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Interleukin-13 as a potential biomarker in the management of pediatric asthma – a longitudinal study

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ABSTRACT

Introduction and aim. Asthma is predominantly a Th2 type hypersensitive disorder, with interleukin (IL) 4 and IL-13 playing a pivotal roles. Interleukin 13 is one of several cytokines that cause persistent inflammation associated with asthma. The aim was to examine the relationship between the response to treatment in asthma and serum IL-13.

Material and methods. This study, conducted at the SRM Medical College Hospital and Research Center, in Tamil Nadu, involved 68 children aged 6 to 12 years of age diagnosed with asthma. The study included medical history, including age of onset of wheezing, history of allergic rhinitis/atopic dermatitis, food allergies, use of inhalational corticosteroids, hospital admissions, and family history. Spirometry was performed, and treatment with inhalational corticosteroids was started according to GINA guidelines. Blood was collected prior to and after 3 months of treatment.

Results. A substantial positive correlation was observed between gender and IL-13 levels. An improvement in forced expiratory volume in the first second (FEV_1) was observed after treatment [(74.72% vs 95.05%)

(p<0.0001)]. A negative correlation was discovered between IL-13 and FEV_1. A statistical significance between IL-13 levels before and after treatment (p=0.005).

Conclusion. Inhalational corticosteroids reduced serum IL-13 levels, indicating its role as a prognostic marker in pediatric asthma.

Keywords. asthma, biomarkers, interleukin-13

Introduction

Chronic asthma is an inflammatory illness of the airways marked by recurrent episodes of airflow restriction brought on by bronchospasm, edema, and increased mucus production. The term "atopic triad" refers to common conditions that are associated with asthma, including eczema (atopic dermatitis) and seasonal allergies (allergic rhinitis).[1]

Asthma is predominantly a Th2 type hypersensitive disorder, with interleukin 4 (IL-4) and IL-13 playing pivotal roles. Among the several cytokines that cause persistent inflammation associated with asthma is IL-13. In response to antigen-specific stimulation, activated Th2 CD4 and CD8 cells generate IL-13.[2-3] In the absence of appropriate preventive therapy, patients with asthma who have elevated serum IL-13 values may experience irreversible airway modelling due to airway hyper-responsiveness, mucus hypersecretion, fibroblast activation, and airway smooth muscle hyperplasia and hypertrophy.[4]

The clinical heterogeneity observed in individuals with poorly managed asthma requires the development of biomarkers that may facilitate the identification of a specific subgroup of patients. It has been demonstrated that in type 2 asthma, the pleiotropic cytokine IL-13 plays a significant role in the etiology of asthma. Induction of goblet cell metaplasia, increased mucus production, increased airway hyperreactivity, and indirectly, chemotaxis-mediated eosinophil trafficking to the site of tissue damage are among the effects of IL-13 in these situations.[5] Mechanistic insights into the involvement of this cytokine in inducing eosinophilia have been unveiled by data from preclinical models and clinical studies including IL-13 inhibitors in humans.

In the era of personalized medicine, to deliver an effective approach to asthma, it is important to identify biomarkers that can predict the course of the disease and response to therapy. A biomarker is a measurable indicator that can evaluate normal or pathological biological processes or the pharmacological response to a therapeutic intervention. Among the few biomarkers studied in asthma, serum IgE lacks specificity, eosinophils show elevated trends in other conditions, such as protozoal infections, serum periostin is elevated in growing children and fractional exhaled nitric oxide requires the availability of specialized equipment.

There is a general lack of literature on the study of biomarkers in assessing therapeutic response in asthma, specifically in the pediatric population.

In the present study, we provide a perspective on the utility of IL-13 as a predictor for evaluating the response to therapy in pediatric asthma patients, as evidenced by an improvement in forced expiratory volume in the first second (FEV_1).

