Activities of Periostin and Inteleukin-13 among Asthma Patients in Comparison with Healthy people in Southern India

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Asthma is a chronic inflammatory airway disease which requires biomarkers that reflect refractoriness to conventional therapy with inhalational steroids. Periostin is an extracellular matrix protein that is secreted in the airway epithelium, in response to stimulation by Interleukin -13 (IL-13). IL-13 is a cytokine that mediates airway inflammation following the Type 2 immune response. Both these biomarkers can be used to signify type 2 inflammatory response, which reflects steroid hypo-responsiveness in asthmatics. The objectives of the study were to: 1. Obtain the reference value of serum Periostin and IL-13 levels in healthy south Indian adult population 2. To compare the reference range of Periostin and IL-13 with that of the asthmatics on inhalational corticosteroids. The study was carried out among 50 asthmatics and 50 healthy volunteers aged between 25 to 65 years. After procuring the informed consent, the Pulmonary Function test assessment was done to recruit the study subjects. The blood sample was collected for assessment of Serum Periostin and IL-13. The median (IQR) baseline level of Serum Periostin among control group and in asthmatics was 13.2 (8.8-28.1) ng/ml and 16.7 (10.9-20.7) respectively. Also, the median (IQR) baseline level of Serum IL-13 among healthy individuals and among asthmatics was 42.9 (37.8-52.4) pg/ml and 73.5 (60.0-91.1) pg/ml respectively, which was statistically significant. The obtained baseline values of Serum IL-13 and Periostin could be of clinical utility in asthmatics. The validity of the data obtained from this study can be tested out on larger study populations.

Keywords: Asthma; interleukin-13; inhalational steroid; Periostin.

Bronchial asthma is a type 2 inflammatory disease of the airways characterized by shortness of breath, wheeze, and airflow obstruction. Globally, asthma ranks as the 14th leading chronic disease affecting 334 billion people worldwide. Though the number of deaths has dropped, the number of people affected based on quality of life and the economic burden has increased, viz asthmatics in Europe, account for huge amount spent on treatment (509 Euro per patient). In India, studies have shown asthma as the 25th leading cause of Disability Adjusted life years (DALYs) and studies

by Barnet et al, Murray et al and Global asthma report 2011 have reported that bronchial asthma contributes to about 1% of the total DALYs. [1-4] People diagnosed with asthma are conventionally treated with inhalational corticosteroids (ICSs) or beta 2 agonists. Based on an estimation in the year 2015, the amount spent on asthma treatment in India is approximately about 139.45 billion INR [H" 2.3 billion USD]. Studies have suggested that if all the asthmatics receive medications as per the evidence-based guidelines, the treatment cost is likely to come down. [5] The modality of asthma treatment is aimed at: the suppression of inflammation associated with asthma, the reduction of bronchial hyper-reactivity with the use of leukotriene antagonists/Long-acting beta-2 sympathomimetic agents in combination with ICSs.

Various studies have concluded that biomarkers could aid in the identification of asthmatics based on phenotype, severity and refractoriness to the conventional management. However, there is great challenge in identification of the asthmatics who are hypo-responsive to steroids. Evidence suggests that biomolecules like Periostin (POSTN) and Interleukin -13 (IL-13) can be used as markers to Type 2 (Th2) inflammatory process that led to eosinophilic recruitment , inflammation and thereby leading to airway remodeling. Studies have also shown that measurement of periostin, IgE, IL-13 and the blood eosinophil count could help in identification of asthmatics who remain as poor responders to steroids and also could possibly express enhanced response to treatment with monoclonal antibodies. [6-15] But, limited data are available with respect to a diagnostic test with better sensitivity in diagnosing steroid hypo-responsive asthma. Hence, the present study was carried out Periostin and IL-13 as a novel biomarker which can be used to develop a new treatment strategy by categorizing the asthmatics.

