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Estimation of pro and anti-inflammatory cytokine levels in asthma during exacerbations and remissions
Pro- and anti-inflammatory cytokines in asthma

Dr Safa Ansari, MD[1]; Dr K Sailaja, MD[2*]Dr Parveen jahan MSc Phd [3]

[1]*Senior Resident, Department of Respiratory Medicine, Princess Esra Hospital, Deccan College of medical sciences. Shalibanda , Hyderabad , 500002 , Telangana, India. Email: safaansari0304@gmail.com*

[2] *Professor and Head of the department, Department of Respiratory Medicine, Mediciti institute of Medical sciences Ghanpur, Medchal 501401, Telangana. India.*

[3] *Associate professor, school of sciences, Maulana Azad National Urdu university, Gachibowli Hyderabad, India.*

[*]*Corresponding author: Dr K Sailaja,*

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Conflicts of interest
The authors of the study have contributed equally in the study and have no conflicts of interest to declare.

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Abbreviations list

- Immunoglobulin E: IgE
- Tumor necrosis factor alpha: TNF-α
- Interleukin-10: IL-10
- Probability value: P value orP
- T-helper type 2: TH2
- B-lymphocytes: B cells

ABSTRACT: Background: Airway inflammation is centrally important in asthma and other lung diseases.One of the striking advances in the last decade has been the recognition that cytokines play a critical role in the inflammatory response in asthma.

Research question: The present study was conducted to estimate the levels of pro-inflammatory and anti-inflammatory, cytokines, and serum immunoglobulin E (IgE) in patients with asthma during exacerbation and remission in comparison with healthy controls.

Study design: The present 2-year case-control study was conducted in the department of Respiratory Medicine. A total of 50 individuals aged 18–65 years were enrolled in the study and were divided into two groups: cases (asthma patients, n = 25) and controls (healthy controls, n = 25). Patients diagnosed with asthma by spirometry showing a post bronchodilator reversibility of > 12%, 200 ml, or clinically diagnosed cases of acute exacerbations were included in the case group.Enzyme-linked immunosorbent assay was performed to estimate the levels of plasma tumor necrosis factor alpha (TNF-α), interlukin-10 (IL-10), and IgE.The data obtained were entered in Microsoft Excel spreadsheet and analyzed using statistical package for social service v.22. A probability of $P < 0.05$ was considered as statistically significant.

Results: In cases, the serum levels of pro-inflammatory cytokine TNF-α (7.38 ± 5.4 vs. 5.01 ± 4.1, $P < 0.05$) and IgE receptor (230.9 ± 148.5 vs. 13.72 ± 11.4, $P < 0.05$) were significantly high during exacerbation when compared to remission phase. However, during remission phase in cases, the levels of anti-inflammatory cytokine IL-10 were significantly high when compared to exacerbation phase. The serum levels of TNF-α (5.01 ± 4.1 vs. 2.43 ± 0.83, $P < 0.05$), IL-10 (13.72 ± 11.4 vs. 3.42 ± 2.79, $P < 0.05$), and IgE (119.4 ± 137.9 vs. 52.3 ± 61.1, $P < 0.05$) were significantly high in cases during remission phase when compared to controls.

Interpretation: Persistently elevated levels of TNF-α and IgE in the exacerbation and remission phases indicates the chronic inflammatory nature of asthma and the exacerbations being acute-on chronic type of inflammation. The higher levels of IL-10 point toward the anti-inflammatory role of IL-10 and its role in inducing a remission.

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Text

Introduction

Airway inflammation is centrally important in asthma and other lung diseases. Currently, asthma is viewed as a chronic inflammatory airway disease, and international guidelines place great importance on treating inflammation in this condition.[1] In contrast, the assessment of disease severity and progression is based on clinical symptoms and lung function tests. Monitoring of inflammation is not included in current asthma guidelines, although it has been demonstrated that monitoring the nature, extent and intensity of inflammation improves asthma control.[2]

Asthma is a heterogeneous disease with multiple phenotypes that have variable risk factors and responses to therapeutics. One of the striking advances in the last decade has been the recognition that cytokines play a critical role in orchestrating, perpetuating, and amplifying the inflammatory response in asthma. It is also characterized by a specific pattern of inflammation, which is largely driven via immunoglobulin E (IgE) dependent mechanisms. The exact functional role of each individual cytokine in the pathogenesis of the disease remains to be fully established.[3]

An important aspect while evaluating the functional role of cytokines in a complex disease, such as asthma is the interaction with the other cytokines in the microenvironment. Increased expression of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) can further enhance the inflammatory process, and is increasingly linked to disease severity. TNF-α may have an important amplifying effect on asthmatic inflammation. There is evidence of increased expression in asthmatic airways and IgE triggering in sensitized lungs leads to increased expression in epithelial cells. Interleukin-10 (IL-10) is a pleiotropic cytokine that can exert either immunosuppressive or immunostimulatory effects on a variety of cell types. It is a potent inhibitor of monocyte/macrophage function, suppressing the production of a number of pro-inflammatory cytokines, including TNF-α.[4] The present study was conducted to estimate the levels of pro-inflammatory (TNF-α) and anti-inflammatory (IL-10) cytokines and serum IgE in patients with asthma during exacerbation and remission in comparison with normal controls.

