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RESEARCH ARTICLE

ROLE OF TH-17 (IL-17) IN THE PATHOGENESIS OF BRONCHIAL ASTHMA: A NEW TARGET FOR THERAPY

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ABSTRACT

Background IL-17F is produced by many cell types such as memory CD4+ T cells, CD8+ T cells, γδ T cells, NKT cells and B cells etc. IL-17F can induce various asthma-related cytokines, chemokines, and adhesion molecules in bronchial epithelial cells, eosinophils, fibroblasts, airway smooth muscle cells, and vein endothelial cells, and thereby contributes to the pathogenesis of asthma.

Objective In our study we tried to find out association of serum levels of IL-17 and total duration of illness in patients of Bronchial Asthma.

Materials and Methods We evaluated prospectively 66 cases of bronchial asthma and 20 controls from normal population with the help of proper history and relevant investigations [to rule out other diseases like COPD, ILD etc.] All Subjects were evaluated for serum IL-17 levels, by using ELISA kit of KOMA Biotech Inc. Obtained data was evaluated statically by using SPSS software.

Results We found Serum IL-17 levels significantly raised in Asthma patients as compared to control with t-value 7.066 and p-value <0.001.

Conclusion Asthma patients tend to have higher serum IL-17 levels than controls from normal population. So it can be a potential target for developing new drugs specially for steroid resistant bronchial asthma.

INTRODUCTION

Asthma is a T cell driven chronic inflammatory disorder of the airways. Both T helper (Th2) and (Th1) lymphocytes play an important role in the path physiology of asthma. Local overproduction of Th2 cytokines (IL-4, IL-5, IL-9 and IL-13) by Th2 cells in the asthmatic airways is well defined and recent studies indicate that Th1 cells, secreting IFN γ, might cause severe airway inflammation.(1)

The relationship between Bronchial Asthma and T-helper 17 has been suggested from various studies; this association may relate to pathogenesis of Bronchial asthma. (2) Asthma is a chronic inflammatory disorder of the lung that is usually associated with airway tissue remodeling. This term is referred to structural changes affecting lung tissue which normally include epithelial detachment, increased airway smooth muscle mass, sub epithelial fibrosis, mucous cell gland and goblet cell hyperplasia, vascular change and edema.(3) Interleukin-17 cytokine seems to be contributing airway tissue fibrosis by enhancing production of eosinophil derived profibrotic cytokines.(4) This role of interleukin-17 is dependent upon on p38 MAPK activation. Therefore upstream activator of p38MAPK within the IL-17 pathway may represent attractive target for corticosteroid unresponsive disease (5). The aim of the present study is to determine the role of Th-17 (IL-17) in the pathogenesis of Bronchial Asthma.

MATERIAL AND METHODS

This study was conducted in Sir Sunderlal Hospital, Institute of Medical Sciences, Banaras Hindu University, Varanasi after approval from ethical committee between August 2013 to July 2015. The hospital serves as tertiary care centre for the patients coming from eastern part of Uttar Pradesh, adjoining area of Bihar, Jharkhand, Madhya Pradesh and Chhattisgarh. Patients selected from those attending Department of Tuberculosis and Respiratory Diseases. We prospectively evaluated all patients who were admitted or attended OPD as a provisionally diagnosed case of bronchial asthma in our department, Department of TB and respiratory diseases, SS Hospital, IMS, BHU. If not previously documented/tested, all such patients were documented for Bronchial Asthma with pulmonary function test and were screened for other causes of

breathlessness like exacerbation of COPD, exacerbation of ILD, worsening of dyspnea due to heart failure etc., by channeling through detailed history, thorough physical examination and a battery of relevant investigations. All patients included in study had undergone Interleukin 17A estimation in department of pathology by using ELISA Kit of Koma biotech inc. supplied by Mind biomeds, New Delhi. Collected data was analyzed by statistical analysis.

Inclusion criteria

Case patient with bronchial asthma

Cases were taken who fulfilled the criteria of bronchial asthma (history suggestive of allergy and spirometry).Control subjects were from normal population.