Role of Periostin and Interleukin in the pathogenesis of Asthma

Periostin is found in healthy individuals as well. It is derived from periodontal ligament and is expressed in epithelial cells, osteoblasts, fibroblasts etc. At the time of disease onset in an individual, an allergen finds its way into the body and mediates type 2 inflammatory response by activating the mast cells, lymphocytes, neutrophils. These immune cells once activated start producing chemical substances like interleukin- 4, 5 and 13 which further causes eosinophil recruitment. The eosinophils in turn produces Periostin which has varied effects. The POSTN thus produced act via autocrine mechanism causing further eosinophil recruitment. It exhibits extracellular inter-linking with fibronectin, tenascin and collagen and act via Transforming growth Factor to bring about sub epithelial fibrosis and airway remodeling leading to refractoriness to routine therapy with inhalational corticosteroids. [16-21] IL-13 is an immune cell cytokine that enhances IgE production and brings about airway inflammation, hyper reactivity and increased mucus production. [22,23] The genetic expression of IL-13 on chromosome 5q31 is strongly linked in individuals with asthma.

METHODOLOGY

This prospective cohort study was conducted among individuals aged 25 to 65 years. The sample size was calculated considering alpha – 5%; power – 80%; Since it was a follow up study, the attrition rate was kept at 20%. Based on this, 50 healthy individuals and 50 asthmatics were enrolled in this study, The study subjects were categorized into two groups, viz asthmatics and healthy controls by subjecting them to pulmonary function test and by following the GINA guidelines. People who were devoid of respiratory symptoms suggestive of asthma and had a normal Pulmonary function test were categorized as healthy individuals. Persons who had symptoms of cough for more than two weeks, a history of wheeze or shortness of breath was considered to have asthma and were subjected to a pre and post broncho-dilator pulmonary function testing using 400 micro- litre of Salbutamol using a spacer. Those persons who showed a 12% and 200 ml reversibility of FEV1% were confirmed as asthmatics and were included in the study group. Following this, 5 ml of blood sample was collected from these individuals in the assessment of serum Periostin and serum Interleukin – 13. People with morbid obesity, diabetes mellitus, pregnant ladies, cardiovascular morbidities and immunodeficiency diseases were excluded from the study.

Assay for Biomarkers

The serum levels of Periostin were measured using the Human Periostin ELISA Kit,

Thermo Scientific, Pierce™ and ab100553-IL-13 (Interleukin-13).

Serum Biomarkers

For the testing of serological markers, the blood samples were collected, centrifuged, sera separated and stored in a deep freezer at -20 degree Celsius. When a sufficient number of samples got collected, the samples were individually tested for serum Periosin and Interleukin-13 respectively.

Serum Periostin

Procedure

All reagents, samples and standards were prepared as instructed in the manual. 100 µL of standard was added to the samples placed in the wells. It was then covered with plate & incubated at room temperature for 2.5 hours. The plate was washed four times. Then 100 µL Biotinylated antibody was added to the wells. Again, it was covered with plates and incubated at room temperature for 1 hour. The plates were washed four times. 100 µL of Streptavidin HRP Reagent was added to each well and was covered with plates and incubated at room temperature for 45 minutes. The plates were washed four times. Then 100 µL TMB Substrate was added to each well. The plates were developed at room temperature in the dark for 30 minutes. 50 µL of Stop Solution was added

to each well. The absorbance was calculated using calorimetry.

Serum IL-13

Procedure

The standard or samples were added to each well. It was then incubated at room temperature. Prepared biotin antibody and then the prepared Streptavidin solution were added to each well and incubated at room temperature. Then TMB One-Step Development Solution was added to each well and incubated at room temperature. The reaction is brought to end by adding stop solution to each well. Reading were obtained using calorimetry at 450nm immediately.

Statistical Analysis

The data were entered in excel and was analyzed using SPSS -21.0. Descriptive Statistics viz Mean, Standard Deviation, Median, IQR were calculated. Mann-Whitney U test was used to compare the mean score of Serum Periostin and Serum IL-13 between healthy individuals and asthmatics.

