Evaluation of Sensitivity and Specificity of Interleukins 25 and 33 in Diagnosis of Pediatric Asthma

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Abstract

The roles of interleukin 25 (a member of the interleukin 17 family) and interleukin 33 (a member of the interleukin 1 family) in asthma and airway hyper responsiveness are yet to be fully understood. The aim of this study was to investigate the roles of IL-25 and IL-33 in the diagnosis of pediatric asthma and their association with severity and treatment of the disease. This was a case-control study comprising 74 children with asthma as the patient group and 75 healthy children as the control group. The age of the participants ranged from 1 to 15 years. Levels of IL-25 and IL-33 in the serum were measured using ELISA kits. The highest positive predictive values (88.9%) occurred in IL-25 with sensitivity and specificity of about 97.3% and 88.0% respectively, while the sensitivity and specificity of IL-33 were about 51.4% and 66.0% respectively, with a positive predictive value of about (60.3%). The present study thus found that IL-25 had higher diagnostic sensitivity and specificity values than IL-33 in children with asthma. In addition, both interleukins were found to have a statistical significance regarding treatment of the disease in children.

Keywords: Pediatric asthma, IL-25 and IL-33, Sensitivity and Specificity
INTRODUCTION
Asthma is a disease characterized by chronic inflammation of the airways. This inflammation is distinguished by recurrent events of breathlessness, wheezing, chest tightness and coughing in the early morning or during the night in susceptible individuals. Several types of cells and cellular elements are associated with this disease and play an important role in its pathogenesis; these include T lymphocytes, neutrophils, epithelial cells, eosinophils and mast cells. The risk factors of asthma are associated with the interactions between environmental and genetic factors. Approximately hundreds of genetic variants are correlated with an elevated risk of asthma disease.

Several viral infections are also considered as risk factors for developing asthma. A study demonstrated that viral infections are associated with the chronic inflammation of airways and exacerbation of asthma in children and adults. Stable and acute exacerbations of asthma are also associated with bacterial infections, while the contribution of fungal infection to asthma is not clear. In addition, the exposure to airborne environmental factors like pollutants and tobacco smoke increases the risk of asthma.

Exposure to environmental and genetic factors, in addition to the alterations in the microbiome and metabolites, are associated with the emergence of lower airway inflammation. The type 2 inflammation, observed in most patients with asthma, is associated with type 2 T helper cells. Specific cytokines such as interleukins 4, 5 and 14, and some inflammatory cells and IgE immunoglobulin are also associated with Type 2 inflammation, which is usually found in allergic diseases and eosinophilic disorders. Moreover, type 2 inflammation can be regulated by epithelial cells of the airway through some cytokines such as interleukins 25 and 33 and thymic stromal lymphopoietin. Both IL-25 and IL-33 are responsible for stimulating the group 2 innate lymphoid cells that participate in the initiation of type 2 immune response.

IL-25 directly stimulates eosinophils via upregulating the expression of ICAM-1, this leads to the production of several proinflammatory chemokines like macrophage inflammatory protein-1α, monocyte chemoattractant protein-1, and interleukins 6 and 8 and ultimately delays apoptosis. The expression of IL-25 was found to increase in the bronchial asthma and also in the patients with eczema. The expression of IL-25 and its receptor that induced by allergen correlated with the disease severity, in atopic asthma as well.

Furthermore, there is significant evidence connecting the effects of tobacco smoke exposure to reduced immune function, resulting in an imbalance in responses of both Th1 and Th2 cells and, consequently, an increase in susceptibility to allergic diseases. A study found that reduction in the number of Th1 adenoidal lymphocytes (IFN-γ-CD8+) in children was related to passive smoke exposure, leading to recurrent respiratory infections. The anti-IgE omalizumab drug represents the major recommended treatment of severe asthma.

MATERIALS AND METHODS
This case-control study was carried out at an outpatients clinic of asthma in the Kerbala Teaching Hospital for Children from September 2019 to December 2019. All the children included in this study were diagnosed according to medical history and clinical examination and also according to the criteria of the American Thoracic Society for asthma.

Ethical approval for the study was acquired from the College of Medicine, University of Kerbala, Medical Research Bioethical Committee. Verbal approval was also taken from the parents of the children before obtaining samples. Written consent was waived by the parents because verbal consent was sufficient, according to the parents’ opinion.

