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Original Research Article

The relationship of serum interleukin-13 to asthma control in Nigerian population

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ABSTRACT

Background: Interleukin-13 (IL-13) is a major contributor to type-2 inflammation in asthma. Asthma control is the extent to which manifestation of disease have been reduced or eradicated by treatment, and it is the main therapeutic goal. However, the link between IL-13 and asthma control has not been extensively studied. We therefore determined if serum IL-13 was associated with asthma control in patients presenting to a tertiary center in Nigeria.

Materials and Methods: Eighty-two adults with physician confirmed asthma, participated in this cross-sectional study. All participants had assessment of asthma control by asthma control test (ACT), and serum IL-13 assay by a commercial ELISA kit; spirometry and skin prick test were also assessed. Correlation between ACT scores and serum IL-13 assay was assessed by spearman's correlation coefficient.

Results: There were 59 females (72%), mean age was 43.4 years (SD16.3) and mean duration of asthma symptoms was 19years (SD=13.4), median (IQR) ACT score was 18.0(13.0-22.0) and median (IQR) serum IL-13 was 4.0(3.3~4.4) pg/ml. Out of the 82 participants, 49(59.8%) had uncontrolled asthma (ACT \leq 20). Serum IL-13 correlated inversely with ACT, rho= -0.218, p=0.025. The serum IL-13 median (IQR) concentration did not differ significantly between non-atopic 4.1(3.7-4.4) pg/ml and atopic 3.8(3.2-4.4) pg/ml; p=0.159 asthmatics.

Conclusion: Serum IL-13 may be a useful biomarker to assess asthma control in asthmatics.

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1. Introduction

Asthma is one of the most prevalent and chronic immunological respiratory diseases. ^{1,2} It is characterized by chronic inflammation of the airways and accumulating evidence indicates that the underlying inflammation in atopic asthma is driven by type 2 CD4⁺ lymphocytes (Th₂ cells) and their cytokines including interleukin-13 (IL-13). IL-13 is a 13KDa, 33 amino acid pleiotropic cytokine, a product of gene on chromosome 5 at 5q31. It is produced by many cells, in particular by Th₂ cells, others are mast cells, eosinophil, monocytes/macrophage and type 2 innate lymphoid cells. It shares a receptor component, signaling pathway and many biological activities with IL-4. ³ IL-

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13 is currently being implicated as a central regulator in Immunoglobulin E (IgE) synthesis, mucus hypersecretion, airway hyper responsiveness, and fibrosis. 4,5

Despite the advances made in the field of asthma pathogenesis and availability of effective therapy, currently, asthma cannot be cured, the main aim of therapeutic management is to achieve and maintain control, ⁶ in fact treatment protocols need to be adjusted according to asthma control levels as specified in many national and international guidelines. ^{7–10} Asthma control was traditionally determined by subjective evaluation of the presence or absence of symptoms, however asthmatics may over or under-estimate their symptoms and oftentimes, symptom perception may not be a direct correlate of the underlying inflammation. ¹¹ Evaluation of asthma control

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using inflammatory biomarkers in the sputum or blood may be a more objective way of measuring airway inflammation and may correlate with disease activity or even predict exacerbation. ¹¹ In addition, patients' outcome may be improved by monitoring inflammatory biomarkers. ¹²

Many studies have demonstrated increase expression of IL-13 in sputum, bronchoalveolar lavage fluid, bronchial mucosa and blood of asthmatics as compared to non-asthmatics. ^{13,14} However, little is known about the association between serum IL-13 and asthma control. Therefore, this study aimed to assess the level of serum IL-13 which is a main driver of inflammation in asthmatics, to asthma control in Nigeria population.

