Serum interleukin-17 as a biomarker in paediatric bronchial asthma: A case control study

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Abstract

Introduction: Bronchial asthma (BA) is a chronic childhood disease causing significant morbidity and mortality. A novel and suitable biomarker is needed for early diagnosis of BA.

Objectives: To establish the association of serum interleukin-17 (IL-17) levels in children with BA and to determine the diagnostic performance of IL-17 in predicting severity of BA.

***Method*:** This was a case control study conducted at the Institute of Child Health and Research Centre, Government Rajaji Hospital & Madurai Medical College, Madurai, India from August 2020 to July 2021. Cases were selected according to the Global Initiative for Asthma (GINA) guidelines and controls were healthy siblings or age and sex matched controls The associations of IL-17 with BA were statistically analysed using Epi info v7 and SPSS 20. Analysis was done using one way ANOVA and t-test. ROC curve was used to find the diagnostic cut-off point. p-value <0.05 was considered statistically significant.

Results: Mean age of the participants was 8.74±2.21 years; 55.8% were males. Mean IL-17 level was significantly higher among cases (2.5±2.7) as compared to controls (1.31±0.96) (p=0.0043). There was a significant increase in mean IL-17 levels with increase in severity (p=0.00)**.** The area under curve for IL 17 levels in diagnosing severe persistent asthma was 0.870 (95% CI=0.708-1.000). The best diagnostic cut-off point was 3.26pg/ml with a sensitivity of 90% and a specificity of 90%. The best diagnostic cut-off point of IL 17 was 1.12pg/ml in predicting asthma with a sensitivity of 60% and a specificity of 50%.

Conclusions: IL 17 level can used as a biomarker for identifying patients with severe persistent asthma.

(Key words**:** Asthma, Interleukin 17, Biomarker)

Introduction

According to the Global Initiative for Asthma (GINA) guidelines, bronchial asthma (BA) is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation[1]. BA is a chronic childhood disease causing significant morbidity and mortality. It lowers the quality of life, curtails daily activities, reduces lung functions and causes school absenteeism. Affected children and their parents experience emotional and functional restraint. The disease also poses a considerable socioeconomic burden and leads to use of a large amount of healthcare resources[2]. In 2015, around 139.45 billion Indian rupees was spent on asthma treatment[3]. Almost 489,000 lives are lost every year globally due to asthma[4]. World Health Organization reports that 15-20 million Indians are asthmatic and of these, 10-15% are children[5]. According to the International Study of Asthma and Allergies in Childhood (ISAAC), estimated prevalence of asthma in children is 6.2-6.8% in the 6-7-year age group and 6.4-6.7% in the 13-14-year age group[3].

Inflammation plays an important role in the development of BA TH2 cells playing a vital role by secreting chemokines like IL4, IL5 and IL23. However, the TH1/Th2 paradigm fails to explain the pathogenic mechanism for various phenotypes of BA and for the full spectrum of BA severity. Recently, a new subset of effector T cells (TH17) that exhibit functions different from TH1 and TH2 cells and preferentially producing IL-17 has been

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identified in the pathogenesis of BA. IL-17 mediates the neutrophilic influx into airways and accentuates the TH2-cell mediated eosinophilic inflammation in BA. IL-17 is also implicated in mechanisms of steroid resistance in BA through induction of GR_β expression and reduced apoptosis[6]. A significant number of studies demonstrate that inhibitors of IL-17 and regulators of IL-17 expression control antigen induced airway inflammation, airway hyper-responsiveness and cytokine levels[6]. Due to lack of a suitable biomarker for early diagnosis of asthma, this study was carried out to estimate serum IL-17 levels in asthmatic and healthy controls and it was assessed whether the change in the level of serum IL-17 in asthmatic could be utilized as biomarker towards early diagnosis and timely start of BA treatment.

Objectives

- To establish the association of serum IL-17 levels in children with BA
- To determine the diagnostic performance of IL-17 in predicting the severity of BA.

Method

A case control study was conducted at the Institute of Child Health and Research Centre, Government Rajaji Hospital & Madurai Medical College, Madurai, India from August 2020 to July 2021.