RESULTS

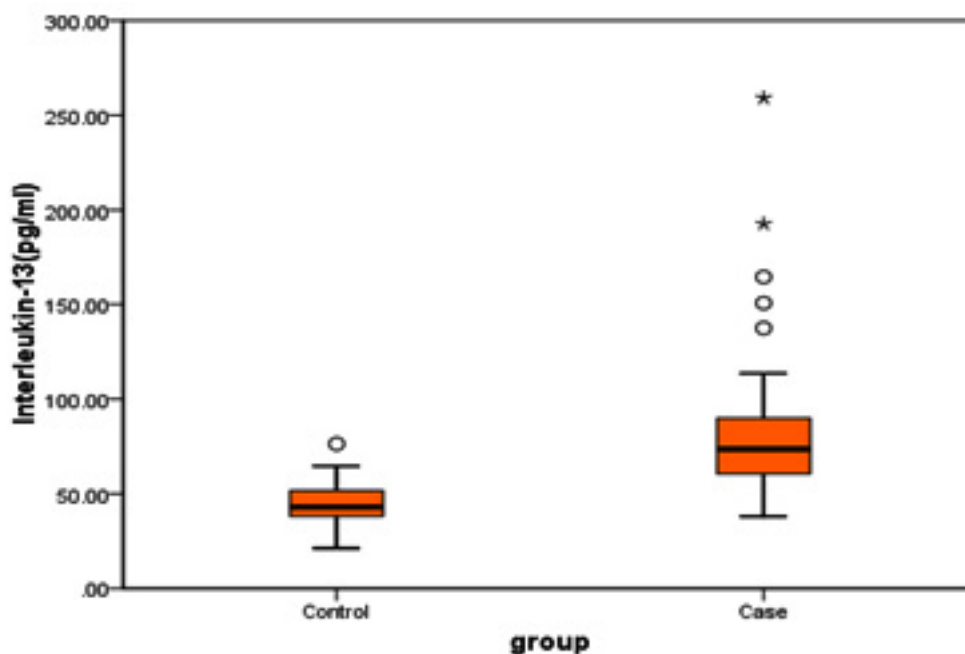The median (IQR) value of serum Interleukin – 13 was 42.9 (37.8-52.4) pg/ml in
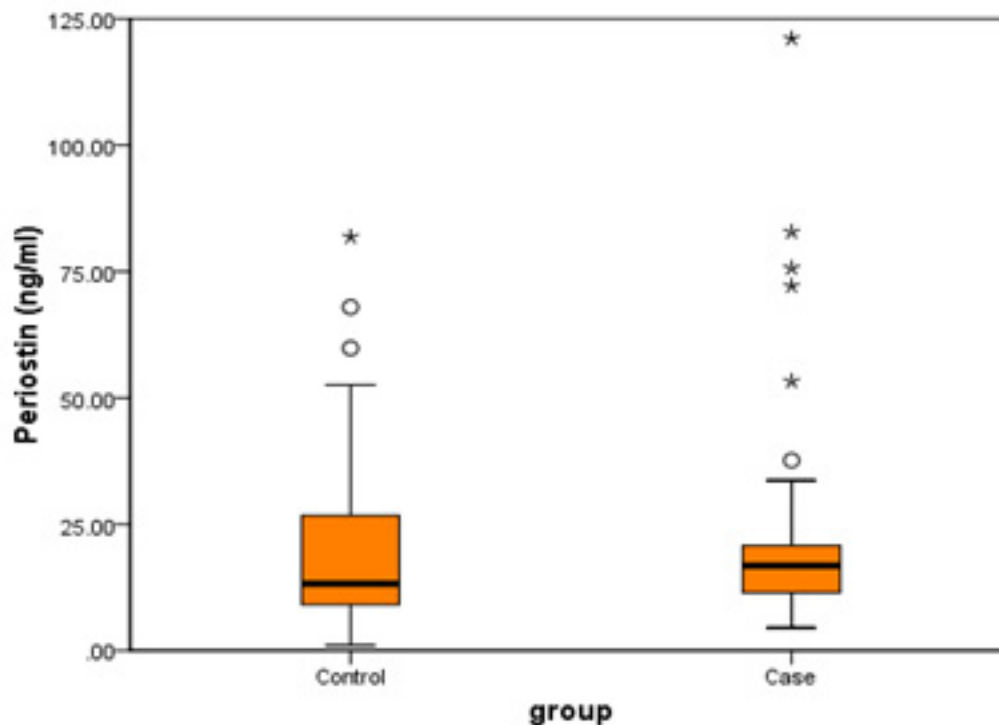