Methods

Sampling and study design

The present 2-year case-control study was conducted in the department of Respiratory Medicine at the Mediciti Institute of Medical Sciences. A total of 50 individuals aged 18–65 years were enrolled in the study. They were divided into two groups: cases (asthma patients, n = 25) and controls (healthy controls, n = 25). Patients diagnosed with asthma by spirometry showing a post bronchodilator reversibility of >12% or 200 ml or clinically diagnosed cases of acute exacerbations were included in the case group. Healthy individuals from the general population were included in the control group. Unwilling individuals, pregnant women, lung pathology like chronic obstructive pulmonary disease, underlying connective tissue disorder, malignancy, active infection elsewhere in the body, and smokers were the exclusion criteria for the case group. Unwilling individuals, pregnant women, smokers, family history of asthma, and individuals with atopy/allergy, any active infection, and any other systematic illness (diabetes mellitus, connective tissue disorder, and malignancy) were classified as exclusion criteria in the control group.

Instrumentation

After recruiting the participants, venous blood (5 ml) was collected from each participant. Whole blood was collected in a tube containing ethylene diamine tetra acetic acid and left to naturally coagulate for about 20 minutes at room temperature. Later, the tubes were centrifuged at 2000 rotations/min for 10 mins. The supernatant was collected carefully and stored at −70°C. Enzyme-linked immunosorbent assay was performed to estimate the levels of plasma TNF-α, IL-10, and IgE.

Exacerbation was defined as acute worsening of cough, breathlessness, or chest tightness in a known asthmatic or a new patient with symptoms consistent of acute asthma.

Remission was defined as symptom alleviation following treatment of asthma, within one or two weeks after initiation of treatment.

Data collection and statistical analysis

Before the commencement of the study, ethical clearance was obtained from the Institutional Ethical Committee. After explaining the purpose of the study, written consent was obtained from the patients before data collection. Data were recorded in a predesigned and pretested

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proforma. The data obtained were entered in Microsoft Excel spreadsheet and analyzed using statistical package for social service (SPSS v. 22). Since, plasma cytokine levels were not in a Gaussian distribution, the Mann–Whitney rank sum test was used to assess the differences in the concentration of cytokines in asthmatic patients (during remission and exacerbation) and controls. The Spearman's rank correlation test was used to ascertain the correlation among plasma cytokine correlations. A probability of $P < 0.05$ was considered as statistically significant.

Results

The sociodemographic and clinical details of the cases are depicted in Tables 1 and 2, respectively.The serum levels of inflammatory cytokines TNF-α, IL-10, and IgE are shown in Table 3.

In cases, the serum levels of pro-inflammatory cytokine TNF-α ($P < 0.05$) and IgE receptor ($P < 0.05$) were significantly high during exacerbation when compared to remission phase (Table 4). However, during remission phase in cases, the levels of anti-inflammatory cytokine IL-10 were significantly high when compared to exacerbation phase ($P < 0.05$; Table 4). The serum levels of TNF-α ($P < 0.05$), IL-10 ($P < 0.05$), and IgE ($P < 0.05$) were significantly high in cases during remission phase when compared to controls (Table 5). The correlation (r) using spearman's rank correlation test between TNF-α and IgE during exacerbation had a positive correlation but a weak one , and IL-10 and IgE during remission had a positive correlation but was statistically insignificant.

Discussion

The present study aimed at evaluating the serum levels of pro-inflammatory and anti-inflammatory cytokines in patients with asthma during exacerbation and remission phases and in healthy individuals.

A weak positive correlation was established between TNF-α and IgE during exacerbation. Further, a positive correlation was observed between IL-10 and IgE during remission, which was statistically insignificant (Table 6). Correlation between duration of asthma in years and these parameters revealed no statistical significance between cases as well as controls.