Inclusion criteria:

- ***Cases****:* These were selected and classified based on the severity of BA according to GINA guidelines[1]. They included children in the 6-12 year-age group with a history of wheezing and shortness of breath during or without concurrent respiratory infections, estimation of peak expiratory flow rate (PEFR) and reversibility with bronchodilator therapy
- ***Controls****:* These were healthy siblings or age and sex matched controls with no history or symptoms of BA, no history or symptoms of any pulmonary disease, no history or symptoms of any allergic / atopic disease and no history or symptoms of chronic inflammatory disease.

Exclusion criteria: Children with any other allergic, infectious or chronic disease and children on prolonged systemic steroids were excluded.

Ethical issues: Approval for the study was obtained from the Institutional Ethics committee of Madurai Medical College and Government Rajaji Hospital, Madurai, India on 14.10.2020. Informed written consent was obtained from parents / guardians of all children after fully explaining the study procedure.

A detailed history was obtained including general examination and systemic examination.

Estimation of IL-17 expression: About 3 ml of a venous blood sample was collected in an EDTA-anticoagulant tube from each participant. All samples collected were centrifuged at 3,000 rpm for 10 minutes at 4°C and the supernatant plasma was separated and stored at -80°C until used. The circulating levels of IL-17 in plasma for patients and controls were measured using enzyme linked immunosorbent assay (ELISA).

***Statistical analysis*:** The associations of IL-17 with BA was statistically analysed using Epi info v7 and SPSS 20. Quantitative data were expressed as mean ±SD. One-way ANOVA was used to compare between groups. Discrete variables were analysed using t-test. Receiver operator characteristic (ROC) curve was used to find the diagnostic cut-off point. $p < 0.05$ was considered statistically significant.

Results

The study included 52 cases and 52 controls. Table 1 shows that there was no significant difference in the gender distribution (p=0.1) or mean age (p=0.22) between cases and controls.

Table 1: ***Baseline characteristics of study population***

Variable	Cases	Controls	Total	p-value
Gender – n (%)				
Female	19 (36.5)	27 (51.9)	46 (44.2)	0.1
Male	33 (63.5)	25 (48.1)	58 (55.8)	
Age in years – Mean ± SD	9.13±2.335	8.59±2.04	8.74±2.21	0.218

Of the 52 cases 60% had shortness of breath with wheeze, 23% had only wheeze and 17% had cough with wheeze; 60% had only nocturnal symptoms and 23% had symptoms both day and night; 60% had symptoms for two to four years; 84.6% had seasonal asthma and 15.4% had perennial asthma. A positive parental history of asthma was present in 80.8% of cases. Only 36.5% had used medications for asthma and among them only 2 had used both oral medications and inhalers, the remaining children using either one. Steroid usage for asthma was seen in 28.8% of cases. Among them, 12 children had used steroids for less than a week and 4 children had used steroids for one to three months. Only 9.6% had atopic findings. Regarding severity of asthma among cases, 12 had mild intermittent asthma, 9 had mild persistent asthma, 20 had moderate asthma and 11 had severe persistent asthma (Table 2)

Table 2: ***Medical history among cases***

Variable	Frequency (%)
Age group (years)	
5-7	17 (32.7)
8-10	14 (26.9)
>10	21 (40.4)
Symptoms	
Shortness of breath and wheeze	31 (59.6)
Cough and wheeze	09 (17.3)
Wheeze	12 (23.1)
Symptom timing	
Daytime	09 (17.3)
Night time	31 (59.6)
Both	12 (23.1)
Total	52 (100.0)
Symptom duration	
1 month	01 (01.9)
2 months	01 (01.9)
6 months	03 (05.8)
1 year	09 (17.3)
2 years	15 (28.9)
3 years	10 (19.2)
4 years	06 (11.5)
5 years	03 (05.8)
6 years	03 (05.8)
8 years	01 (01.9)
Total	52 (100.0)
Type	
Perennial	08 (15.4)
Seasonal	44 (84.6)
Total	52 (100.0)
Family history	
Yes	42 (80.8)
No	10 (19.2)
Total	52 (100.0)
Medication used	
Yes	20 (36.5)
No	32 (63.5)
Total	52 (100.0)
Route of drug	
Inhalation	09 (17.3)
Oral	09 (17.3)
Oral + Inhalation	02 (03.9)
Total	20 (36.5)
Steroids taken	
No	36 (71.2)
Yes	16 (28.8)
Total	52 (100.0)
Steroids duration	
≤ 1week	12 (23.1)
1 month	01 (01.9)
2 months	02 (03.9)
3 months	01 (01.9)
Total	16 (28.8)
Atopic findings	
No	47 (90.4)
Yes	05 (09.6)
Total	52 (100.0)
Severity of asthma	
Mild intermittent	12 (23.1)
Mild persistent	09 (17.3)
Moderate persistent	20 (36.5)
Severe persistent	11 (21.2)
Total	52 (100.0)

