ORIGINAL RESEARCH

A Study on Estimation of Pro and Anti-Inflammatory Cytokine Levels in Asthma During Exacerbations and Remissions in Rural Tertiary Care Centre

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ABSTRACT

Background:Asthma 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. AIM: To estimate the pro inflammatory cytokine (TNFα), anti-inflammatory cytokine (IL-10) and serum IgE levels during exacerbation and remission of asthma and to compare with normal controls.

Materials and Methods: Study Design: Prospective comparative study. Study area: Department of. Pulmonary Medicine and General Medicine, M. V. J Medical College and Research Hospital, Hoskote, Bengaluru. Study Period: 1st march 2021 to 28th February 2022. Study population: Patients who had symptomatology of Asthma attending and admitted in the Dept. of. Pulmonary Medicine and General Medicine. Sample size: study consisted a total of 50 patients. (25 cases and 25 controls) Sampling method: Simple Random sampling method. Ethical consideration: Institutional Ethical committee permission was taken prior to the commencement of the study. Study tools and Data collection procedure: The study included a total of 50 individuals of which 25 were patients of asthma and 25 normal healthy controls from general population. The subjects included in the study were explained the purpose of enrolment and informed consent was taken. Statistical Analysis: Statistical analysis was done using Microsoft excel sheet and analysed statistically using SPSS (statistical package for social service) version 22. Since plasma cytokine concentrations were not in a Gaussian distribution, the Mann–Whitney rank sum test was used to assess the differences.

Results: 8(32%) cases where in the age range of 20-30 years, 9(36%) in 31-40 years range and 8(32%) were above 40 years. 6(24%) participants of the study group were males and 19(76%) were females. The serum levels of the pro inflammatory cytokine TNFα during exacerbation of asthma was 7.38± 5.4 pg/ml and during remission was 5.01±4.1 pg/ml, while the controls had a mean of 2.43± 0.83 pg/ml. The serum levels of the anti-inflammatory cytokine IL- 10 were 4.36±5.9 pg/ml during exacerbation, 13.72±11.4 pg/ml during remission and 3.42±2.8 pg/ml in controls.

Conclusion: Persistently elevated levels of the pro inflammatory cytokine (TNFα) and IgE in the remission phase points to the chronic inflammatory nature of asthma and the exacerbations being acute on chronic type of inflammation. The higher levels of IL-10 point out towards the anti-inflammatory role of IL -10 and its role in inducing a remission.

Keywords: Asthma, pro inflammatory cytokine (TNFα) and IgE, exacerbations.

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INTRODUCTION

Asthma 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.

It affects people of all ages and imposes a substantial burden on patients, their families, and the community.[1] 334 million people worldwide suffer from asthma. Among them 8.6% of young adults (aged 18-45) experience asthma symptoms. The burden of asthma is greatest for children aged 10-14 and the elderly aged 75-79.[2]

Asthma is a heterogeneous disease with multiple phenotypes that have variable risk factors and responses to therapeutics.[3] 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 immunoglobin E (IgE) dependent mechanisms.[4]The exact functional role of each individual cytokine in the pathogenesis of the disease remains to be fully established. An important aspect when evaluating the functional role of cytokines in a complex disease such as asthma is the interaction with other cytokines in the microenvironment.

Increased expression of pro-inflammatory cytokines such as tumor necrosis factor-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.[5] There is evidence of increased expression in asthmatic airways and IgE triggering in sensitized lungs leads to increased expression in epithelial cells. 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.[6]

So the present study was undertaken to estimate the pro and anti-inflammatory cytokine levels in asthma during exacerbations and remissions and compare these values with the control population.

AIM:

To estimate the pro inflammatory cytokine (TNFα), anti-inflammatory cytokine (IL-10) and serum IgE levels during exacerbation and remission of asthma and to compare with normal controls.

MATERIALS & METHODS

Study Design: Prospective comparative study.

Study area: Department. of. Pulmonary Medicine and General Medicine, M. V. J Medical College and Research Hospital, Hoskote, Bengaluru.

Study Period: 1st march 2021 to 28th February 2022.

Study population: Patients who had symptomatology of Asthma attending and admitted in the Department of. Pulmonary Medicine and General Medicine.

Sample size: study consisted a total of 50 patients. (25 cases and 25 controls)

Sampling method: Simple Random sampling method.

