Original article

Elevated serum KL-6 in pediatric asthma exacerbation: a proof of alveolar injury

Background: *Asthma is one of the most popular chronic diseases in children. It is defined as a complicated inflammatory disorder in which the patient suffers from chronic and persistent inflammation of the airways. The sialylated glycoprotein Krebs von den Lungen-6 (KL-6), one of the lung epithelium-specific proteins, has been recognized as a significant biomarker which directly associates with interstitial lung disease (ILD) pathogenesis, indicating the extent of damage and regeneration of type II pneumocytes.* ***Objective:*** *the aim of this study is to investigate the degree of alveolar damage in asthmatic children with acute exacerbation as reflected by serum KL-6 levels.* ***Methods:*** *This cross-sectional controlled study included 50 patients with acute asthma exacerbation diagnosed as per the GINA guidelines definition and 50 age- and sex-matched healthy children as controls. Spirometry was done for all participants. Serum KL-6 level was estimated by Enzyme Linked Immunosorbent Assay (ELISA), and total serum IgE level was measured via the electrochemiluminescence technology.* ***Results:*** *The asthma patients included 35 (70%) males and 15 (30%) females with mean age of 10.76 ±1.9 years. Forty-seven patients (94%) had a positive family history of bronchial asthma and 32 (64%) had other atopic manifestations The mean serum KL-6 level in patients was more than double the mean level of the control group (115.79 vs 55.64). No significant relation was observed between KL-6 serum level and age, family history of asthma, seasonal variation, or atopic manifestation among the cases. Serum total IgE levels were significantly higher in cases compared to controls (P<0.05).* ***Conclusion:*** *Serum KL-6 levels in pediatric asthma patients may be a useful diagnostic tool for detecting and monitoring the severity of airway inflammation. The use of serum KL-6 alone may help to differentiate between asthmatic patients in exacerbation and healthy controls.*

Keywords: pediatric asthma, KL-6, alveolar injury.

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INTRODUCTION

One of the most popular chronic diseases, particularly in children, is asthma. This disease is defined to be a complicated inflammatory disorder in which the patient suffers from chronic and persistent inflammation of the airways.[1,2] In 2014, the number of patients with this disease reached 300 million people worldwide. Asthma is more frequent in the developing countries than the developed ones.[3] The frequency of this disease is increasing sharply in spite of the significant progress in pharmacological therapy. Several previous studies in literature investigated the mechanisms underlying asthma pathogenesis, recognizing the epithelial cell injury to be a major cause of asthma.[4]

The sialylated glycoprotein Krebs von den Lungen-6 (KL-6), one of the lung epithelium-specific proteins, has been studied and recognized as a significant biomarker that directly associates with the pathogenesis of interstitial lung disease (ILD). It is indicative of the extent of damage and regeneration of type II pneumocytes.[5] In addition, high serum KL-6 levels have been recently presented by many studies to be a severity indicator of systemic sclerosis disease which is measured through pulmonary function test (PFT) as well as a predictor of its early progression.[6,7] Kl-6 splits off the S-S bond close to the epithelial membrane surface. Then, it becomes distributed in pulmonary epithelial lining fluid.[8] The expression of this glycoprotein always takes place on alveolar type 2 cells in the lung. The rate of KL-6 expression is higher in the proliferating, regenerating, or injured type 2 cells compared to the normal ones.[9]

Some studies reported the correlation between KL-6 plasma levels and the indices of alveolar capillary permeability; this suggests an association between serum KL-6 and alveolar epithelial barrier dysfunction.[10,11] Moreover, the circulating levels of this protein have been recognized and used as a

diagnostic as well as prognostic tool in a variety of interstitial pneumonitis, sarcoidosis, and alveolar proteinosis. However, KL-6 was not studied extensively as a biomarker for pediatric asthma.[12]

The aim of this work is to investigate the degree of alveolar damage in asthmatic children with acute exacerbation as reflected by serum KL-6 expression.