Mean IL-17 level was significantly higher among cases (2.5±2.7) compared to controls (1.31±0.96) (p=0.0043). There was an increase in mean IL-17 levels with increase in severity and there was a significant difference in mean IL-17 levels between patients with different severity levels (p=0.00) (Table 3)

Table 3: ***Interleukin-17 levels among study participants***

Variable	Number	Mean	SD	p-value
Cases	52	2.5	2.7	
Controls	52	1.31	0.96	0.0043
Mild intermittent	12	0.48	0.27	
Mild persistent	09	1.28	0.97	
Moderate persistent	20	2.56	2.40	0.0000017
Severe persistent	11	5.61	2.90	

ROC curve was used to find out the diagnostic performance of IL17 level in predicting severe persistent asthma. Area under the curve was 0.870 (p=0.000). Best diagnostic cut-off point was 3.26pg/ml. It gave a high Youden's J statistic in predicting severe persistent asthma with a sensitivity of 90% and specificity of 90% (Figure 1 and Table 4).

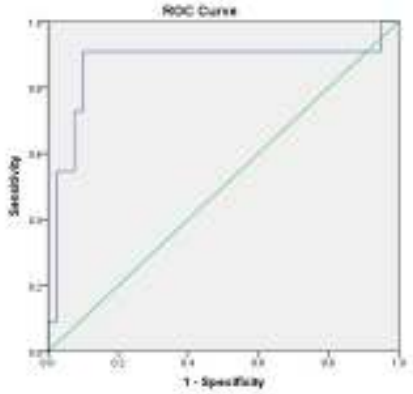

Figure 1: ***ROC curve for IL-17 in predicting severe persistent asthma***

Table 4: ***Diagnostic performance of IL-17 in predicting severe persistent asthma and asthma***

Area under curve	Area	Standard error[a]	Asymptotic Sig.[b]	Asymptotic 95% confidence interval	
				Lower Bound	Upper Bound
Severe persistent asthma	0.870	0.083	0.000	0.708	1.000
Asthma	0.588	0.056	0.000	0.512	0.676

We tried to plot ROC curve to find out the best diagnostic cut-off point for IL-17 level in predicting asthma. Area under the curve was 0.588 (p=0.056). Best cut-off point was 1.12pg/ml with high Youden's J statistic. It can predict asthma with a sensitivity of 60% and specificity of 50% (Figure 2).

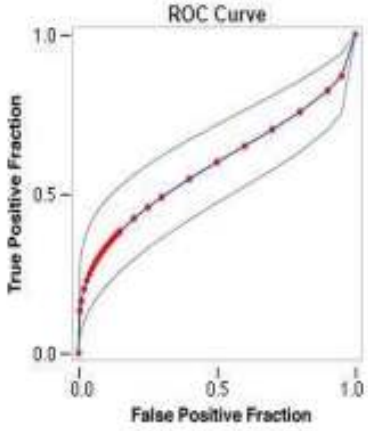

Figure 2: ROC curve for IL-17 in predicting asthma

Discussion

A total of 52 asthmatic children and 52 healthy children (controls) were studied over a period of 1 year in this case control study. Prevalence of asthma was 63% among boys as compared to 37% among girls. This accords with previous studies by Chakaravarthy S, *et al*[7] and Kumar GS, *et al*[8]. The mean age of presentation of asthma was 9.13 years and 51% of affected children were in the 10-12-year age group. This is similar to previous studies by Ashwathi S, *et al*[9] and Kumar GS, *et al*[8]. The predominant symptoms of asthma in children were shortness of breath with wheezing (60%), only wheezing (23%) and cough with wheezing (17%) and 60% had symptoms nocturnally. Thus, wheezing is the predominant symptom of childhood asthma which is in accordance with the study by Chakaravarthy S, *et al*[7].