The maximum amount of air expelled during the first second of a forced expiration from a position of fully expressed inspiration is known as the FEV_1. The degree of airway obstruction in asthma is determined by the FEV_1 marker. Reversibility with bronchodilator administration is indicated by a 12% or 200 mL increase in FEV_1.[6-7]

A receiver operating characteristic (ROC) curve, a graphical illustration and fundamental tool used for the evolution and comparison of diagnostic systems that allow comparisons of sensitivity and specificity.[8]

Aim

Examine the relationship between children's response to treatment in asthma and serum IL-13 levels.

Material and methods

The current longitudinal study was conducted at the SRM Medical College Hospital and Research Center, a tertiary hospital in Tamil Nadu, with the approval of the Institutional Ethics Committee (IEC No: ST0922-797). The research project started in September 2022 and spanned six months. In total, 68 patients were recruited for the investigation (n=68).

After receiving informed consent from parents and a duly signed consent form from the research participant, a total of 68 children aged between 6 and 12 years, recently diagnosed with asthma and starting inhalational corticosteroids as defined in the guidelines of the Global Initiative for Asthma (GINA) were recruited into the study.[9] They were followed up after 3 months.

Children with acute exacerbations of asthma that required systemic steroids in the previous 3 months, those already on asthma treatment, those unwilling to give consent, children on steroid therapy for any other conditions such as dermatological and endocrine disorders, those on anti-allergic medications, children <6 years of age and those who developed acute exacerbations during the study period were excluded from the study.

A comprehensive medical history with information on the age of onset of wheezing, history of allergic rhinitis/atopic dermatitis, food allergies if any, prior use of inhalational corticosteroids, hospital admissions related to wheezing and familial history of wheezing or asthma was acquired from the parents.

Children were instructed not to engage in physical activity since the morning of the study. The procedure was carried out with the patient in an upright position, dressed in lightweight clothing, and with their legs uncrossed. Dentures that caused any disruption of the procedure were removed. Occluding the nares manually with nose clips limited air leakage via the nasal passages. The calibration of the spirometer was verified on the day of examination. The patient is required to introduce the mouthpiece into his mouth.

After verifying the absence of any leaks and ensuring that the patient is not obstructing the mouthpiece. The process is executed in the following manner:

1. The patient should inhale deeply, taking in as much air as possible, and hold their breath for less than 1 second at the maximum amount of air their lungs can hold.
2. The mouthpiece is placed within the oral cavity, specifically between the teeth, immediately after deep inhalation. To prevent any air leakage, it is important to tightly seal the lips around the mouthpiece. Exhalation should have a minimum duration of 6 seconds, or a duration recommended by the instructor. To solely measure forced expiratory volume, the patient should place the mouthpiece after completing step 1 and should refrain from breathing through the tube.
3. If any of the techniques are executed improperly, the technician must stop the patient's activity to prevent exhaustion and provide the patient with a renewed explanation of the operation.
4. The process is repeated at regular intervals of 1 minute until two matching results are obtained and satisfactory results are obtained.
5. To assess reversibility, following the above procedure, the child was administered 400 micrograms of bronchodilator (salbutamol).
6. The same procedure was repeated after 15 minutes of administration of bronchodilators.

A change in baseline FEV_1 by >12% was suggestive of a positive response, indicating reversibility, thus establishing the diagnosis of asthma.

Based on the medical history and the pulmonary function test, the children were classified into intermittent, mild persistent, moderate persistent and severe persistent asthma and treatment with inhalational corticosteroids was initiated accordingly according to guidelines.

Blood samples were obtained for the estimation of serum levels of IL-13 from the children in EDTA collecting tubes, without endotoxin / pyrogen and centrifuged at 1000 rotations/min for 10 minutes. The supernatant serum (250–500 µL) was stored in deep freezers at -70°C in the Molecular Biology Laboratory, SRM Institute of Science and Technology.

The subjects of the study were evaluated in the outpatient department after three months of adherence to the treatment regimen. In addition, a history of the frequency of symptoms and the need for reliever therapy was obtained. A repeat spirometry was performed and the average of three readings was tabulated against the initial reading. Blood samples were collected to analyze serum levels of IL-13 after therapy and preserved in a deep freezer at -70°C.