Fig. 1. Interleukin 13 levels

Fig. 2. Periostin levels

healthy individuals and 73.5 (60.0-91.1) pg/ml among asthmatics. There was significant difference between both the groups. (p < 0.0001). (Figure 1) Also, the median (IQR) value of serum Periostin was 13.2 (8.8 - 28.1) ng/ml in healthy individuals and 16.7 (10.9–20.7) ng/ml among asthmatics. There was no significant difference between both the groups. (p=0.269). (Figure 2).

DISCUSSION

The gold standard treatment that is being followed to treat asthmatics is the use of inhalational glucocorticoids. However, it doesn't always infer complete relief in roughly around 5 to 10% of the asthmatics. This type of refractory asthma can be identified using various tools like FENO, blood or sputum eosinophil count, biomarkers like IgE, Periostin and IL-4, 5,13. Despite the availability of these tests, the biopharmaceutical medication targeting the asthmatics using a newer modality of management had not yielded promising results due to the underutilization of the biomarker assays.

The refractoriness to asthma management varies based on the pathogenesis. Eosinophil mediated hyper responsiveness of airway would require targeted therapy using biomolecule. This suggests that there is a need of assessment of more sensitive biomarkers that is highly feasible to identify the level of control of asthma activity. [23-31] This would pave the way for better asthma control based on genotyping and severity.

Among them, studies recommend the assessment of serum Periostin mediated by IL-13 as a multicellular protein that terminates in the formation of sub epithelial fibrosis. [32] Research that utilizes the pathogenetic role of POSTN and IL-13 in the categorization of asthmatics remains as an area of active research during the last decade. [21,33-34]

The present study projected the distribution of serum Periostin among asthmatics: 16.7 (10.9–20.7) ng/ml and healthy individuals: 13.2 (8.8 - 28.1) ng/ml. This was similar to other studies. [11,12,21] Though the raw data seems to show that there is a difference in the periostin levels

between both the groups, probably due to an insufficient number of study population, we were unable to yield statistically significant result. The study was carried out by adjusting for the possible errors that could happen. However, the process of sample collection, storage, processing and immunoassay were done using a standardize protocol.

The study found the levels of IL-13 among the asthmatics: 73.5 (60.0-91.1) pg/ml and healthy individuals was 42.9 (37.8-52.4) pg/ml. There was significant difference between the serum IL-13 levels between both the groups. Various studies that have been attempted to find the link between the IL-13 and asthma have yielded conflicting results. Few studies have shown IL-13 as a molecule that is responsible for the raised IgE levels and hyper-reactivity whereas few studies have shown IL-13 to have either a protective role or no association in the pathogenesis of asthma. [35-39] Studies have shown that persons with elevated interleukin levels showed a good response to treatment with monoclonal antibodies. Identification of such phenotypes who would respond better to targeted therapy, would mediate improvement in airway inflammation, thereby, decrease the number of exacerbations and provide a better quality of life.

CONCLUSION

The baseline values of serum IL-13 and Periostin were obtained from healthy individuals and was compared with asthmatics. Serum periostin and IL-13 can be considered as biomarkers to identify asthmatics who are steroid resistant. The study can be conducted on a large scale and values, thus obtained could be clinically utilized in targeting refractory asthma and thus change the management strategy.

Limitations of the study

The study was carried out among a less number of people and the result procured cannot be extrapolated to the entire population. Also, serum periostin is elevated only in a small subset of asthmatics. But in the present study, phenotype subgroup with equal number of subjects in each group was not considered. The recruitment of study subjects based on reversibility testing in PFT was challenging. Hence it is recommended to carryout study on a larger scale and considering

equal number of study subjects in each phenotype subgroup.

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