age of 12.1 years (12.1±2.77SD) were enrolled. Children with asthma had significantly higher periostin levels than the controls [23.5 (22, 26) vs 22 (19.40, 22.96), p-value 0.04]. The median serum periostin level in children with uncontrolled asthma [25.8 (23.2, 33.5)] was significantly higher than in children with well-controlled asthma [23 (21,

24] (p-value 0.01). On multivariable logistic regression analysis, serum periostin levels were associated with poor asthma control in children (OR, 1.12; 95% CI, 1.01-1.24, p-value 0.02).

10.6 Conclusion: Serum periostin levels are higher in children with asthma, and elevated serum periostin levels are associated with poor asthma control. However, more research is needed to fully understand the role of periostin in asthma pathophysiology and clinical value for physicians dealing with asthma.

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11. BIBLIOGRAPHY

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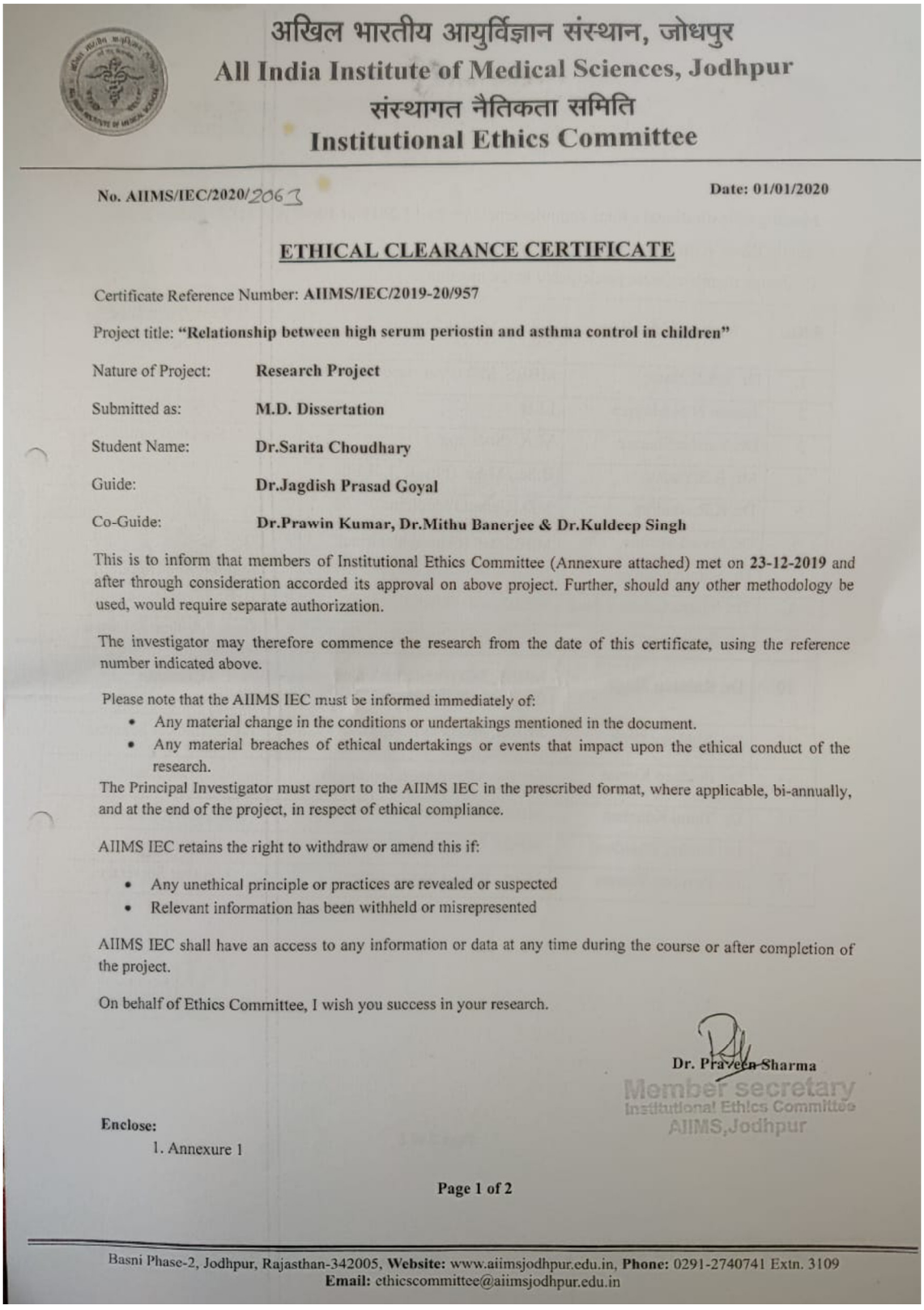
APPENDIXES