Inclusion Criteria for Cases

- Willing
- Both males and females
- Age – 18 years and above
- Diagnosed cases of asthma by spirometry showing a post bronchodilator reversibility of > 12% or 200 ml or clinically diagnosed cases in acute exacerbations.

Exclusion Criteria for Cases

- Unwilling
- Age : <18 years, >65years
- Pregnancy
- Lung pathology like COPD
- Underlying connective tissue disorder
- Malignancy
- Active infection elsewhere in the body.
- Smokers
- Patients with Renal and Cardiac failure

Inclusion Criteria for Controls

- Age between 18 to 65 years
- Willing to take part
- Both males and females

Exclusion criteria for Controls

- Unwilling
- Age < 18yrs >65yrs
- Pregnancy
- Smoking
- Family history of asthma
- Atopy / allergy
- Any active infection
- Any other systemic illness: diabetes mellitus, connective tissue disorders, malignancy.

Ethical consideration: Institutional Ethical committee permission was taken prior to the commencement of the study.

Study tools and Data collection procedure

The study included a total of 50 individuals of which 25 were patients of asthma and 25 normal healthy controls from general population. The subjects included in the study were explained the purpose of enrolment and taken informed consent.

Venous blood (5 ml) was taken from each participant. Whole blood was collected using ethylene diamine tetra acetic acid (EDTA) tubes and naturally coagulated for about 20 minutes at room temperature, and centrifuged at 2000 r/min for 10 minutes. The supernatant was collected carefully and stored at –70°C. Enzyme-linked immunosorbent assay (ELISA) was used to detect plasma TNF- α, IL 10 and IgE levels.

A total of twenty-five patients were enrolled during exacerbation and the corresponding samples during remission. Twenty-five controls were enrolled.

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 symptom alleviation following treatment of asthma, within one to two weeks after initiation of treatment.

The serum which was stored appropriately at -700C in the college laboratory was retrieved. ELISA was performed using commercially available kit as per the manufacturer's instructions and serum levels of TNF α, IL-10 and IgE was estimated.

Diaclone ELISA kit was used for the estimation of TNFα (950.090 Human TNFα ELISA kit-version 9) and IL 10 (950.060 Human IL 10 ELISA kit Insert Version11) and Monobind Inc. Accu Bind ELISA microwells kit(product code 2525-300) was used for IgE estimation. The procedure was performed as per manufacturer's instructions.

Statistical Analysis:

Statistical analysis was done using Microsoft excel sheet and analysed statistically using SPSS (statistical package for social service) version 22. Since plasma cytokine concentrations 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 control subjects. The Spearman's rank correlation test was used to ascertain the correlation among plasma cytokine concentrations. A probability of $P < 0{\cdot}05$ was considered as significant association.

RESULTS

Table 1: Age and Sex Distribution of Cases

Age in years	**Cases n = 25**	**Controls n=25**
20-30	8 (32%)	8 (32%)
31-40	9 (36%)	8(32%)
>40	8 (32%)	9 (36%)
Males	6 (24%)	6 (24%)
Females	19 (76%)	19 (76%)

8(32%) cases where in the age range of 20-30 years, 9(36%) in 31-40 years range and 8(32%) were above 40 years. 6(24%) participants of the study group were males and 19(76%) were females.