From a total of 149 participants in this study, 74 children (56 boys and 18 girls represented the patient group with an age range of 1-15 years, and 75 healthy children represented the control group with the same sex and age distribution as the patient group but with no history of allergies, asthma or inflammatory diseases. Patients with inflammatory conditions other than asthma were excluded from this study. The stages of asthma severity were diagnosed as per the NAEPP/ EPR 3 guidelines by the specialist pediatricians.

From each participant about 5 cc of venous blood was taken. Serum sample was separated and stored in deep freeze at -20°C until
analyzed. Levels of interleukins 25 and 33 were assessed with commercial ELISA kits (CUSABIO) with catalog numbers CSB-E11715h and CSB-E13000h, respectively, by the using quantitative sandwich enzyme immunoassay technique. 

**Statistical analyses**

Statistical Package for the Social Sciences (SPSS: version 20, IBM, Chicago, IL, USA) program was used for data entry and analysis. Nonparametric tests, Mann-Whitney U test and Kruskal-Wallis test, were used for the comparison of the mean and standard error of interleukins with the socio-demographic data in the patient group. Receiver-operating characteristic (ROC) curves were used to evaluate the diagnostic utility of interleukins as estimated by the area under the curve. Furthermore, the sensitivity and specificity in association with positive predictive value (PPV) and negative predictive value (NPV) were calculated for the patients relative to the healthy controls. $P < 0.05$ was considered to be statistically significant.

**RESULTS**

The differences in the concentration of IL-25 and IL-33 according to sociodemographic and clinical variables in the patient group are demonstrated in Table 1. There are significant differences in the level of IL-25 according to type of treatment and higher concentration was found among patients receiving montelukaste ($P < 0.029$), Meanwhile the same significant

| Variable                  | N     | Interleukin-25 | P value | Interleukin-33 | P value |
|---------------------------|-------|----------------|---------|----------------|---------|
|                           |       | Mean ±SE       |         | Mean ±SE       |         |
| Gender                    |       |                |         |                |         |
| Male                      | 56    | 167.50±40.28   | 0.112   | 1.30±0.06      | 0.198   |
| Female                    | 18    | 295.27±69.60   | 0.081   | 1.16±0.05      | 0.383   |
| Eczema                    |       |                |         |                |         |
| Positive                  | 8     | 25.32±15.33    |         | 1.16±0.11      |         |
| Negative                  | 66    | 172.96±38.43   |         | 1.26±0.06      |         |
| Allergic rhinitis         |       |                |         |                |         |
| Positive                  | 40    | 163.23±41.92   | 0.965   | 1.26±0.08      | 0.879   |
| Negative                  | 34    | 149.68±57.88   |         | 1.24±0.07      |         |
| Allergic conjunctivitis   |       |                |         |                |         |
| Positive                  | 20    | 167.06±62.24   | 0.933   | 1.39±0.08      | 0.33    |
| Negative                  | 54    | 149.94±41.89   |         | 1.20±0.06      |         |
| Family history of eczema  |       |                |         |                |         |
| Positive                  | 16    | 49.05±18.55    | 0.247   | 1.31±0.12      | 0.617   |
| Negative                  | 58    | 186.78±43.25   |         | 1.23±0.06      |         |
| Family history of asthma  |       |                |         |                |         |
| Positive                  | 50    | 138.68±40.38   | 0.945   | 1.15±0.06      | 0.013*  |
| Negative                  | 24    | 195.17±66.71   |         | 1.45±0.07      |         |
| Family history of rhinitis|       |                |         |                |         |
| Positive                  | 58    | 188.38±43.30   | 0.293   | 1.26±0.06      | 0.599   |
| Negative                  | 16    | 43.26±13.02    |         | 1.20±0.10      |         |
| Family history of smoking |       |                |         |                |         |
| Positive                  | 34    | 107.61±35.58   | 0.447   | 1.23±0.07      | 0.845   |
| Negative                  | 40    | 198.98±56.22   |         | 1.27±0.08      |         |
| Aggravating by flu        |       |                |         |                |         |
| Positive                  | 50    | 167.24±45.94   | 0.229   | 1.29±0.06      | 0.392   |
| Negative                  | 24    | 135.68±48.87   |         | 1.17±0.10      |         |
| Aggravating by dust       |       |                |         |                |         |
| Positive                  | 40    | 200.38±60.65   | 0.602   | 1.31±0.07      | 0.501   |
| Negative                  | 34    | 105.96±23.17   |         | 1.18±0.08      |         |
| Treatment                 |       |                |         |                |         |
| Montelukaste              | 30    | 217.36±71.45   | 0.029*  | 1.46±0.08      | 0.002** |
| Corticosteroid#           | 4     | 1.77±0.14      |         | 1.50±0.09      |         |
| Mixed                     | 2     | 1.77±0.00      |         | 1.33±0.00      |         |
| No treatment              | 38    | 133.86±35.67   |         | 1.05±0.06      |         |
| Severity                  |       |                |         |                |         |
| Mild                      | 62    | 166.07±40.88   | 0.196   | 1.27±0.06      | 0.217   |
| Moderate                  | 12    | 110.12±34.06   |         | 1.41±0.13      |         |