Once the research samples (both before and after treatment) were acquired, they were defrosted at room temperature and subjected to analysis using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Diaclone, Besancon Cedex, France). The IL-13 kit utilizes a solid phase sandwich ELISA. The wells of the microtiter strips have been coated with a monoclonal antibody that

specifically targets IL-13. The wells are filled with samples, including known IL-13, control specimens, and unknowns, using a pipette. In the initial incubation, a biotinylated monoclonal antibody specific for IL-13 and the IL-13 antigen are incubated together. Following the washing process, the enzyme (streptavidin-peroxidase) is introduced. After incubation and extensive washing to remove any residual enzyme, a substrate solution is added to induce a chromogenic product of reaction in the enzyme that is attached to the substrate. The amount of IL-13 contained in the samples is directly correlated with the color of this product.

A linear standard curve was generated by plotting the average absorbance of each standard on the vertical axis versus the corresponding human IL-13 standard concentration on the horizontal axis. IL-13 levels in each sample were calculated by extrapolating optical density measurements against standard IL-13 concentrations based using the standard curve.

Statistical analysis

Statistical analysis was performed using SPSS version 22.0 (IBM, Armonk, NY, USA) to compare blood IL-13 concentrations before and after 3 months of therapy using a paired t-test. A p-value of 0.05 or less was deemed statistically significant. Spearman's correlation analysis was used to analyze the relationship between serum IL-13 levels and treatment response indicated by an improvement in FEV_1 on spirometry, and also between IL-13 and all other demographic and clinical indicators. The ROC curve was used to assess the ability of a diagnostic test, specifically interleukin 13 in our case, to reliably differentiate between two patient conditions: mild asthma and moderate to severe asthma. In addition, to determine an adequate threshold value that will be beneficial for accurately diagnosing the presence or absence of a condition.

Results

In total, 68 patients were recruited for the investigation (n=68).

A significant positive correlation was observed between gender and circulating levels of IL-13 ($r=0.396$; $p<0.05$) was noted; that is, men had higher concentrations of IL-13 in their bloodstreams than females.[10] Serum IL-13 did not correlate significantly with the other clinical and demographic characteristics in our investigation. Table 1 provides an overview of individual baseline characteristics and their correlation with serum IL-13 before and after treatment.

Table 1. Baseline characteristics of subjects their correlation with serum IL-13 before and after treatment

Baseline characteristics	**Study subjects**	**Correlation coefficient of IL-13 before treatment (r value)**	**Correlation coefficient of IL-13 after treatment (r value)**
Age in months			
Mean (SD)	101.4±25.2	0.180	-0.027
Gender n (%)			
Male	54 (79%)	0.396	0.135
Female	14 (21%)		
Height (cm)	127.8±16.9		
Weight (kg)	27.8±10.9		
Body mass index (kg/m^2)	16.3±3.8	-0.040	-0.060
Family history n (%)			
Yes	59%	0.052	-0.086
No	41%		
History of atopic dermatitis n (%)			
Yes	29%	-0.205	0.012
No	71%		
History of allergic rhinitis n (%)			
Yes	44%	-0.211	-0.110
No	56%		
Previous hospital admission n (%)			
Yes	33%	-0.075	0.196
No	67%		
FEV_1 (%)	74.72%	-0.274	-0.085
Severity based on spirometry			
Mild asthma	85%		
Moderate asthma	6%	-0.274	-0.085
Severe asthma	9%		

The pulmonary function test (FEV_1) classified the researchers into three groups: mild asthmatics (85%), moderate asthmatics (6%), and severe asthmatics (9%).[11] Children were initiated on inhaled corticosteroids according to starting therapy, blood samples were collected to determine serum IL-13 levels.

The average FEV_1 at the beginning of therapy was 74.72%, and after three months of treatment, the value was 95.05% (Fig. 1) .