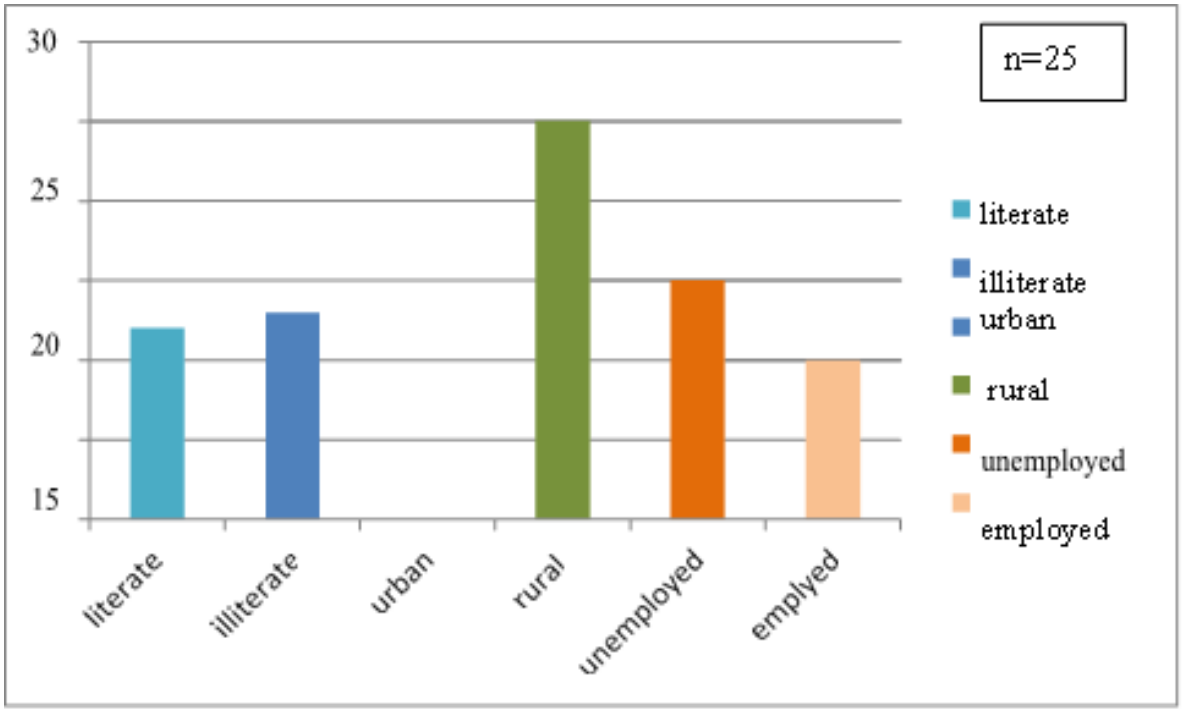

Figure 1: the socio demographic distribution of the cases.

All the participants were from the rural population, maximum being illiterate (52%) and (60%) were unemployed, most of them being females. The participants were predominantly illiterate, unemployed and from rural background.

Table 2: Exposure and Family History

Factors	**Frequency (n =25)**
Allergen exposure	15 (60%)
Biomass fuel exposure	8 (32%)
Family history	6 (24%)

60% of the participants have allergen exposure at home or work place in the form of dust and smoke.32% had a history of significant biomass fuel exposure. 24% had a family history of first degree relative with asthma.

Table 3: Treatment History

Treatment history	**n = 25**
Regular treatment	7 (28%)
Symptomatic treatment	17 (68%)
Not on any treatment	1 (4%)

28% of the patients were on regular treatment with ICS/LABA combination twice a day. 68% of the patients were not on any regular treatment; used medication ranging from oral corticosteroids, salbutamol inhalers or tablets on symptomatic basis.one patient was not on any treatment and had a prior self-limiting episode

Table 4: Duration of Asthma in Years

Duration in years	**n=25**
<10 years	11 (44%)
10-20 years	6 (24%)
>20 years	8 (32%)

11 patients had duration of illness less than 10 years, 6 had duration of illness between 10-20 years and 8 had duration of illness more than 20 years.

Table 5: estimated pro and anti-inflammatory cytokine levels in asthma during exacerbation, remission and in controls.

Cytokine	Controls Mean ±SD	During exacerbationMean± SD	During remission Mean ±SD
TNF α (pg/ml)	2.4316± 0.83	7.38±5.4	5.01±4.1
IL 10(pg/ml)	3.4204±2.8	4.36±5.9	13.72±11.4

The serum levels of the pro inflammatory cytokine TNFα during exacerbation of asthma was 7.38± 5.4 pg/ml and during remission was 5.01±4.1 pg/ml, while the controls had a mean of 2.43± 0.83 pg/ml. The serum levels of the anti-inflammatory cytokine IL- 10 were 4.36±5.9 pg/ml during exacerbation, 13.72±11.4 pg/ml during remission and 3.42±2.8 pg/ml in controls.

The serum levels of TNFα were elevated during exacerbation and reduced in remission but still higher than controls. IL -10 levels were found to be elevated in remission when compared to exacerbation and at a lower level in controls.