APPENDIX- 1



अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2020/2063 Date: 01/01/2020

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2019-20/957**

Project title: **"Relationship between high serum periostin and asthma control in children"**

Nature of Project: **Research Project**

Submitted as: **M.D. Dissertation**

Student Name: **Dr.Sarita Choudhary**

Guide: **Dr.Jagdish Prasad Goyal**

Co-Guide: **Dr.Prawin Kumar, Dr.Mithu Banerjee & Dr.Kuldeep Singh**

This is to inform that members of Institutional Ethics Committee (Annexure attached) met on **23-12-2019** and after through consideration accorded its approval on above project. Further, should any other methodology be used, would require separate authorization.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Enclose:
1. Annexure 1

Page 1 of 2

Basni Phase-2, Jodhpur, Rajasthan-342005, **Website:** www.aiimsjodhpur.edu.in, **Phone:** 0291-2740741 Extn. 3109
Email: ethicscommittee@aiimsjodhpur.edu.in

APPENDIX - 2

PARTICIPANT INFORMED CONSENT FORM (PICF)

Protocol / Study number: _____

Participant identification number for this trial: _____

Title of project: Relationship between high serum periostin and asthma control in children.

Name of Principal Investigator: Dr. Sarita Choudhary Tel. No- 9958739387

The contents of the information sheet dated that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

The nature and purpose of the study and its potential risks/benefits and expected duration of the study and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from AIIMS, Jodhpur. I permit for these individuals to have access to my records.

I agree to take part in the above study.

---------- Date:

(Signatures / Left Thumb Impression) Place:

Name of the Participant: _____

Son / Daughter / Spouse of: _____

Complete postal address: _____

This is to certify that the above consent has been obtained in my presence.

Signatures of the Principal Investigator Date:
Place:

1) Witness – 1

Name:
Signature
Address:

2) Witness – 2

Name:
Signature
Address:

परिशिष्ट - 3

आंशिक सूचित सहमति फार्म (PICF)

प्रोटोकॉल / अध्ययन संख्या: _____

इस परीक्षण के लिए प्रतिभागी की पहचान संख्या: _____

परियोजना का शीर्षक: अस्थमा नियंत्रण के साथ सीरम पेरीओस्टिन का संघ।

प्रधान अन्वेषक का नाम: डॉ सरिता चौधरी Tel. No- 9958739387

उपलब्ध कराई गई सूचना पत्र की सामग्री को मेरे द्वारा ध्यान से पढ़ा गया है / मुझे विस्तार से समझाया गया है, जिस भाषा में मैं समझता हू,और मैंने सामग्री को पूरी तरह से समझ लिया है। मैं पुष्टि करता हूं कि मुझे सवाल पूछने का अवसर मिला है। अध्ययन की प्रकृति और उद्देश्य और इसके संभावित जोखिम/लाभ और अध्ययन की अपेक्षित अवधि, और अध्ययन के अन्य प्रासंगिक विवरण मुझे विस्तार से बताए गए हैं। मैं समझताहूं कि मेरी भागीदारी स्वैच्छिक है और मैं बिना किसी कारण के, बिना किसी चिकित्सीय देखभाल या कानूनी अधिकार के प्रभावित हुए बिना किसी भी समय वापस लेने के लिए स्वतंत्र हूं। मैं समझता हूं कि इस शोध में मेरी भागीदारी और मेरे किसी भी मेडिकल नोट के वर्गों के बारे में मेरे द्वारा एकत्रित जानकारी को एम्स, जोधपुर के जिम्मेदार व्यक्तियों द्वारा देखा जा सकता है। मैं इन व्यक्तियों को अपने रिकॉर्ड तक पहुंचने की अनुमति देता हूं। मैं उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूं।

(हस्ताक्षर / बाएं अंगूठे का निशान) **दिनांक:**

जगह:

प्रतिभागी का नाम: _____

पुत्र / पुत्री / पति / पत्नी: _____

पूरा डाक पता: _____

यह प्रमाणित करना है कि मेरी उपस्थिति में उपरोक्त सहमति प्राप्त हुई है।

प्रधान अन्वेषक के हस्ताक्षर

दिनांक:

जगह:

APPENDIX - 4

PARTICIPANT INFORMATION SHEET (PIS)

Title of the Study/Project: Relationship between high serum periostin and asthma control in children.