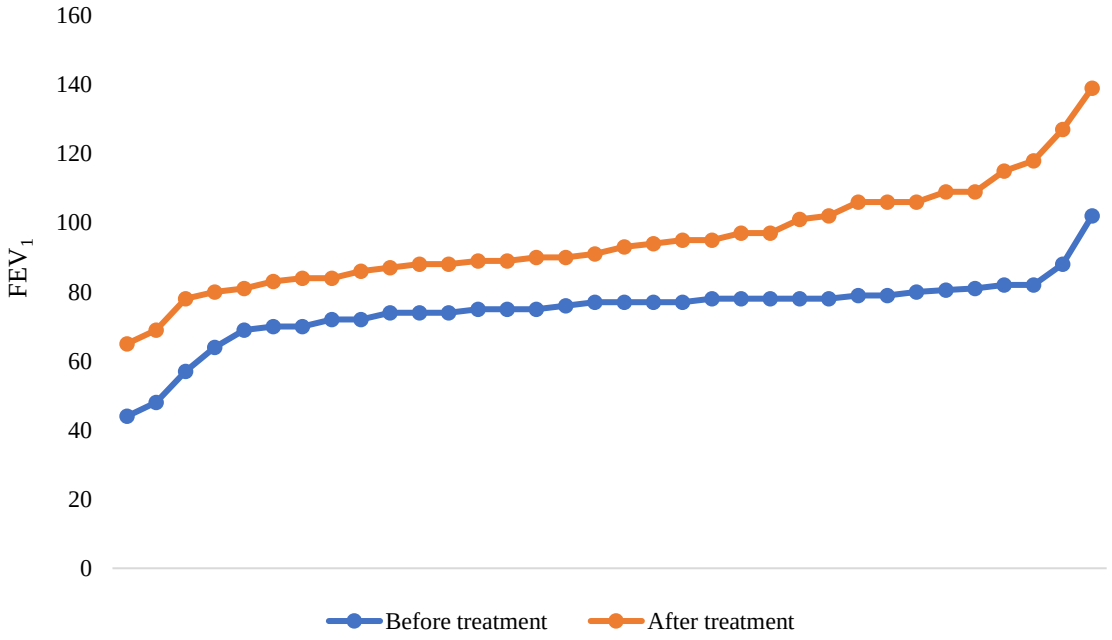

Fig. 1. Graphical representation of the trend of FEV_1 before and after treatment in children with asthma

Table 2. Descriptive statistics of asthma patients – FEV1, treated with ICS

	Mean (%)	Std. deviation	Min–max.	Median	p
Before treatment	74.72	10.18	44–102	77	<0.0001
After treatment	95.05	15.36	65–139	92	

Following therapy, serum IL-13 levels showed a downward trend.

The average serum IL-13 concentration prior to the start of treatment was 2.7±1.2 pg/mL. Following three months of appropriate treatment, the mean value decreased to 1.43±0.9 pg/mL. A difference of statistical significance was identified, as indicated by a p=0.005 (Fig. 2).