Table 6: Estimated IgE levels in the control group and the asthmatic group (during exacerbation and during remission)

IgE levels IU/ml	Control group	Asthma patients duringExacerbation	Asthma patients duringRemission
Mean	52.3±61.1	230.9 ± 148.5	119.4 ± 137.9

The serum level of IgE in the control group was 52.3±61.1 IU/ml, while in the cases during exacerbation it was 230.9 ± 148.5 IU/ml and during remission it was 119.4 ± 137.9IU/ml. Elevated IgE levels in cases during exacerbation than in remission and higher levels in remission when compared to the control group.

Table 7: Comparison of Pro and Anti-Inflammatory Cytokine Levels in Patients with Asthma During Exacerbation and Remission

Cytokine	DuringexacerbationMean ±SD	During remission Mean ±SD	Z score	P value
TNF-α(pg/ml)	7.38±5.4	5.01±4.1	2.13	<0.05
IL10 (pg/ml)	4.36±5.9	13.72±11.4	-4.57	<0.05

A statistically significant difference is seen between the two parameters during remission and exacerbation.

Table 8: Comparision of pro and anti-inflammatory cytokine levels in patients with asthma during remission and in controls.

Cytokine	During RemissionMean ±SD	In controls Mean ±SD	Z score	P value
TNF-α(pg/ml)	5.01±4.1	2.43 ± 0.83	2.78	<0.05
IL 10 (pg/ml)	13.72±11.4	3.42± 2.79	5.02	< 0.05

A statistically significant difference is seen in these cytokine levels during remission and in control group.

Table 9: Comparision of serum IgE levels in exacerbation, remission and controls.

IgE during exacerbation	IgE during remission	Z score	P value
230.9 ± 148.5	119.4 ± 137.9	2.91	<0.05
IgE during remission	IgE in controls	Z score	P value
119.4 ± 137.9	52.3±61.1	1.061	<0.05

A statistically significant difference is seen in the IgE levels during exacerbation and remission and also during remission and the control group.

DISCUSSION

Of the twenty-five patients in the case group, 6(24%) participants of the study group were males and 19(76%) were females. 8(32%) cases where in the age range of 20-30 years,9(36%) in 31-40 years range and 8(32%)were above 40 years.The age range of cases was 19 to 66 years, the mean age of the cases was 39.96 years. All the participants were from rural background.12(48%) of them were literates and 13 (52%) of them were illiterates.10 (40%) of them were employed. Most of them being farmers and 15 (60%) of them were unemployed most of them being housewives.

The results of the present study showed that serum IgE level were significantly higher in the asthma group (during exacerbation and after remission) compared with the control group. Our study is in agreement with the study by Thirunavukkarasu et al,[7] who investigated serum IgE level in 60 asthmatic patients between 18 and 60years of age, classified according to the GINA classification (31 male and 29 female), and 13 healthy controls between 18 and 60 years of age. They found that the mean IgE level in the control group was 151 IU/ml and that in the asthmatic group ranged from 404 to 1045 IU/ml. They attributed this to the fact that there is a link between total IgE and asthma, which appears to be independent of allergen sensitization. One of the typical aspects of airway inflammation of asthma is the infiltration of the airway wall by T-helper type 2 cells. These cells are attracted to inflammatory sites by adhesion molecules and chemokines, among which CCR3 and CXCR4 receptors appear to be of importance. Differentiation of B-cells into IgE secreting plasma cells is a complex cascade of events in which cytokines play a crucial role. Both interleukin (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 and B cells, involving a number of surface and adhesion molecules such as CD40-CD40L and CD28/CD80. Production of T-helper type 2-cytokines is not restricted to T-cells, as basophils and mast cells can produce them, indicating that these cells may be of importance in the synthesis of IgE.[8] 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 affinity and hence the circulating IgE may not give conclusive evidence of the severity of inflammation.[9]

The results revealed the pro inflammatory cytokine TNFα to have a mean value of 7.38±5.4 pg/ml (S.E 1.08) (range -2.48-23.26) during exacerbation of asthma and 5.01±4.1pg/ml (S.E 0.824) (range - 1.49-14.31) during remission. TNFα being a pro inflammatory cytokine was elevated during the exacerbation phase of asthma and the levels decreased significantly in remission ($p<0.05$). The TNFα level in the control group was 2.4316± 0.83 pg/ml (S.E 0.16) (range -1.09-3.98). It was also found that the TNF α levels in remission period of the asthmatics was higher than compared to controls and this difference was statistically significant ($p<0.05$).

