Original Article

Serum Interleukin-33 and Soluble Suppression of Tumorigenicity 2 in Bronchial Asthma

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Abstract

Background. Interleukin-33 (IL-33) is a member of IL-1 family of cytokines and is a ligand to the receptors of soluble suppression of tumorigenicity 2 (sST2). The IL-33/sST2 axis is involved in the promotion and maintenance of allergic inflammatory pathway in asthma; hence serum levels of these two biomarkers were studied.

Methods. This cross-sectional and observational study was conducted in Government Medical College and Hospital, Chandigarh, India. A total of 220 subjects were recruited and divided into four groups: Group A: asthmatic patients with exacerbation, Group B: asthmatic patients without exacerbation, Group C: patients with other lung diseases, like chronic obstructive pulmonary disease, community acquired pneumonia, lung cancer, etc and Group D: age and gender matched apparently healthy individuals and serum IL-33 and sST2 levels were studied.

Results. Present study showed that IL-33 and sST2 levels were significantly raised in asthmatic patients with acute exacerbation. Mean serum values of IL-33 were 37.9, 27.1, 15.2 and 14.5pg/mL in groups A, B, C and D, respectively and those of sST2 were 7.91, 7.03, 3.98 and 3.56 ng/mL in the groups A, B, C and D, respectively.

Conclusion. Serum IL-33 and sST2 can be considered as novel biomarkers in asthma severity.
[Indian J Chest Dis Allied Sci 2019;61:171-174]

Key words: Asthma, IL-33, sST2, COPD, Cancer.

Introduction

Asthma is a common chronic respiratory disease affecting 1% to 20% of the people in different populations.[1,2] It is thought to be T-helper 2 (Th2) cell-mediated immune disease. Two of the most important cytokines responsible for Th2 immune response are interleukin-33 (IL-33) and thymic stromal lymphopoietin. IL-33 is released by injured or necrosed epithelial cells of bronchus and small airways in response to triggers, such as virus, allergen and smoke and this expression is increased in bronchial epithelial cells of patients with asthma.[3]

One of the ligands of IL-33 is ST2, which is a member of the IL-1 family and exerts its function through a group of receptors that belong to the toll-like receptor-IL-1 receptor super family, which is defined by the presence of an intracellular Toll-IL-1R domain. ST2 is highly expressed on mast cells along with macrophages and it is a highly selective marker of Th2 cells.[3-5]

IL-33 is a member of the IL-1 family of cytokines and binds to two receptors: soluble suppression of tumorigenicity 2 (sST2) and IL-1 receptor accessory protein. There are two isoforms of ST2 proteins: ST2L, a transmembrane form, and soluble ST2, a secreted form that can serve as a decoy receptor of IL-33.[4]

The IL-33/ST2 axis is involved in the promotion and maintenance of allergic inflammation via a number of cell types and also activates airway eosinophils that exacerbate airway inflammation.[5] Certain studies state that inhibitors of Th2 driven inflammatory pathways in asthma have shown promising results in the management of asthma and prevention of future exacerbations.[6-8] Search of available literature on the levels of these inflammatory markers in asthmatics with and without exacerbation has revealed limited data.[9-20] Being important mediator of inflammatory pathways in asthma, the present study was planned to evaluate IL-33 and sST2 as biomarkers in asthmatic patients with and without exacerbation and compare their levels in patients with other lung diseases and healthy volunteers.

Material and Methods

This cross-sectional and observational study was conducted in the Department of Pulmonary Medicine in collaboration with the Department of Biochemistry, Government Medical College and Hospital, Chandigarh, India, after approval from the Institute's Ethical Committee. Because of lack of literature in Indian context, sample size was calculated on the basis of the mean levels of serum IL-33 and sST2 and their respective deviations in the Egyptian population.[14] Assuming 95% level of significance and 10% relative precision, sample size came out to be 55 in each

[Received: April 20, 2018; accepted after revision: January 14, 2019]

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group. The period of study was from November 2016 to September 2017.

A total of 220 subjects were recruited in the study and divided into four groups. Group A: 55 cases of asthma with exacerbation, defined according to the guidelines of Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2015[21], Group B: 55 cases of asthma without exacerbation, defined according to GINA guidelines 2015, Group C: 55 patients with other lung diseases, *e.g.* chronic obstructive lung disease diagnosed according to Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines 2015,[22] community-acquired pneumonia diagnosed according to British Thoracic Society guidelines for the management of community-acquired pneumonia in adults 2009,[23] histologically proven lung cancer, tuberculosis diagnosed according to Revised National Tuberculosis Control Programme (RNTCP) guidelines,[24] and Group D: 55 age- and gender-matched apparently healthy controls from the hospital staff, students, and colleagues who voluntarily participated in the study. Patients less than 18 years of age and patients presenting with respiratory failure were excluded.