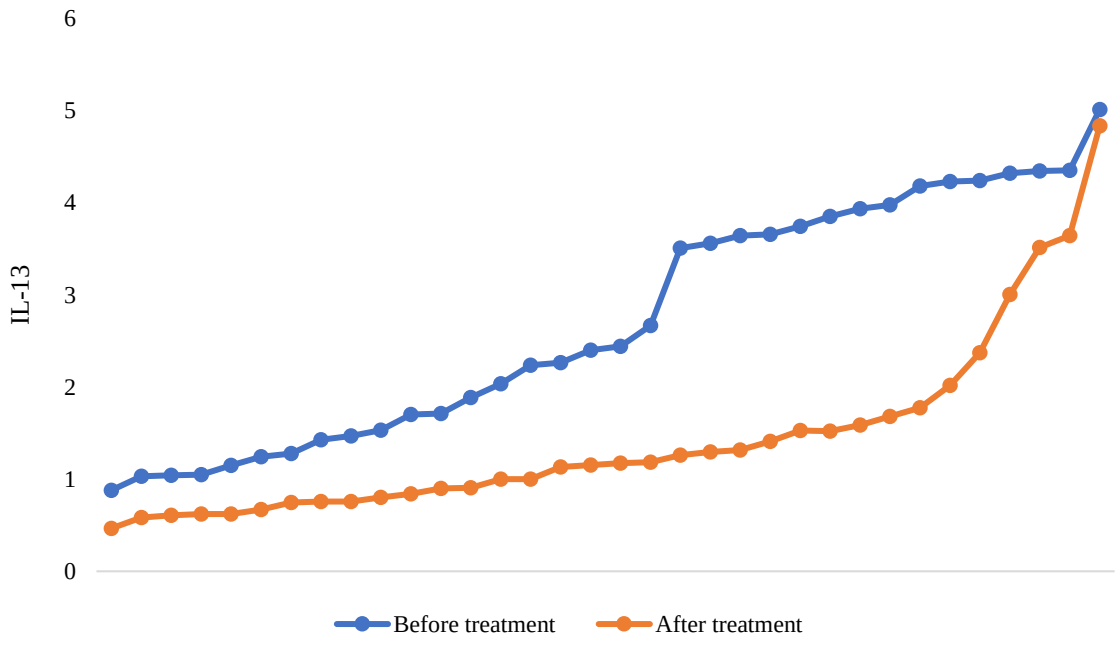

Fig. 2. Graphical representation of the trend of IL-13 before and after treatment in children with asthma

Table 3. Serum IL-13 concentrations in asthma patients treated with ICS

	Mean (pg/mL)	**Std. deviation**	**Min–max.**	**Median**	**p**
Before treatment	2.7	1.2	0.8–5.0	2.4	0.005
After treatment	1.43	0.9	0.4–4.8	1.1	

It is worth noting that a negative correlation was identified between IL-13 and FEV1. Specifically, after treatment, there was a reduction in serum IL-13 concentrations in conjunction with an improvement in FEV_1. However, the correlation between IL-13 and FEV1, as determined by the Spearman correlation table, did not have statistical significance (r=-0.274; p>0.05) (Fig. 3).