2. Materials and Methods

2.1. Study population

It was an observational cross-sectional study conducted from December 2015 to November 2016. Stable asthmatics (patients who had not required hospital admissions, emergency room visits or timely use of oral corticosteroids within the last month) on follow-up at the respiratory clinic of Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife were consecutively recruited. The diagnosis of asthma was based on physician's assessment and must have had episodic respiratory symptoms, such as wheeze, cough, chest tightness, or dyspnoea that are consistent with asthma and reversible airflow obstruction as documented in the casefile by reversibility of FEV1 of >12% and at least 200 mls from the baseline with the use of 400 mcg salbutamol. Excluded were pregnant ladies, subjects with any other concomitant diagnoses such as Chronic Obstructive Pulmonary Disease, bronchiectasis, lung cancer, autoimmune diseases and cardiac illness.

A minimum of 81 participant would be needed to provide 80% power and alpha of 0.05 with moderate effect size of 0.3 in a correlational study. ¹⁵

Ethical approval was obtained from ethical committee of Obafemi Awolowo University Teaching Hospitals Complex (ERC/2015/12/04). Approved written informed consent was obtained from all subjects before any study related procedures were done.

2.2. Procedure

All participants filled questionnaire which included sociodemographics variables, duration of symptoms, family history of asthma, history of current drug therapy and Asthma Control test (ACT). Anthropometry and spirometry were also done for the participants.

2.3. Asthma control

Asthma control was measured by ACT. ACT is short, simple, self-reporting, and the most widely validated of all asthma control composite scores. ¹⁶ It is a 5-item questionnaire used to assess asthma symptoms (daytime and nocturnal), use of rescue medications and the effect of asthma on daily functioning limitations in work or home activities, and patients' self-perception of asthma control during the past 4 weeks. Each item includes 5 response options corresponding to a 5-point rating scale. Scores range from 5 to 25 with higher scores indicating better asthma control, Scores from 5 to 19 indicated uncontrolled asthma and scores \geq 20 were indicated well controlled asthma. ACT is an efficient and reliable test (test-retest reliability of 0.77 and Cronbach's α of 0.84-0.85 cross-sectionally; 0.79 longitudinally). ¹⁷

2.4. Interleukin 13 assay

Blood samples were drawn by venepuncture of the cubital vein on the fore arm. 5ml of blood was drawn into plain bottles, it was allowed to clot for 1 hour at room temperature. The clotted blood was centrifuged for 10 minutes at 2500 rpm. The serum obtained were stored at -80° C until further analysis for measurement of serum IL-13.

The serum concentration of IL-13 was measured using a specific enzyme linked immunoassay (Aviscera Bioscience, Santa Clara, CA, USA). This employs the quantitative sandwich ELISA technique. The manufacturer's instruction was followed. All samples were run in duplicates with all reagents and samples are brought to room temperature before the start of the assay. 100µl of the IL-13 standards, control and sera samples were pipette into polystyrene microplate wells coated with an antibody against IL-13. The microplate was covered with a plate sealer and incubated for 2hours. Plates were emptied and washed 4 times with 300µl of wash buffer to remove unbound antibody and the blotted by absorbent paper towel. 100 µl of detection antibody were also added and incubated at room temperature for another 2 hours, after which the plates were emptied and washed 4 times again with $300\mu l$ of wash buffer. After this $100\mu l$ of avidin -horse radish peroxidase was added to all the wells and incubated for 45 minutes at room temperature, protected from light. The plates were emptied and washed again 4 times, then $100\mu l$ of substrate solution were added to each well and this results in formation of a blue colour. The resulting colorimetric reaction was stopped by addition of 100 μ l per well of the stop solution (1 M sulphuric acid), causing yellow colour to develop which is directly proportional to the concentration of IL-13 in the sample. The optical density of the wells was read at 450 nm using a microplate reader (DNM-9602 microplate reader, Beijing Perlong New Technology Co, Ltd, China). A curve-fitting on excel software program was used to quantitate IL-13 concentrations.