All study subjects were given necessary information and informed consent was obtained on a standard proforma before the study. Detailed history was taken from each subject and a thorough clinical examination was done. Besides the routine investigations, 3mL of venous blood sample was collected under strict aseptic conditions in plain vial for serum IL-33 and sST2 level estimation from every participant of this study. Serum was separated and stored at -70 °C till analysis. Serum levels of IL-33 were determined by enzyme-linked immunosorbent assay (ELISA) using reagent kits (Weldone, Diclone) and sST2 was quantified by ELISA using reagent kits from Biosource, according to the recommendations of the manufacturer. Intra-assay and inter-assay coefficient of variation for IL-33 and sST2 were less than 7%.

Statistical Analysis

Statistical analysis was done using IBM-SPSS statistics (version 22.0). Discrete categorical data was presented as number (per cent). Continuous data was written in the form of mean and standard deviation. The normality of data was checked by measures of Kolmogorov-Smirov test of normality. For normality distributed data, statistical comparison was carried out by using Student's 't' test. A p-value of <0.05 was taken as statistically significant. For categorical data, comparisons were done using Pearson's Chi-square test as appropriate. Correction was done using Spearman's rho test. Post-hoc-Levence test and Tukey-Honest significant difference test were applied to confirm where the differences occurred between the groups.

Results

All the groups were age- and gender-matched. The mean age of the patients was 46.2, 44.9, 47.4 and 41.4 years in the groups A, B, C and D, respectively. Mean serum IL-33 levels (in pg/mL) were found to be 37.9, 27.1, 15.2 and 14.5 in groups A, B, C and D, respectively. The difference in mean values of IL-33 in group A and group C as well as in group B and C was found to be highly significant (p=0.001 in both). When the mean values of IL-33 in group B were compared with group C and D, the values were also found to be highly significant (p=0.001 in both). However, the difference in mean values of IL-33 in group A and group B were found to be non significant (p=0.1).

Mean serum sST2 levels (ng/mL) were found to be 7.9, 7.0, 4.0 and 3.6 in groups A, B, C and D, respectively. On comparison of mean values of sST2 in group A with group C and D, the same were found to be highly significant (p=0.001 in both). The difference in mean values of sST2 in group B were also found to be highly significant when compared with group C and D (p=0.001 in both). The difference in mean values of sST2 in group A and B was also statistically significant (p=0.002).

IL-33 levels correlated significantly with sST2 levels (p<0.001) in patients of group A and B, respectively (Figure 1). For IL-33, comparison of asthmatics with controls revealed area under the curve (AUC) to be 0.711 on receiver-operating characteristic (ROC) analysis (Figure 2). For sST2, comparison of asthmatics with controls revealed AUC to be 0.99 on ROC analysis (Figure 3).

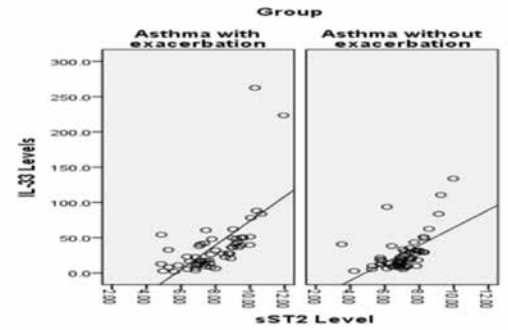

Figure 1. Correlation of IL-33 with sST2 levels.

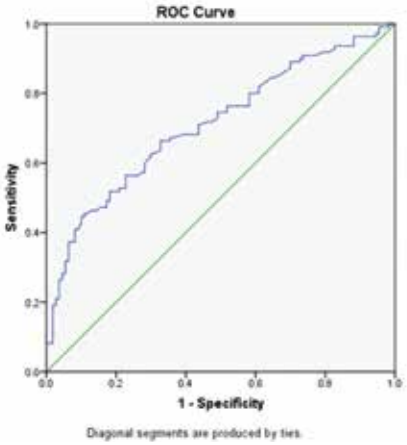

Figure 2. Area under the curve (0.711) in receiver-operating characteristic (ROC) analysis for IL-33.