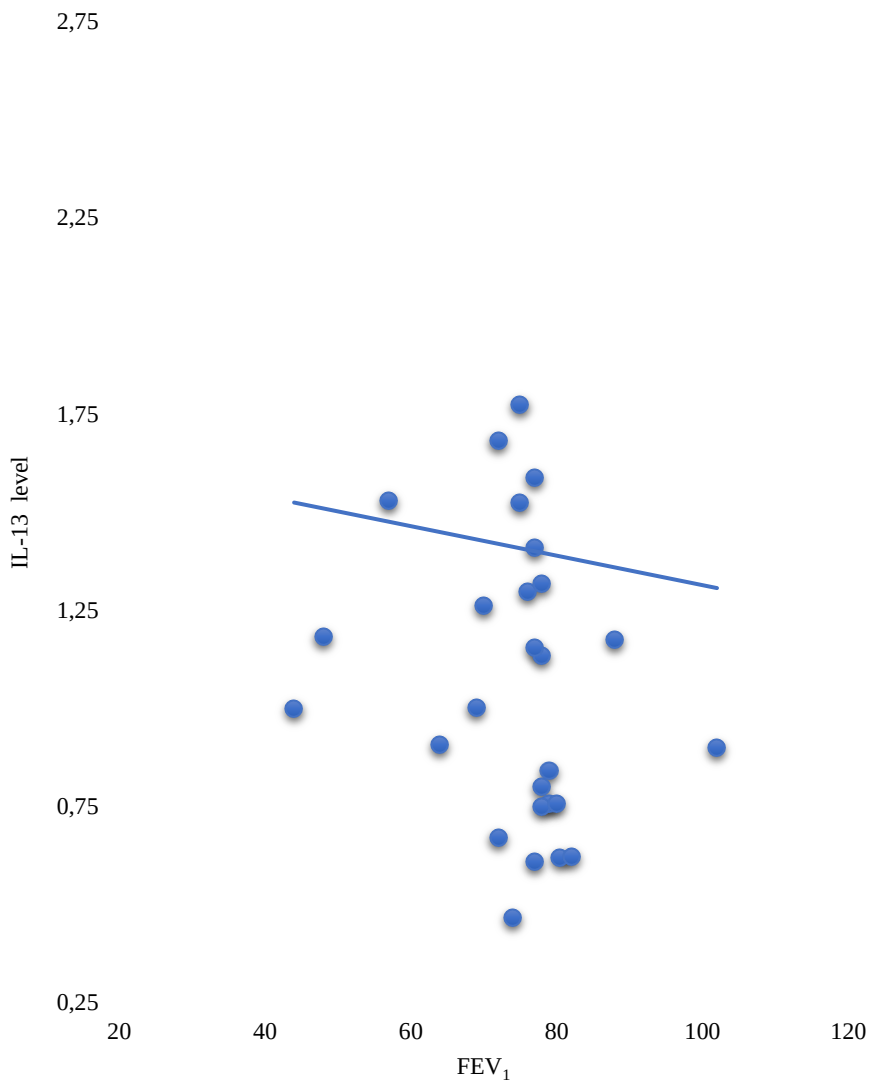

Fig. 3. Graphical representation of the correlation between serum IL-13 and FEV_1

Our study demonstrated that IL-13 can effectively differentiate between mild asthmatics and those with moderate to severe asthma, with a sensitivity and specificity of 100%. The cut-off value to differentiate the groups was 2.6 pg / mL (p=0.001) as depicted in Figure 4.

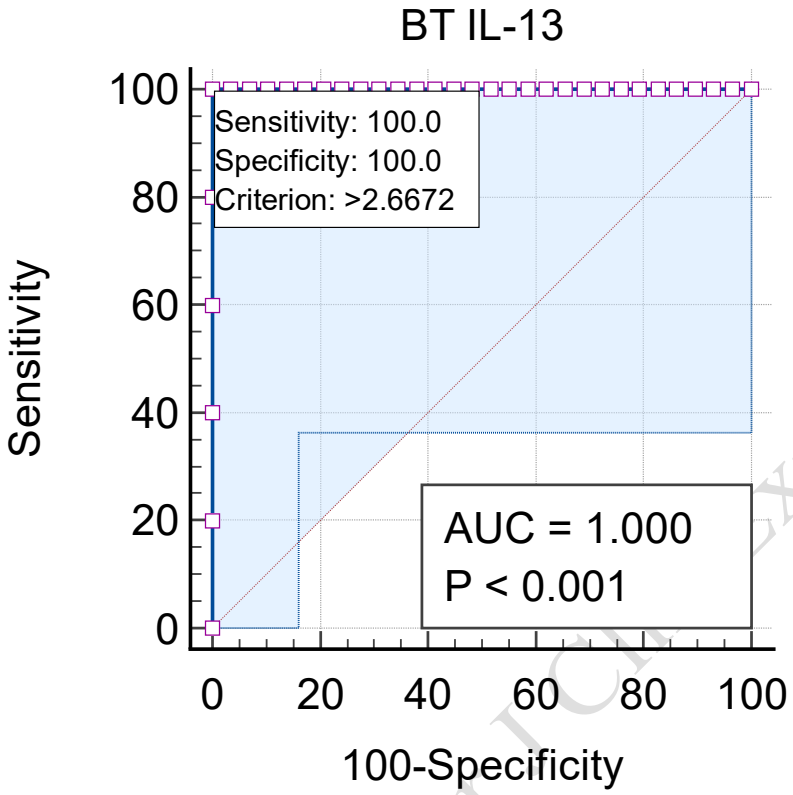

Fig. 4. ROC curve for IL-13 – determination of the cut-off point

Discussion

Asthma, one of the most prevalent noncommunicable significant diseases, substantially impacts the quality of life10. Bronchial asthma is a chronic inflammatory disease in which many produced cells and substances produced participate. Special significance is ascribed to eosinophils and lymphocytes of type 2 T helper, which induce IL-4, IL-5, IL-13. A deeper understanding of the mechanisms of the disease made possible by fundamental scientific investigations over the past twenty years has led to the development of highly specific treatments.11