In allergic reactions, IL-10 production and release by monocytic cells is upregulated by TNF-α and by negative feedback regulation. Importantly in the context of asthma, IL-10 inhibits eosinophil through suppression of IL-5 and granulocyte-macrophage colony stimulating factor, direct effect on eosinophil apoptosis, and effects on cell proliferation through down-regulation of IL-1. The cytokine suppressive characteristics of IL-10 occur through its upregulation of suppressor of cytokine signaling-3. In addition, IL-10 is a suppressor of nitric oxide production, which may have implications for its role in airway inflammatory diseases.[5]

Initial clinical trials have demonstrated relative safety and few clinically adverse events at doses of recombinant human 1L-10 below 50 µg/kg with a mixed success in the treatment of patients with inflammatory bowel disease and psoriasis. However, steroid therapy and allergen-specific immunotherapy are known to elevate endogenous IL-10 levels, which may account for their efficacy. This suggests that further study of IL-10 as a target for treatment of airway inflammatory diseases, such as asthma is warranted.[5]

IL-10 has important anti-inflammatory properties in immune diseases and it is speculated that diminished IL-10 production in asthma would permit the unopposed synthesis of pro-inflammatory cytokines, contributing to the development and severity of asthma. Borish et al. demonstrated constitutive secretion of IL-10 into bronchoalveolar lavage fluid of normal, non-asthmatic subjects (130 ± 61 pg/ml; $n = 8$). The bronchoalveolar lavage fluid of asthmatic patients was characterized by diminished concentrations of IL-10 (9 ± 18 pg/ml, $n = 8$; $P < 0.01$) as compared to normal subjects. By using the RNA-based polymerase chain reaction, the study demonstrated that diminished IL-10 occurred as a result of inhibition of transcription.[4] Therefore, IL-10 can be considered as an important agent in the resolution of inflammation.

The present study reflects that serum IgE levels were significantly higher in the cases (during exacerbation and remission) as compared to the control group. This is in agreement with the study conducted by Thirunavukkarasu et al.,[7] who investigated serum IgE level in 60 asthmatic patients and 13 healthy controls aged between 18–60 years. They observed that the mean IgE levels in the control and the case groups were 151 IU/ml and 756 IU/ml. These observations were attributed to the fact that the link between IgE and asthma are independent of allergen sensitization.[6] The airway inflammation in asthma is due to the infiltration of the airway wall with T-helper type 2 (TH2) cells,

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which are attracted to inflammatory sites by adhesion molecules and chemokines, such as chemokine receptor type 3 and chemokine receptor type 4 receptors. Differentiation of B lymphocytes (B-cells) into IgE secreting plasma cells is a complex cascade of events in which cytokines play a crucial role. Both IL-4 and IL-13 induce IgE synthesis, whereas interferon-γ and IL-12 block IgE synthesis. IgE production by B-cells not only requires the presence of IL-4 or IL-13, but also a physical interaction between T lymphocytes and B cells. Production of TH2 cells is not only accredited to T-cells but also to eosinophils and mast cells, indicating the importance of these cells in the synthesis of IgE.[8]

Hence, it may be proposed that the levels of IgE are quite high locally at the site of inflammation and the serum levels do not necessarily reflect the levels in lungs and bronchus. It is also known that IgE is bound to mast cells with rather high affinifty,[9] and therefore the circulating IgE may not give a conclusive evidence of the severity of inflammation.

TNF-α increases the expression of cellular adhesion molecules and facilitates the passage of leukocytes into the airway in response to allergen and bacterial products. In addition, it increases the airway smooth muscle cell contractility, expression of eotaxin, and increases IL-5 secretion. In asthma, TNF-α may have apoptotic activity, although this specific question has not been addressed within the airway but perhaps it could be responsible for airway epithelial shedding. A polymorphism in the TNF-α promoter resulting in increased generation of this cytokine has been linked to asthma in genotypic studies. Further, there are data to implicate TNF-α in airway remodeling and fibrosis. These facts make TNF-α,a logical target for intervention, and studies are underway to determine if inhibition of this multifunctional cytokine may improve the range of drugs available in asthma therapy.[11]

Glucocorticosteroids are the most effective anti-inflammatory agents in the treatment of asthma and possess a wide range of activity across various cytokine networks and other mediators.[8]Inhibition of TNF-α production is no exception to this activity; however, this leads to unwanted side effects at higher doses and during prolonged treatment period. Novel methods of inhibiting TNF-α are currently under investigation in diseases other than asthma (Rheumatoid arthritis) wherein an excess in TNF-α contributes to morbidity and mortality (Malaria, gram-negative sepsis, and Jarisch–Herxheimer reaction). A variety of components under study are postulated to have different mechanisms, including inhibitors of TNF-α mRNAtranscription (Pentoxifylline and Phosphodiesterase inhibitors).[11'12]