Aims and purpose of the research

Your child is suffering from asthma. It has been proved from observational studies that the level of serum Periostin plays an important role in asthma management.

Procedure

Your child is invited to join the study. It is planned to include around 64 such children in this study. The child will undergo an investigation serum periostin. The assessment shall include the recording of relevant history and clinical examination findings. Total ~ 5 mL venous blood will be drawn for assessing the serum periostin.

The benefits to be expected from the research to the subject or to others: Serum periostin has an important role in asthma and it has been associated with low responsiveness to inhalation corticosteroid therapy.

i. **Any risk to the subject associated with the study:** Potentially none. Drawing a 5 mL blood sample is not expected to inflict any significant physiological derangements in a school going child

ii. **Maintenance of confidentiality of records:** The medical records of the patient shall be kept confidential and accessed only by the treating physician or, if necessary, by the Ethics Committee of the All India Institute of Medical Sciences, Jodhpur.

iii. **Provision of free treatment, compensation for research-related injury:** Serum periostin will be provided free of cost.

iv. **Freedom of the individual to participate and to withdraw from research at any time without penalty or loss of benefits to which the subject would otherwise be entitled:** You are free to participate in and withdraw from this study at any time you so desire. This will in no way affect your ongoing treatment at the Institute.

v. **Amount of blood sample to be taken:** ~5 mL (1 teaspoon full)

vi. **Costs and source of investigations, disposables, implants, and drugs:** Investigations mentioned will be done in AIIMS; you are not expected to pay for the cost of these investigations.

vii. **Telephone number/contact number of Principal Investigator and Co-investigator:** In case of any concerns related to your child's treatment, you should contact:

Dr. Jagdish Prasad Goyal, Additional Professor, Department of Paediatrics, All India Institute of Medical Sciences, Jodhpur, Rajasthan; 342001; Phone 8475000270, email: jpgoyal@rediffmail.com

APPENDIX - 5

आंशिक सूचना शीट (पीआईएस)

अध्ययन / परियोजना का शीर्षक: अस्थमा नियंत्रणके साथ सीरम पेरीओस्टिन का संघ।

i)**अनुसंधान का उद्देश्य और उद्देश्य: आपका बच्चा अस्थमा से पीड़ित है। अवलोकन अध्ययनों से यह साबित हुआ है कि सीरम पेरीओस्टिन का स्तर अस्थमा प्रबंधन में महत्वपूर्ण भूमिका निभाता है।**

प्रक्रिया: आपके बच्चे को अध्ययन में शामिल होने के लिए आमंत्रित किया जाता है। इस अध्ययन में लगभग 64 **ऐसे बच्चों को शामिल करने की योजना है। बच्चे की जांच सीरम पेरीओस्टिन के तहत होगी। मूल्यांकन में प्रासंगिक इतिहास और नैदानिक परीक्षानिष्कर्षों की रिकॉर्डिंग शामिल होगी। सीरम पेरीओस्टिन का आकलन करने के लिए कुल** ~ 5 **मिलीलीटर शिरापरक रक्त खींचा जाएगा।** ii) **विषय भागीदारी की अपेक्षित अवधि-** 6 **महीने**

iii) **शोध से विषय या दूसरों के लिए अपेक्षित लाभ: सीरम पेरीओस्टिन अस्थमा में एक महत्वपूर्ण भूमिका है और यह साँस लेना कोर्टिको-स्टेरॉयड थेरेपी के लिए कम जवाबदेही से जुड़ा हुआ है।**

iv) **अध्ययन से जुड़े विषय पर कोई जोखिम: संभावित रूप से कोई नहीं। स्कूल जानेवाले बच्चे में** 5**मिलीलीटर रक्त के नमूने को आकर्षित करने के लिए किसी भी महत्वपूर्ण शारीरिक व्युत्पन्नता की संभावना नहीं है**

v) **अभिलेखों की गोपनीयता का रख-रखाव :अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर की आचार समिति द्वारा रोगी के मेडिकल रिकॉर्ड को गोपनीय रखा जाएगा और उपचार चिकित्सक के पास या यदि आवश्यक हो, तभी पहुँचा जा सकता है।**

vi) **नि:शुल्क उपचार का प्रावधान, अनुसंधान से संबंधित चोट का मुआवजा: सीरम पेरीओस्टिन नि: शुल्क प्रदान किया जाएगा।**