2.5. Atopic status

Atopic status was determined by skin prick testing (SPT) performed after discontinuation of antihistamine medications for at least 5 days on the volar aspect of the forearm, using percutaneous multi test method (Multi-Test II Device Lincoln Diagnostics, Decatur, IL, USA) with a panel of aero-allergens common in our environment. This includes the following extract: mould mix (Alternaria alternata, Aureobasidium sorokiniana, Cladosporium Drechslera pullulans, sphaerospermum, Aspergillus niger, and Penicillium notatum), standardized house dust mite mix (Dermatogoides pteronyssinus/farinae), mixed feather (chicken, duck and goose), cockroach (Periplanata americana and Blattella germanica) dog epithelium (Canis familiaris) and 7 standardized grass mix (Timothy, Orchard, June, Redtop, Meadow Fescue, Perennial Rye and Sweet Vernal). Histamine (1.0mg/ml) was used as positive control while glycerinated -saline (NaCl 0.9% glycerin 50%) was used as negative control. All extracts are from ALK ABELLO, Port Washington, New York, USA. A wheal dimension of at least 3mm greater than the negative control was considered to be a positive reaction. Atopy was defined as a positive skin test reaction to at least one of the applied allergens.

2.6. Spirometry

Spirometry was performed according to the European Respiratory Society (ERS) and American Thoracic Society (ATS) protocol, ¹⁸ using an automated, flow sensing, desktop spirometer (Spirolab III MIR Roma, Italy). Participants were given detailed instructions and also the procedure was demonstrated before asking them to perform the spirometry. They were asked to inspire to total lung capacity and afterwards performed a forced expiratory manoeuvre.

Each spirogram was carefully reviewed by the lead author according to the ATS guideline for acceptability and reproducibility. Only spirometry tests meeting these criteria were included in the analysis. The largest values of forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV1) were retained. The following were recorded; forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/FVC, force expiratory flow between 25% and 75% of FVC (FEF25–75), and peak expiratory flow (PEF). Predicted values were calculated by the reference equations from NHANES (National Health and Nutrition Examination Survey) III with a 12% reduction for African ethnic group. ¹⁹

2.7. Statistical analysis

Participants' characteristics and outcome were described using frequencies with percentages for categorical variables, means with standard deviations (SDs) or medians with inter quantile range (IQRs), and descriptive statistics were presented for the overall study population. To determine significant differences between participants that were well controlled and non-controlled asthmatic, we used Mann-Whitney test, t-tests, and chi-square tests, as appropriate. In addition, we used Spearman's Rank correlation coefficient to determine power and direction of linear relationships between asthma control score and IL-13. All reported p-values were two-sided with p<0.05 considered statistically significant. Statistical analysis was performed with IBM-SPSS software V.20 (IBM, Armonk, New York, USA).

3. Results

The mean age of the participants was 44.3 + 16.3 years with age range between 18 to 75 years. 59 of them were females (72%) and 57(69%) of them had post-secondary education. Clinically, 55 (67%) of them about half of them had no emergency visit in the last one year. Allergic rhinitis is the commonest allergic comorbidities. Fifty-six (68%) of the participant were positive for at least one out of the six inhalant aeroallergens and were classified as atopic.

The median (IQR) of serum IL-13 was 4.0(3.3-4.4) pg/ml and. Other social demographics and clinical parameters are shown in Table 1 . The range of ACT scores ranged from 5 to 25. The mean score+ SD was 17.83+5.01, while the median (IQR) score was 18.0(13.0-22.0). Participants with well controlled asthma ACT score \geq 20 was 33 (40%) and participant with uncontrolled asthma were 49(60%).