Mean duration of asthma in children in our study was 2 years with 60% being between 2 to 4 years. Whilst 44 (85%) children had seasonal symptoms 8 (15%) had symptoms throughout the year. Thus, asthma is a seasonal disease with the majority presenting during winter (November to January). This is explained by the fact that airway hyperresponsiveness is triggered by an exposure to cold wind which is seen in winter. In our study, the prevalence of asthma was significantly more among those with a family history of asthma or atopic disorders (80%) which is similar to observations in studies by Kumar GS, *et al*[8] and Jain A, *et al*[10]. In contrast to the findings of Kumar GS, *et al*[8]and Jain A, *et al*[10], where atopic findings were common in children with asthma, in our study only 9% had atopic findings like allergic rhinitis or eczema.

The main objectives of this study were to find the association of serum IL-17 levels with childhood asthma and to find correlation with the severity of asthma. From this study, it was observed that among 52 asthmatic children, 38% had moderate persistent asthma, 23% had mild intermittent asthma, 21% had severe persistent asthma and 17% had mild persistent asthma. Mean comparison of IL-17 levels between cases and controls in our study showed that cases had significantly higher levels of IL-17 (mean = 2.5pg/ml, SD=2.7) when compared with controls (mean = 1.31pg/ml, SD=0.96) and this was statistically significant ($p=0.0043$. $p<0.005$). Similarly, a study by Bazzari *et al*[11] in 50 asthmatic children showed that IL-17 was significantly higher ($p<0.001$) in cases and they also gave a cut-off value of IL-17 >5.39pg/ml can be used as a diagnostic level of asthma in children.

Among asthmatics, mean value of IL-17 was highest in severe persistent asthma (mean=5.61pg/ml, SD=2.90) followed by moderate persistent asthma (mean=2.56pg/ml, SD=2.40), mild persistent asthma (mean=1.28pg/ml, SD=0.97) and intermittent asthma (mean=0.48pg/ml, SD=0.27). By using ANOVA test, it was found that IL-17 level was significantly higher in children with increasing severity of asthma ($p<0.05$). This was similar to the study by Bazzari *et al*[11] and Alyasin S, *et al*[12]. In her study she concluded that IL-17 and IL17mRNA expression can be used to predict the severity of asthma.

It has been shown that IL-17 is clearly expressed in the airways of asthmatics and its expression level correlates with disease severity[13]. In some recent studies, the serum IL-17 levels were significantly higher in children with asthma than in healthy controls[14,15]. A study conducted on 120 asthmatic children has concluded that CD4+IL-17A+ T cell counts and serum IL-17 levels in conjunction with augmented FeNO levels are systemic markers of childhood asthma[16]. There are also reports of significant differences in the serum IL-17 concentrations during asthmatic exacerbations between mild and meso-severe attack groups with healthy controls[14]. In another study conducted on adult asthmatics, serum IL-17 concentrations had been considered as an independent risk factor for severe asthma[15].

The second objective of the study was to derive a cut-off value that can be used to diagnose asthma and predict severity of asthma. ROC curve analysis was done and it was found that serum 17 levels of 1.12pg/ml can diagnose asthma with sensitivity of 60% and specificity of 50% and IL 17 levels of 3.32pg/ml can predict severe persistent asthma with sensitivity of 90% and specificity of 90%. IL 17 level can used as a marker for identifying patients with severe persistent asthma.

Conclusions

IL-17 levels can be used as a biomarker for early diagnosis of asthma and can also be used for assessing severity and diagnosing steroid resistance.