The anti-inflammatory cytokine IL-10 levels was found to have a mean value of 4.36±5.9 pg/ml (S.E- 1.18) (range -0.57-29.12) during exacerbation and 13.72±11.4 pg/ml (S.E - 2.29)

(range -2.37-49.77). IL 10 was found to be significantly higher in remission than in exacerbation ($p<0.05$). The value of IL 10 in controls was 3.4204±2.8 (S.E -0.558) (range -0.73-12.79) and it was significantly higher in remission when compared to controls ($p<0.05$).
The serum IgE levels during exacerbation was 230.9 ± 148.5IU/ml (range - 4.06 - 441.05) and in remission it was 119.4 ± 137.9 IU/ml (range - 2.33 -397.67). The serum IgE levels were found to be significantly higher in exacerbation than in remission ($p<0.05$), the control group the mean value was 52.3±61.1IU/ml (range - 52.3±61.1). The difference between the control group and remission was statistically significant. ($p<0.05$)
The correlation (r) using spearman's rank correlation test between TNFα and IgE during exacerbation had a positive correlation but a weak one, and IL10 and IgE during remission had a positive correlation but was statistically insignificant.
Thus we can conclude that the levels of TNFα are 3.03 folds higher in exacerbation and 2.06 folds higher in remission when compared to the controls. IL- 10 levels were 1.27 folds higher in exacerbation and 4.01 folds higher in remission when compared to controls. IgE levels were 4.41 folds higher in exacerbation when compared to controls and 2.28 folds higher in remission in comparison to controls.
Tumor necrosis factor-α increases the expression of cellular adhesion molecules and facilitates the passage of leucocytes into the airway in response to allergen and to bacterial products. In addition, it would appear to increase airway smooth muscle cell contractility and expression of eotaxin, and also to increase IL-5 secretion. In asthma, as in other situations, 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. There is also data to implicate TNF-α in airway remodelling and fibrosis. A polymorphism in the TNF-α promoter resulting in increased generation of this cytokine has also been linked to asthma in genotypic studies. 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.
Glucocorticosteroid (GCS) treatment of asthma is the most effective anti- inflammatory agent and has a broad range of activity across many cytokine networks and other mediators.[10] Inhibition of TNF-α production is no exception to this activity. The breadth of this inhibition and activity also leads to unwanted side-effects at higher doses, and when the treatment period is prolonged. There is therefore a need for increasing the range of GCS-sparing treatments that can be used to reduce the dose of these highly effective drugs. Novel methods of inhibiting TNF-α are currently under investigation in diseases other than asthma, for example, Rheumatoid arthritis and conditions where an excess of TNF-α contributes to morbidity and mortality (e.g. Malaria and gram-negative sepsis, and the jarish herxheimer reaction). A variety of candidates are being studied. These are postulated to have different mechanisms, including inhibitors of TNF-α mRNA transcription (e.g. Pentoxifylline and phosphodiesterase Inhibitors).[11,12] Entzian et al.[12] studied Three xanthines and showed both inhibition of IFN-ɣ and TNF-α release with the novel compound a802715 demonstrating greater potency than pentoxifylline or theophylline.
Other types of pharmacological TNF-α inhibitors include accelerators of TNF-mRNA degradation (e.g. Thalidomide);[13,14]inhibitors of TNF protein translation (e.g tetravalent guanylhydrazone) and the metalloproteinase Inhibitors that prevent the cleavage of the 26 k da membrane bound Protein to the active 17 kda molecule.[15,16] Other approaches include TNF receptor fusion proteins and monoclonal antibodies.[17] Monoclonal antibodies have also been raised against TNF-α and have reached trials in human subjects who have rheumatoid arthritis, usually as a humanized murine antibody Generally,[18] these studies have shown encouraging results, although the problems associated with this type of therapy may limit its use to certain categories of disease.

CONCLUSION

Persistently elevated levels of the pro inflammatory cytokine (TNFα) and IgE in the remission phase points to the chronic inflammatory nature of asthma and the exacerbations being acute on chronic type of inflammation. The higher levels of IL-10 point out towards the anti-inflammatory role of IL -10 and its role in inducing a remission.

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