Comparing some of the clinical and lung function indices between those who are not controlled and those who were well controlled: there is no significant difference between the mean age in those who were well controlled and those who were not $(47.6\pm16.5\text{vs}\ 42.0\pm15.9\ \text{p}=0.137)$, however more females were well controlled compared to males $(84.4\%\ \text{vs}\ 63.5\%\ \text{p}=0.033)$. Percentage of the predicted PEF is significantly more in those who are well controlled. $(67.5\pm25.2\ \text{vs}\ 53.3\pm26.2,\ \text{p}=0.018)$. Although the serum IL-13 was lower in well controlled asthma, this did not reach statistical significance p=0.095 2Table 2)

4. Discussion

The main finding of our study is that serum IL-13 inversely correlate with level of asthma control as measured by the ACT questionnaire implying that, the more controlled asthma, the less the serum level of IL-13.Although, the correlation is modest, it is statistically significant(r=0.218 p=0.025). In this study, we measured serum IL-13 because it is one of the central regulators of type II response in asthma pathogenesis, more so, monoclonal antibodies are

Table 1: Demographical and Clinical characteristics of the participants

Variables	Participants (n=82)
Anthropometrics	
Age (years)	44.3+16.3
BMI (kg/m2)	22.3+5.4
Gender	
Female	59 (72%)
Male	23 (28%)
Age of asthma diagnosis (years)	$25.4{\pm}15.7$
Duration of asthma symptoms (years)	19.0 ± 13.4
Atopy	56(68%)
Highest level of Education	
Primary	10(12%)
Secondary	15(19%)
Post-secondary	57(69%)
Emergency Visit in the last One year	
0	55(67%)
1	18(22%)
≥2	9(11%)
Comorbidities	
None	41(50%)
Allergic rhinitis	30(37%)
Clinic sinusitis	5(6%)
Allergic conjunctivitis	5(6%)
Gastroesophageal reflux	14(17%)
Asthma Medications	
Inhaled salbutamol	78(92%)
Inhaled steroid/ LABA	43(52%)
Oral prednisolone	14(17%)
Lung function test	
PEF (L/s)	5.0+2.3
PEF (% pred)	58.9 +26.6
FEV1 (L)	2.2+1.3
FEV1(% pred)	73.2 +27.5
FVC(L)	2.7 + 0.9
FVC (% pred)	84.0+24.0
FEF 25-75(L/s)	2.3+1.4
FEF 25-75(% pred)	49.9+31.4
FEV1/FVC	0.73+0.17
FEV1/FVC <0.7	28(34%)

Table 2: Comparison of Clinical and Lung function of the participants according to asthma control status

Variables	Well controlled n=33	Not controlled n=49	p value
Age (years)	47.6 ± 16.5	42.0 ± 15.9	0.137
BMI (kg/m^2)	24.6 ± 5.1	25.7 ± 5.7	0.398
Duration of symptoms(years)	15.0(8.0-27.5)	15.0(8.0-30.5)	0.856
Gender(female)	28(84.4%)	31(63.5%)	0.033*
Inhaled corticosteroid use	19(57.6%)	24(49.0%)	0.445
No emergency visit in the last one year	24(72.7%)	31(63.3%)	0.839
PEF (% pred)	67.5 ± 25.2	53.3 ± 26.2	0.018*
FEV ₁ (% pred)	78.8 ± 22.6	69.5 ± 29.9	0.115
FVC (% pred)	85.3 ± 20.7	83.2 ± 26.1	0.692
FEF ₂₅₋₇₅ (% pred)	57.8 ± 33.2	44.43 ± 28.7	0.070
Serum IL-13 (pg/ml)	3.7(3.0-4.4)	4.1(3.4 -4.3)	0.095

Data are shown as means (SD) or medians (interquartile range), or numbers (percentages of respondents) Student's t-test or Mann Whitney test for continuous variables; Pearson chi-square for categorical variable *The significant ones are asterisked

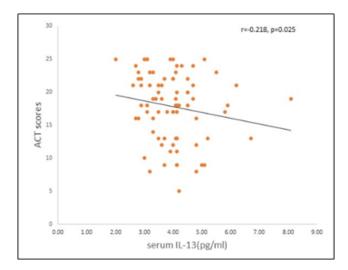


Fig. 1: Correlationbetween ACT scores and Serum IL-13

currently being developed against IL-13 that may be useful as add- on therapy in the management of uncontrolled asthma. Although the relationship between serum IL-13 and asthma control has not been extensively studied, our study corroborates other studies that assessed IL-13 in the sputum.