Entzian et al. studied three xanthenes and demonstrated inhibition of both interferon-γ and TNF-α with the novel compound a802715 exhibiting greater potency than pentoxifylline and theophylline. [13] Other pharmacological inhibitors of TNF-α include accelerators of TNF-mRNA degradation[14-16] inhibitors of TNF protein translation[14] and metlloprotinease inhibitors that prevent the cleavage of the 26 kda membrane bound protein to the active 17 kda molecule.[18]

Interpretation

Persistently elevated levels of pro-inflammatory cytokine (TNF-α) and IgE in the exacerbation and remission phases indicates the chronic inflammatory nature of asthma and the exacerbations being acute-on chronic type of inflammation. The higher levels of IL-10 point toward the anti-inflammatory role of IL-10 and its role in inducing a remission. Further studies are warranted to explore the potential of these parameters for therapeutic purposes and their role as biomarkers of the disease.

Although the study has proven to be statistically significant, it is not devoid of certain limitations. The first being a small sample size and all the participants enrolled were from rural background. The categorization of cases based on the phenotype of asthma was not performed.

Treatment strategies to enhance the inhibition of pro-inflammatory cytokines are warranted. In addition, a spirometric correlation of the severity of the disease and associated changes in the cytokine levels along with sputum levels of the parameters would give a better insight of the local inflammatory response and extrapolation to the present study objectives.

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Take home point: Future studies are warranted to explore the potential of these parameters for

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therapeutic purposes and their role as biomarkers of disease.

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Tables

Table 1: Sociodemographic distribution of participants in the study

Variable	**n (%)**
Cases	
Gender	
Males	6 (24)
Females	19 (76)
Age (years)	
20–30	8 (32)
31–40	9 (36)
40–65	8 (32)
Background	
Rural	25 (100)

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Urban	0
Literacy	
Literate	12 (48)
Illiterate	13 (52)
Employment	
Employed	10 (40)
Unemployed	15 (60)
Controls	
Gender	
Males	6 (24)
Females	19 (76)
Age (years)	
20–30	8 (32)
31–40	8 (32)
40–65	9 (36)

Table 2: Clinical details of the cases in the study

Variable	**n (%)**
Duration of asthma (years)	
<10	11 (44)
10–20	6 (24)
>20	8 (32)
Symptoms	
Wheeze	22 (88)
Chest tightness	22 (88)
Breathlessness	24 (96)
Cough	25 (100)
Sneezing	15 (60)
Rhino sinusitis	16 (64)
Seasonal variation	25 (100)
Diurnal variation	25 (100)
Exposure	
Allergen	15 (60)
Biomass fuel	8 (32)
Family History	
Yes	6 (24)
No	19 (76)
Treatment history	
Regular	7 (28)
Symptomatic	17 (68)
Not on any treatment	1 (4)

Table 3: Estimated serum levels of TNF-α, IL-10, and IgE

Cytokine	**Controls (mean±SD)**	**During exacerbation (mean±SD)**	**During remission (mean±SD)**
TNF-α (pg/ml)	2.431 ± 0.83	7.38 ± 5.4	5.01 ± 4.1
IL-10 (pg/ml)	3.420 ± 2.8	4.36 ± 5.9	13.72 ± 11.4
IgE (IU/ml)	52.3 ± 61.1	4.06 ± 441.05	119.4 ± 137.9

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Table 4: Comparison of serum levels of TNF-α, IL-10, and IgE in cases during exacerbation and remission phases

Cytokine	Exacerbation (Mean±SD)	Remission (Mean±SD)	Z score	P score
TNF-α	7.38 ± 5.4	5.01 ± 4.1	2.13	< 0.05
IL-10	4.36 ± 5.9	13.72 ± 11.4	-4.57	< 0.05
IgE	230.9 ± 148.5	119.4 ± 137.9	2.91	< 0.05

Table 5: Comparison of serum levels of TNF-α, IL-10, and IgE in cases during remission phase and in controls

Cytokine	Remission (Mean±SD)	Controls (Mean±SD)	Z score	P score
TNF-α	5.01 ± 4.1	2.43 ± 0.83	2.78	< 0.05
IL-10	13.72 ± 11.4	3.42 ± 2.79	5.02	< 0.05
IgE	119.4 ± 137.9	52.3 ± 61.1	1.061	< 0.05

Table 6: Pro and anti-inflammatory parameters in asthmatics in comparison with controls

Parameter	Exacerbation vs. Control	Remission vs. Control
TNF-α	3.03 folds higher	2.06 folds higher
IL 10	1.274 folds higher	4.01 folds higher
IgE	4.41 folds higher	2.28 folds higher