vii)**किसी भी समय दंड या लाभ के नुकसान के बिना किसी भी समय अनुसंधान से पीछे हटने की स्वतंत्रता, जिसके लिए विषय अन्यथा हकदार होगा: आप इस इच्छा से किसी भी समय इस अध्ययन से भाग लेने और वापस लेने के लिए स्वतंत्र हैं। यह किसी भी तरह से संस्थान में आपके चल रहे उपचार को प्रभावित नहीं करेगा।**

viii) **लिए जाने वाले रक्त के नमूने की मात्रा: ~ ५मिली (१ चम्मच भरा हुआ)**

ix) **लागत और जांच के स्रोत, डिस्पोजल, प्रत्यारोपण और ड्रग्स: उल्लिखित जांच एम्स में की जाएगी, आपको इन जांचों की लागत का भुगतान करने की उम्मीद नहीं है।**

x) **प्रधानअन्वेषक और सहअन्वेषकका टेलीफोन नंबर / संपर्क नंबर**: **आपके बच्चे के उपचार से संबंधित किसी भी चिंता के मामले में, आपको संपर्क करना चाहिए**:

डॉ। जगदीश प्रसाद गोयल, **अतिरिक्त प्रोफेसर**, **बाल रोग विभाग**, **अखिल भारतीय आयुर्विज्ञान संस्थान**, **जोधपुर**, **राजस्थान**; 342001; **फोन** 8475000270, **ईमेल**: jpgoyal@rediffmail.

APPENDIX - 6

Screening form: Date: -

Screening no.-

Name: -

Father/Mother's name -

UHID/CR No.-

Date of birth: or Approximate Age (years):

Children diagnosed with moderate persistent asthma as per GINA guideline (Yes=1, No=2)

Age 6-18 years (Yes=1, No=2)

Diagnosis:

Consent taken: Yes=1, No=2

If not given, specify reason_____

Enrolled in study: Yes=1 No=2

If not enrolled, specify reason_____

Unique identification number

Relationship between Serum Periostin with Asthma Control in Children

Enrolment Form

STICKER

Enrolment number	
UHID	
Date:	

PURPOSE OF THE FORM: • This form is meant to be filled only if the patient satisfies all the criteria mentioned in the screening form		
Part A: Baseline characteristics of the patient		
S. No.	**Items**	**Response**
1.	Name	
2.	Date of birth (dd/mm/yy)	
3.	Age (completed years)	
4.	Gender (Male=1/female=2)	
5.	Father's name	
12.	Address 1:	Address 2:
	Pin code	Pin code
	Mobile 1	
	Mobile 2	

Part A 1: History and Details of disease		
S.	**Items**	**Response**

No.		(yes=1/No=2)
1.	History of cough?	
	Current status? Present=1/absent=2	
	Duration of symptoms (months) < 1 year=1, 1-5 year= 2, 5 -10 years=3 >10 years =4	
2.	History of breathlessness?	
	Current status? Present=1/absent=2	
	Duration of symptoms (months) < 1 year=1, 1-5 year= 2, 5 -10 years=3 >10 years =4	
3.	History of wheeze?	
	Current status? Present=1/absent=2	
	Duration of symptoms < 1 year=1, 1-5 year= 2, 5 -10 years=3 >10 years =4	
4	History of chest tightness?	
	Current status? Present=1/absent=2	
	Duration of symptoms < 1 year=1, 1-5 year= 2, 5 -10 years=3 >10 years =4	
5	History of nasal block/sneezing/nasal discharge?	
	Current status? Present=1/absent=2	
	Duration of symptoms < 1 year=1, 1-5 year= 2, 5 -10 years=3 >10 years =4	

6.	History of eczema?	
	Current status? Present=1/absent=2	
	Duration of symptoms < 1 year=1, 1-5 year= 2, 5 -10 years=3 >10 years =4	
7	History of snoring?	
	Current status? Present=1/absent=2	
	Duration of symptoms < 1 year=1, 1-5 year= 2, 5 -10 years=3 >10 years =4	
8	Association with fever?	
9.	Seasonal variation?	
10	**Medications (Y=1/N=2)**	
	Use of inhaled beta 2 agonists?	
	Current status?	
	Use of inhaled steroids?	
	Current status?	
	Use of systemic steroids?	
	Current status?	
	Use of mast cell stabilizers?	
	Current status?	
	Use of nebulization?	
	Current status?	
	Use of IV medications	