significant p-value, ** highly significant p-value, # inhaled corticosteroid
SE=standered error of the mean, P=probability value
difference was reported in the level of IL-33 according to type of treatment, highest readings of IL-33 was showed among patients with corticosteroids ($P < 0.002$). The variable of asthma family history also showed a notable statistical significance ($P < 0.013$) with regard to the concentration of IL-33.

ROC curve analysis of IL-25 and IL-33 showed cutoff values of $\geq 1.6350$ and $\geq 1.3100$, respectively. The sensitivity and specificity of IL-25 and IL-33 in association with PPVs and NPVs are listed in Table-2.

**DISCUSSION**

The present study revealed that IL-25 was more sensitive than IL-33 in the diagnosis of asthma. One study showed increased IL-25 expression in the mucosa of patients with bronchial asthma and in the skin of patients with eczema\textsuperscript{13}.

Another study found that cigarette smoke was responsible for stimulating the airway epithelial cells for the production of thymic stromal lymphopoetin (TSLP) and, consequently, the dendritic cells that activate the polarization of Th2\textsuperscript{14}. The epithelial cells in the airway are the major exporters of IL-25, IL-33 and TSLP, which act upstream of the cytokines of Th2 that include interleukins 4, 5 and 13 and thus activate the immune response type of Th2\textsuperscript{15}. Therefore, cigarette smoke is considered to be a powerful and prime factor for airway inflammation, brought about by increased the TSLP production\textsuperscript{14}. The inflammatory process and bronchoconstriction can be inhibited using montelukast\textsuperscript{16}. Other studies also found that montelukast add-on therapy to the inhaled corticosteroids enabled effective control of persistent asthma\textsuperscript{17,18}.

A study reported the association between elevated of IL-25 expression due to allergen

| Variable   | Cutoff value | Sensitivity | Specificity | PPV    | NPV    |
|------------|--------------|-------------|-------------|--------|--------|
| IL-25 pg/ml| $\geq 1.6350$| 97.30%      | 88.00%      | 88.90% | 97.10% |
| IL-33 pg/ml| $\geq 1.3100$| 51.40%      | 66.00%      | 60.30% | 58.10% |

**Fig. 1.** ROC curves for optimal cutoff points at which IL-25 and IL-33 discriminate asthmatic patients from non asthmatic controls.
exposure in patients with asthma and the disease severity. According to the ROC analysis, this study revealed a low sensitivity and specificity of IL-25 in the differentiation between patients with and without asthma. These results are inconsistent with that of the present study that revealed a high diagnostic sensitivity of IL-25 and also a non-significant relationship between IL-25 serum level and severity of disease. Another study reported high sensitivity (93.5%) and specificity (60.0 %) of IL-25 in the diagnosis of airway hyperresponsiveness and this result is compatible with the results of our study.

To conclude, this study highlights the need for more cellular and molecular investigations and extensive studies to recognize the role of interleukins 25 and 33 in pediatric asthma, which could improve the diagnostic and therapeutic strategies for this disease.

CONCLUSION
This study suggests that IL-25 shows higher diagnostic sensitivity and specificity than IL-33; therefore, IL-25 represents a significant marker for the diagnosis of pediatric asthma.

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DATA AVAILABILITY
All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT
Not applicable.

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