Exclusion criteria

- Patients who presented not due to bronchial asthma but because of other disease like COPD, bronchiectasis, HIV, alcohol intake, churg-strauss syndrome and pulmonary tuberculosis etc.
- Patient who associated with other chronic disease like diabetes, hypertension, renal disease, hepatic disease, neurological disease, coronary artery disease etc.
- Patients with multiple organ failure
- Those patients who did not consent.

Study size 86 (66 case+ 20 control)

All selected patients underwent following investigations like Complete blood count, renal and liver function tests, random blood sugar ,X-ray chest PA view, ECG , Absolute eosinophil count, Arterial blood gas analysis, Pulmonary function test with post bronchodilator reversibility, SerumIg E level, CECT thorax (to rulled out other differential diagnosis) And Interleukin 17A estimation by using ELISA Kit.

Estimation of Interleukin-17A (IL-17A)

Estimation of Interleukin-17A was done by using ELISA Kit of Koma biotech inc. supplied by Mind biomed New Delhi India.

Principle of test

It is an enzyme-linked Immunosorbent assay for the quantitative measurement of human IL-17 in serum. This assay employs antigen-affinity purified mouse anti-human IL-17A antibody specific for human IL-17A coated on 96 well plate. Standard and samples are pipetted into the wells and IL-17A present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated monoclonal antibody is added together with streptavid in- peroxidase conjugate. The biotinylated antibody bind to the solid phase antibody antigen complex and in turn, binds to the conjugate. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IL-17A bound.

Analysis

All data analysis was performed using SPSS statistical package for windows. For comparing data on IL-17 in cases and controls, Student's t- test was used. ANOVA test was used to determine association between total duration of illness and serum IL-17 levels.

RESULTS

A total of 86 patients were included in the study of which 66 were cases Bronchial Asthma and 20 were controls. Age-wise distribution of the patients revealed that most of the patients (47) were in the age of 20-40 years; 12 patients were >40 years of age; and 7 patients were <20 years of age as shown below:

Table 1 Age distribution of the total patients studied, males constituted 57.6% (38) and females constituted 42.4% (28).

Age (years)	Cases		Controls	
	No.	%	No.	%
<20	7	10.6	3	15.0
20-40	47	71.2	14	70.0
>40	12	18.2	3	15.0
Total	66	100	20	100

Table 2 Sex distribution of the patients

Sex	Frequency	Percent
Male	38	57.6
Female	28	42.4
Total	66	100.0

29 patients had a clinical duration of illness between 2-5 years; 17 patients had a duration of <2 years and 20 patients had >5 years of total duration of illness.

Table 3 Total Duration of illness

Duration illness (years)	Frequency	Percent
<2	17	25.8
2-5	29	43.9
>5	20	30.3
Total	66	100.0

On comparing the severity of disease, the study revealed that maximum number of patients (29) had mild intermittent disease; 20 had mild persistent disease; 14 patients had moderate persistent disease; and only 3 patients had severe persistent disease.

Table 4 Severity of disease

Severity of disease	Frequency	Percent
1	29	43.9
2	20	30.3
3	14	21.2
4	3	4.5
Total	66	100.0

Post-bronchodialator reversibility testing, 39 patients showed <12% change in FEV1 and 27 patients showed ≥12% change in FEV1 as sown below:

Table 5 Change in FEV1

Change in FEV1 (%)	Frequency	Percent
<12	39	59.1
≥12	27	40.9
Total	66	100.0

Serum Absolute Eosinophil Count Levels were found to be higher in cases (770.47±843.957) as compared to controls (217.05±86.886) and were found to be statistically significant (p-value 0.005).

Though serum Ig E levels were also found to be higher in cases (140.56±59.728) as compared to controls (108.00±30.810) but were found to be statistically insignificant (p-value 0.022).

Serum IL-17 levels too, were higher in cases (882.85±175.216) as compared to controls (581.00±137.076) and were found to be statistically significant (p-value 0.000).