	Current status?	
	Use of cough syrup	
	Current status?	
	Use of homeopathy/ayurvedic?	
	Current status?	
11	**Past History (Yes=1/No=2)**	
	LRTI (bronchiolitis/pneumonia)	
	Tuberculosis	
	Number of previous excerbations	
12	**Perinatal History (Yes=1/No=2)**	
	Prematurity	
	Meconium stained liquor	
	Exclusive breast feeding for 6 months	
13	Symptom frequency (seasonal=1/Perennial=2) If seasonal mention month [Summer(march-June)/monsoon (July to October)/winter (Nov to Feb)}	
14	Missed school (Y=1/N=2) If yes then number:	
15	History of smoke exposure? (Y=1/N=2)	
16	**ENVIRONMENTAL**	
	Rural=1/urban =2	
	Cooking fuel used (biomass=1/ Kerosene=2/LPG=3)	

	Pets at home (Y=1/N=2)	
17	**TRIGGERS**	
	Exercise (Y=1/N=2)	
	FOOD (Y=1/N=2)	
	Infection/URI (Y=1/N=2)	
	Mosquito coils (Y=1/N=2)	
	Pollens (Y=1/N=2)	
	Drugs (Y=1/N=2)	
	Cockroaches in house (Y=1/N=2)	
	House Dust (Y=1/N=2)	
	Smoke (Y=1/N=2)	

Part A2: Family History (Yes=1/No=2)				
	Father	**Mother**	**Sibling**	**Grandparents**
Age				
Asthma				
Atopy				
Other specify				
H/O smoking at home (Yes=1/No=2)				

Examination		
Part B1: Vitals and GPE		
1.	Pulse Rate	

2.	Respiratory Rate	
3.	SpO_2	
4.	Blood Pressure	
5.	Pallor(Y=1/N=2)	
6.	Lymphadenopathy(Y=1/N=2)	
7.	Clubbing (Y=1/N=2)	
6	Nasal polyp(Y=1/N=2)	
7.	Hypertrophied tonsil (Y=1/N=2)	
8.	Ear discharge(Y=1/N=2)	
9.	Sinus tenderness (Y=1/N=2)	
10.	DNS (Y=1/N=2)	

Part B 2: Anthropometry

	Items	Response
1.	Height (nearest 0.1 cm)	
	IAP Z score	
2.	Weight (nearest 0.1 kg)	
	IAP Z score	
3.	BMI (kg/m^2)	
	IAP Z score	
4.	Waist circumference (cm)	
5.	Hip circumference (cm)	

6.	Waist/Hip ratio	

Part B3: Respiratory System Examination	
Respiratory rate (per minute)	
Chest wall deformity (Yes=1, No=2)	
Auscultation (Clear=1, wheeze=2, Crepitations=3, both 2& 3=4)	

Part C: Spirometry						
	Observed (pre)	**Observed (post)**	**Expected (pre)**	**Expected (post)**	**Percentage (pre)**	**Percentage (post)**
Forced Expiratory Volume-1 second (FEV_1)						
Forced Vital Capacity (FVC)						
FEV_1/FVC						
Peak Expiratory Flowrate (PEFR)						
Forced Expiratory Flow25 (FEF_{25})						
Forced						

Expiratory Flow50 (FEF_{50})						
Forced Expiratory Flow75 (FEF_{75})						

Part D: C-ACT score	
C-ACT score	
Part F: Blood values	
Sample collected on: Date- **Time-**	
Sample ID:	
	Report
Serum periostin (ng/mL)	
Hemoglobin (g/dl)	
Total leukocyte count($\times 10^3/\mu l$)	
Neutrophil count (%)	
Eosinophil count (%)	
Platelet count ($\times 10^3/\mu l$)	
IgE levels	
SPT	

Part G Diagnosis: Asthma	
Symptoms pattern	**Yes=1, No=2**

More than one symptom (wheeze, cough, shortness of breath or chest tightness)	
Symptoms are often worse at night or early morning	
Symptoms vary over time and in intensity	
Symptoms triggered by cold, exercise, change in weather, laughter, smoke, dust, or strong smell	
Positive bronchodilator reversibility test (Increase in $FEV_{1\ of}$ > 12 % predicted)	

Part: H	
Asthma comorbidities (Yes=1, No=2)	
If yes write the comorbidities:	

Part: I	Treatment step	
Step 1	SABA SOS	
Step 2	Low to moderate dose ICS SABA SOS	
Step 3	low to moderate dose ICS+LABA SABA SOS	
Step 4	High dose ICS + LABA ± LTRA SABA SOS	
Step 5	High dose ICS + LABA + Molecular therapy SABA SOS	