A biomarker-driven methodology is being used to define a severe asthma endotype. There is no ideal biomarker, and unlike glycated hemoglobin, or HbA1c, in diabetes, or other certain conditions, the precise nature of asthma biomarkers is yet unknown.[12,13] Further evidence for the involvement in the pathophysiology of airway hyperresponsiveness comes from the overexpression of the protein in asthmatics' sputum, peripheral blood, bronchial submucosa, and mast cells in the airway smooth muscle bundle.[14]

Prevalent studies report elevated levels of IL-13 in asthmatics compared to healthy age matched controls, thereby implicating the role of IL-13 in the pathogenesis of asthma; however, there is paucity of literature

among the pediatric population where severity of asthma and response to treatment have been followed up.[15]

In 2019, Saleh Jebur et al. studied 150 asthmatics and 50 healthy controls, aged 10 to 65, in order to estimate the levels of serum IL-13 and serum IgE levels in the blood of patients suffering from allergic asthma. Before starting inhalational corticosteroids, the total serum levels of IgE and IL-13 were measured in both the patients and the control group. Following completion of treatment, blood samples were collected to calculate the total level of serum IgE and IL-13. Consistent with our investigation, a statistically significant decrease in serum IL-13 concentrations was observed after treatment ($p<0.001$).[16]

Jovanovska Janeva et al. conducted a trial to identify the difference in serum IL-13 and FEV1, before and after treatment with ICS/LABA (or) Montelukast in addition to ICS/LABA, in 56 patients with uncontrolled severe persistent asthma in 2015. After therapy, FEV1 improved considerably ($p<0.001$). In conjunction with our study, after 6 months of medication, a substantial decrease in serum levels of IL-13 was found compared to the initial values in both groups ($p<0.0014$, $p<0.001$ respectively). However, the Montelukast group showed a more pronounced benefit.[17]

Gemou-Engesaeth and colleagues proposed that serum levels of IL-13 and surfactant protein D (SP-D) could reflect a disease status in children with asthma. A study was carried out on 20 asthmatic children and 15 controls of the same age. Serum levels of SP-D and IL-13 were initially determined. Children were administered inhaled glucocorticoids or sodium cromoglycate according to the criteria of the American Thoracic Society and European Consensus Guidelines. Serum levels of SD-P and IL-13 were monitored again 4-6 months after treatment. In this research, inhaled glucocorticoids had no significant impact on IL-13 levels, unlike our current study. Patients treated with sodium cromoglycate exhibited a tendency toward a lower level of IL-13, although this difference was not statistical significance.[18]

Recent research indicates that targeting the interleukin-13 pathway may be crucial in treating various asthma subtypes19. However, these studies are limited, especially in the pediatric population, and the role of IL-13 as a biomarker in asthma treatment has rarely been studied. A statistically significant difference in IL-13 levels was observed before and after treatment, suggesting the possibility of it being a potential biomarker in pediatric asthma management.

Study limitations

The study was carried out with a limited sample size, and the findings must be reconfirmed using a larger cohort. Also, the study must be analyzed in conjunction with a control population that is matched by age and sex. Furthermore, the incorporation of additional prospective biomarkers and their correlation would provide more insight.

Conclusion

In conclusion, after inhalational corticosteroids, a substantial reduction in serum IL-13 levels and an evident negative relationship between FEV_1 and IL-13 were observed, thereby supporting the role of IL-13 as a marker in predicting response to treatment in pediatric asthma.

Declarations

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Authors' contributions

Conceptualization, P.R.; Methodology, P.R. and P.S.; Software, V.A; Validation, S.S.; Formal Analysis, P.S. and L.K.V.; Investigation, P.S. and L.K.V.; Resources, P.R. and S.S.; Data Curation, P.R., P.S. and V.A.; Writing – Original Draft Preparation, P.S.; Writing – Review & Editing, P.R.; Visualization, V.A.; Project Administration, S.S.; Funding Acquisition, P.R.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval

The current longitudinal study was conducted at the SRM Medical College Hospital and Research Center, a tertiary hospital in Tamil Nadu, with the approval of the Institutional Ethics Committee (IEC No: ST0922-797).

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