Table 6 Comparison of mean AEC, Ig E and IL-17 between cases and controls

Serum levels	Cases Mean±SD	Controls Mean±SD	t-value	p-value
AEC	770.47±843.957	217.05±86.886	2.916	0.005
IgE	140.56±59.728	108.00±30.810	2.339	0.022
IL-17	882.85±175.216	581.00±137.076	7.066	<0.001

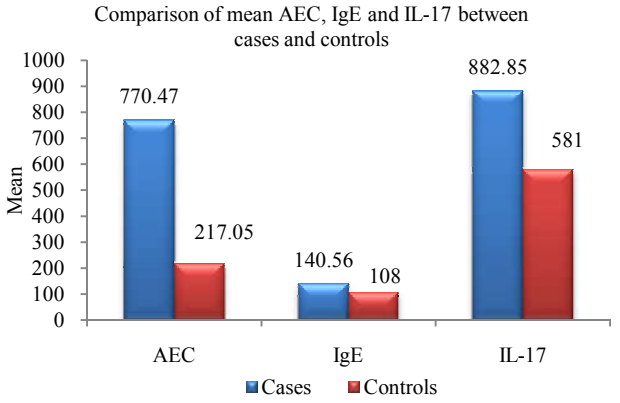

Figure 1

Comparison of levels of AEC, IgE, IL-17 levels and pre-treatment FEV1 with respect to the total duration of illness showed that Serum AEC levels were not related to total duration of disease and were incidentally found to be higher in patients with duration in between 2-5 years (1064.28±1168.041) as compared to duration of <2 years (590.59±394.983) and >5 years (497.35±235.612).

Similarly serum IgE levels and pre-treatment FEV1 also didn't show any correlation with total duration of disease as shown in the table below.

However, Levels of IL-17 were found to be significantly associated with total duration of disease with highest levels seen in patients with total duration>5 years (999.40±148.463) as compared to duration of < 2 years (728.06±108.872) and 2-5 years (893.21±158.678) respectively (P-value <0.001).

Table 7 Total duration of illness

	<2 year (Group A) Mean±SD	2-5 year (Group B) Mean±SD	>5 year (Group C) Mean±SD	f-value	p-value
AEC	590.59±394.983	1064.28±1168.041	497.35±235.612	3.429	0.039
IgE	147.35±59.036	146.17±63.371	126.65±55.299	0.775	0.465
FEV1	61.35±10.087	68.86±16.628	67.30±15.881	1.390	0.257
IL-17	728.06±108.872	893.21±158.678	999.40±148.463	16.359	<0.001

In our study we found that IL-17 has positive correlation with duration of disease(r=.581, p<0.001) and severity of disease (r=.703, p<0.001).

We also found that % change in FEV1on bronchodilator reversibility test has significant negative correlation with FEV1 (r=-0.472, p <0.001), as shown in table 12.

DISCUSSION

Bronchial asthma affects approximately 8% of the adult population and as many as 20% of children worldwide. Most of the studies have shown airway inflammation to be associated with T-cell activation, eosinophil accumulation, and Th2- type cytokine production. Furthermore, the subpopulation of patients with severe asthma appears to manifest a different pattern of airway inflammation that is not associated with either classic Th1 or Th2 cells. Th17 cells produce a number of cytokines, but in particular IL-17A and IL-17F Functional studies have suggested that IL-17F is involved in asthma pathology, hence, increased understanding of the significance of IL-17F would help to uncover the molecular mechanisms of asthma(1). Asthma is a disease of young patients. We found during our study as most of the patients (71.2%) were belong to age group between 20-40 years.

Childhood asthma occurs more frequently in boys than in girls. It's unknown why this occurs although some experts find a young male's airway size is smaller when compared to the female's airway, which may contribute to increased risk of wheezing after a cold or other viral infection. Around age 20, the ratio of asthma between men and women is the same. At age 40, more females than males have adult asthma (6). Findings of our study were slightly deviated as we found slight male predominance over female (57.6% vs. 42.4%).

As we know asthma is a chronic disease, in our study 29 patients had a clinical duration of illness between 2-5 years; 17 patients had a duration of <2 years and 20 patients had >5 years of total duration of illness. On comparing the severity of disease; as defined by guidelines from the National Asthma Education and Prevention Program (NEPP); the study revealed that maximum number of patients (29) had mild intermittent disease; 20 had mild persistent disease; 14 patients had moderate persistent disease; and only 3 patients had severe persistent disease. Bronchodilator reversibility is an important feature of bronchial asthma but it is not present in all patients. Most of the study claims presence of post-bronchodilator reversibility in FEV1 in between 25- 50% (7).