References

1. Global Strategy for Asthma Management and Prevention: GINA guideline updated 2019

2. Linden A, Dahlen B. Interleukin-17 cytokine signalling in patients with asthma. *European Respiratory Journal* 2014; **44**: 1319-31. https://doi.org/10.1183/09031936.00002314 PMid: 24925921

3. Jain N, Satish K, Abhyankar N, Velayudhan N, Gurunathan J. Repeated exacerbation of asthma: An intrinsic phenotype of uncontrolled asthma. *Lung India* 2019; **36**(2): 131–8. https://doi.org/10.4103/lungindia.lungindia_434_17 PMid: 30829247 PMCid: PMC6410599

4. Koul PA, Patel D. Indian guideline for asthma: Adherence is the key. *Lung India* 2015; **32**(Suppl 1): S1–S2. https://doi.org/10.4103/0970-2113.154511

5. Park S, Lee YC. Interleukin-17: an attractive therapeutic approach for asthma. *Respiratory Research* 2010; **11**(1): 78. https://doi.org/10.1186/1465-9921-11-78 PMid: 20565710 PMCid: PMC2894770

6. Loftus PA, Wise SK. Epidemiology and economic burden of asthma. *International Forum of Allergy & Rhinology* 2015; **5**(Suppl 1): S7-S10. https://doi.org/10.1002/alr.21547 PMid: 26010063

7. Chakravarthy S, Singh RB, Swaminathan S, Venkatesan P. Prevalence of asthma in urban and rural children in Tamil Nadu. *National Medical Journal of India* 2002; **15**: 260-3

8. Kumar GS, Roy G, Subitha L, Sahu SK. Prevalence of bronchial asthma and its associated factors among school children in urban Puducherry, India. *Journal of Natural Science, Biology and Medicine* 2014; **5**: 59-62. https://doi.org/10.4103/0976-9668.127289 PMid: 24678199 PMCid: PMC3961954

9. Awasthi S, Kalra E, Roy S, Awasthi S. Prevalence and risk factors of asthma and wheeze in school-going children in Lucknow, North India, *Indian Pediatrics* 2004; **41**:1205-21

10. Jain A, VinodBhat H, Acharya D. Prevalence of bronchial asthma in rural Indian children: a cross sectional study from South India. *Indian Journal of Pediatrics* 2010; **77**(1): 31-5. https://doi.org/10.1007/s12098-009-0308-6 PMid: 20091364

11. Bazzari M, Aly I, Hassib D. Study of serum interleukin-17 level in children with bronchial asthma. *International Journal of Advanced Research* 2016; **4**(11): 2220-1. https://doi.org/10.21474/IJAR01/2329

12. Alyasin S, Karimi MH, Amin R, Babaei M, Darougar S. Interleukin-17 gene expression and serum levels in children with severe asthma. *Iranian Journal of Immunology* 2013; **10**(3): 177-85.

13. Kawaquchi M, Kokubu F, Fujita J, Huang SK, Hizawa N. Role of IL-17F in asthma. *Inflammation and Allergy Drug Targets* 2009; **8**(5): 383-9. https://doi.org/10.2174/187152811090805 0383 PMid: 20025586

14. Xin W, Chun-Yan MA, Ya-Jung Z, Li-Hua N. Expressions of serum IL-17 and LTB4 in childhood asthma and their clinical significance. *International Journal of Pediatrics* 2012: **39**(4): 428-31.

15. Agache I , Giobanu C, Agache C, Anghel M. Increased serum IL-17 is an independent risk factor for severe asthma. *Respiratory Medicine* 2010; **104**: 1131-7. https://doi.org/10.1016/j.rmed.2010.02.018 PMid: 20338742

16. Chien JW, Lin CY, Yang KD, Lin CH, Kao JK, Tsai YG. Increased IL-17 secreting CD4+T cells, serum IL-17 levels and exhaled nitric oxide are correlated with childhood asthma severity. *Clinical and Experimental Allergy* 2013; **43**(9): 1018-26. https://doi.org/10.1111/cea.12119 PMid: 23957337

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