Saha et al., ²⁰ in their study, sought to assess IL-13 expression in sputum and bronchial biopsy specimens from subjects with mild-to-severe asthma. They documented that sputum IL-13 levels exhibited a significant positive correlation with Asthma Control Questionnaire (ACQ) scores (r=0.35, p=.04). The positive correlation observed in their study was explainable by instrument used to measure asthma control. For ACQ the lower score, the better the control so higher score means uncontrolled asthma.

In addition, Tsilogianni et al., ²¹ assess the diagnostic performance of sputum IL-13 for evaluation control and also to compare this performance to that sputum eosinophils and fraction of exhaled Nitric Oxide (FeNO). They found out that IL-13 levels in the sputum supernatant differed significantly between patients with well-controlled asthma and those with not well-controlled asthma; in addition, the diagnostic performance of IL-13 was superior to sputum eosinophil and FeNO.

Furthermore, Jia and colleagues reported that the percentages of IL-13⁺ Type 2 innate lymphoid cells in peripheral blood were significantly higher in patients with uncontrolled asthma (49.7%) and partly controlled asthma (30.8%) than in those in the well-controlled group (16.7%) and healthy control subjects (18.7%). ²²

Taken the above studies together, it does appear that serum IL-13 may be a useful biomarker for assessing asthma control, however, other studies did not find an association between a panel of serum cytokines including IL-13 and asthma control, ¹² neither was there any statistical difference between serum IL-13 in asthmatics and non-asthmatics. ²³

To inform therapies and characterise patients with asthma, there has been a concerted effort to discover and evaluate various biomarkers of inflammation generally. Initial attempts to characterise inflammation showed that proportion of eosinophils may associated with asthma. Later, the measurement of total serum IgE and then specific immunoglobulins targeting particular antigen were also investigated. Most recently, detailed and specific components of the inflammatory and immune response are being explored. Our finding of modest correlation with serum IL-13 with validated measure of asthma control could possibly suggest that IL-13 may be as useful biomarker for asthma control. Although the serum IL-13 level was lower in those who are well controlled, this does not reach significance. This may be that our study was not actually powered to detect the difference between those who are well controlled and those who are not.

Although, more studies are needed to substantiate this finding in clinical setting. It is pertinent to note some limitation to this study, the study population may not be representative of the general population of adults with asthma are they are largely drawn from hospital-based sample. We also measured IL-13 in the serum, it may be argued that sputum IL-13 may reflect more accurately the inflammation in the airway and sputum specimen levels may be more helpful in characterizing airway inflammation, predicting response to treatment, and perhaps identifying patients at risk of exacerbations. However, sputum cytokine measurements require specialized training to collect, process, and analyse and is not generally available in clinical settings, more so, it can lack reproducibility. Bronchoalveolar lavage specimen may be an alternative; however, it is an invasive procedure requiring bronchoscopy in specialized center and time consuming. These methods, may not be feasible for studying biomarkers in our environment for now. The measurement of cytokine serum level may be a valuable alternative to sputum collection, as standardized collection of peripheral blood is practical among participants of all ages for large epidemiological studies and for routine clinical practice. ²¹ Despite the above limitation, our findings that serum IL-13 level correlates negatively with the level of asthma control would serve as exploratory study to stimulate further research.

5. Conclusions

IL-13 levels in serum negatively correlate with ACT score. Serum IL-13 may be a useful biomarker for asthma control. Also, our finding may serve as a rationale for more identification of other serum biomarkers that associated with asthma control status.

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8. Conflict of Interest

The authors declare they have no conflict of interest.

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