Table 8 Correlation between various study parameters

		AEC	IgE	FEV1	Age onset	IL 17	Change Percent	Durationillness	Severity disease
AEC	R	1.000	.181	.039	.002	-.013	-.095	-.076	-.036
	p	.	.147	.757	.985	.921	.446	.543	.774
IgE	R	.181	1.000	-.029	.080	-.142	-.012	-.172	-.128
	p	.147	.	.816	.524	.254	.923	.167	.307
FEV1	R	.039	-.029	1.000	-.106	.208	-.472**	.194	.216
	p	.757	.816	.	.395	.094	.000	.119	.082
Age of onset	R	.002	.080	-.106	1.000	.138	.065	-.058	.276*
	p	.985	.524	.395	.	.271	.605	.645	.025
IL-17	R	-.013	-.142	.208	.138	1.000	-.176	.581**	.703**
	p	.921	.254	.094	.271	.	.159	.000	.000
Percent Change in FEV1 after bronchodilator	R	-.095	-.012	-.472**	.065	-.176	1.000	-.254*	-.092
	p	.446	.923	.000	.605	.159	.	.040	.461
Duration of illness	R	-.076	-.172	.194	-.058	.581**	-.254*	1.000	.688**
	p	.543	.167	.119	.645	.000	.040	.	.000
Severity of disease	R	-.036	-.128	.216	.276*	.703**	-.092	.688**	1.000
	p	.774	.307	.082	.025	.000	.461	.000	.

Post-bronchodilator reversibility testing, 39 patients showed <12% change in FEV1 and 27 patients showed ≥12% change in FEV1.

While there is little data to support the view that eosinophils ameliorate the allergic process, although they could have an important role in the disordered repair that leads to permanently impaired function in some allergic diseases, the evidence that they cause many of the path physiological features of allergic disease (8). In our study serum Absolute Eosinophil Count Levels were found to be higher in cases (770.47±843.957) as compared to controls (217.05±86.886) and were found to be statistically significant (p-value 0.005). Immunoglobulin E and associated cellular responses are responsible for allergic airway diseases. A hypersensitivity reaction initiated by immunologic mechanisms, mediated by IgE antibodies occurs in allergic asthma (9). Though serum IgE levels were also found to be higher in cases as compared to controls but were found to be statistically insignificant this finding was also in accordance to previous study done by Honghua Lu *et al*. (2012). (10)

Serum IL-17 levels too, were higher in cases as compared to controls and were found to be statistically significant (p-value <0.001). IL-17 also correlate positively with the total duration of illness and IL-17 level were found more raised in patient with more duration of disease were found to have higher level of IL-17.in this context Serum AEC levels were not related to total duration of disease and were incidentally found to be higher in patients with duration in between 2-5 years (1064.28±1168.041) as compared to duration of <2 years (590.59±394.983) and >5 years (497.35±235.612). Similarly serum IgE levels and pre-treatment FEV1 also didn't show any correlation with total duration of disease.

Inter-group comparison of AEC, IgE, IL-17 levels and FEV1 between patients with different duration of disease also revealed statistically significant association only with IL-17. When study correlation between with post bronchodilator reversibility in FEV1, absolute eosinophilic count, serum IgE and IL-17 levels, we didn't find much correlation with p-values being 0.141, 0.564 and 0.171 respectively. But the patient having low pre-treatment FEV1 had higher degree of reversibility in terms of higher present change.

CONCLUSION

Asthma is a heterogeneous disease with several phenotypes. For treatment of these different phenotypes understanding of molecular mechanism of pathogenesis is essential. With increased understanding of the significance of IL-17 would help to uncover the molecular mechanisms of asthma and it has a key role in asthma pathology and is a novel drug target for asthma.

In our study we found serum IL-17 levels to be significantly higher in cases than controls and these were strongly associated with disease of longer clinical history. Absolute eosinophil counts were also found to be significantly higher in cases. On post bronchodilator reversibility testing only 41% cases showed more than 12% improvement in FEV1

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