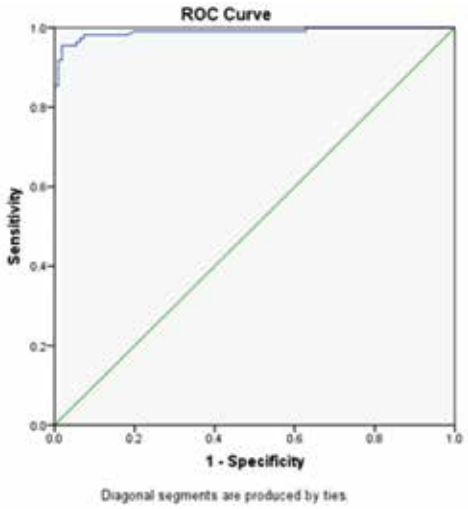

Figure 3. Area under the curve (0.99) in receiver-operating characteristic (ROC) analysis for sST2.

When absolute eosinophil counts were compared with IL-33 and sST2 levels, there was a positive correlation in patients of asthma with exacerbation, but not in patients of stable asthma.

Discussion

Asthma is a chronic inflammatory disease, classically characterised by the airway hyper-responsiveness, allergic inflammation, and increased Th2 cytokine production. IL-33, a member of the IL1-cytokine family, is considered to be crucial for the induction of T-helper type 2 cell dominant immune responses, such as bronchial asthma.[25] ST2 is an interleukin-1 receptor family member and exists in both the membranes — bound isoform and a soluble isoform (sST2).[4] IL-33, the functional ligand for ST2 and IL-33/ST2 signalling pathway is involved in regulating inflammation and immunity in bronchial asthma. Majority of the literature is focussed on the levels of these biomarkers in patients with differing severity of asthma. Some studies have shown that IL-33 expression was higher in endobronchial biopsies of asthmatic patients when compared with controls.[10,13] However, limited literature is available on the levels of these biomarkers in the serum of asthmatic patients in comparison with the normal healthy population and the difference between patients of asthma with acute exacerbation and without exacerbation. Hence, we studied serum levels of IL-33 and sST2 in asthmatic patients with acute exacerbation and stable asthma patients compared them with diseased and healthy controls.

Mean age of the patients in our study was slightly higher than in the previous studies.[9,14] A meta-analysis amongst the role of IL-33 in children states that gender and disease progression may be influencing serum levels of these biomarkers.

Serum IL-33 and sST2 levels were significantly raised in group A compared to group C and D. Similar results were seen with group B *versus* group C and D. Our results for IL-33 and sST2 were in agreement with other studies.[12-14] The rise of sST2 in asthmatic patients with exacerbation was significantly higher than in stable asthmatics. IL-33 was also raised in the patients of asthma with exacerbation than stable asthmatics though the difference was not statistically significant. Serum IL-33 levels correlated with sST2 levels in patients of asthma with and without exacerbation in both group A and B and the results are in agreement with other studies.[12-14] In another study, substantially high levels of IL-33 and sST2 in induced sputum were observed, in addition to serum levels, during exacerbation in asthma patients compared with levels in stable asthmatics and had found that their levels were associated with the disease severity.[16] The rise in levels of these biomarkers can be explained by the fact that IL-33 and sST2 regulate important biological processes, such as the immune responses. Therefore, any increase in their concentrations suggests activation of pathways involved in an inflammatory response or disease development though their concentrations in biological fluids and tissues are undetectable or very low in physiological state. Since cytokine profile in acute phase of the disease is different from chronic phase, hence, there could be a difference in the levels of these biomarkers in the patients of asthma with exacerbation *versus* stable asthmatics.

Our results are in comparison to another study[18] which stated that the serum levels of IL-33 were raised in asthmatic patients and not affected with the administration of inhalational corticosteroids. We observed increased levels of IL-33 in patients with bronchial asthma having acute exacerbation and in stable asthmatics most of whom were already on inhaled corticosteroids, though the differences were not statistically significant. Also since many of these patients were on long-acting beta-2 agonist, leukotriene receptor antagonist etc, the exact medication used, their dose and duration of the treatment which the patients have already taking were not recorded, and hence, their influence, (if any), on the levels of serum IL-33 and sST2 cannot be commented. Data is generating which suggests that IL-33 is involved in lung inflammation and supporting the hypothesis that sST2 can be potential therapeutic targets in the treatment of asthma. The results of our study support this hypothesis, however, with the limited time and resources. Further, the levels of IL-33 and sST2 were measured at a single time, hence temporal correlation with the course of the disease and the effect of frequency of exacerbation could not be determined.

Conclusions

The serum levels of IL-33 and its receptor sST2 were markedly elevated in patients of bronchial asthma with acute exacerbation. Serum IL-33 and sST2 levels can be considered as novel biomarkers in asthma and their marked elevation supports the concept that these can be used as a tool for further understanding of the disease and as future potential therapeutic targets.

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