METHODS

This controlled cross sectional study included 50 patients with acute asthma exacerbation who were diagnosed as per the GINA guidelines' definition [13] and 50 age- and sex-matched healthy children who have neither history nor symptoms of bronchial asthma, pulmonary diseases, other allergies or atopic dermatitis nor first-degree relatives suffering from bronchial asthma or atopic dermatitis as a control group.

The aim and nature of the study were explained to all the participants' parents/care givers. and an informed consent was obtained from them before children enrollment. The ethical committee of the Pediatrics' Department, Faculty of Medicine, Cairo University, approved the work. This study conforms to the provisions of the Declaration of Helsinki in 1964 and its later amendments or comparable ethical standards.

Exclusion criteria of this study were acute respiratory distress that necessitates prompt intervention and latent pulmonary diseases (including but not limited to cystic fibrosis, congenital heart disease, congenital respiratory disease, and thoracic deformity). In addition, children of age more than 18 years or less than 6 years were excluded (as Spirometry cannot be performed for the children less than 6 years) and to avoid cases of non-asthmatic early life wheeze

Thorough history taking and physical examination were conducted for all patients upon study inclusion, including respiratory symptoms, other atopic manifestations, and disease severity.

Spirometry (Master Scope-PC) was done for all participants. Measurements included forced expiratory volume in one second (FEV1), forced vital capacity (FVC), FEV1/FVC%, and FEF25-75%. Readings were correlated with age, sex, and the standing height of children. Results were expressed as percentages of predicted values for sex and height. Prior to each testing session, calibration was done on site according to the manufacturer's instructions. Three technically accepted maneuvers at least were obtained with less than 0.15 L variability for FEV1 and FVC between the highest and second highest result.[14,15]

Measurement of serum Kerbs von Lungren 6 antigen: Serum Kerbs von Lungren 6 antigen (KL-6) levels were estimated by Enzyme Linked Immunosorbent Assay (ELISA) kit (Bioassay technology laboratory Cat. No E1980Hu) that uses a double-antibody sandwich to assay the KL-6 level in blood samples. The results were calculated by the straight line regression equation of the standard curve of the standard density and the optical density (OD) values of the provided standard and its serial dilutions. With the sample OD value in the equation, the tested sample serum concentration of KL-6 was calculated.

Total serum IgE level was measured via the electrochemiluminescence technology using the cobase e 411 immunoassay analyzer (Roche, USA) according to the manufacturer's instructions.

Statistical analysis

Data were coded and entered using the statistical package for Social Sciences (SPSS) version 25 (IBM Corp., Armonk, NY, USA). Data were summarized using mean, standard deviation, median, minimum and maximum in quantitative data. Comparisons between quantitative variables were done using the non-parametric Mann-Whitney test.[16] P-values less than 0.05 were considered as statistically significant.

RESULTS

Results

A total number of 100 participants were recruited in this study. The case group included 35 (70%) males and 15 (30%) females with mean age of 10.76 ±1.9 years. Forty-seven patients (94%) had a positive family history of bronchial asthma and 32 patients (64%) had atopic manifestations such as conjunctivitis, allergic rhinitis, or skin allergy. The patients' demographic data are listed in Table (1).

Table (2) demonstrates the mean spirometric parameters (the percentage predicted) in studied participants which were statistically lower in patients than in controls. These statistical data were highly significant. Serum KL-6 levels were significantly higher in cases than in controls. The mean serum KL-6 level in cases was more than double the mean level in controls (115.79 vs 55.64) table (4), figure (1).

No correlation was observed between KL-6 serum level and age, family history of asthma, seasonal variation, or atopic manifestation among the cases. Serum total IgE levels were significantly

higher in cases compared to controls (P<0.05). The cases studied during acute exacerbation had asthma for a mean duration of 4.21 years. No significant correlation was found between the duration of asthma and KL-6 level.

Table 1. Demographic data of the studied sample.

	Cases	**Controls**
Age (years)	10.76+-1.9	10.12+-2.3
Sex (male %)	35(70%)	37 (74%)
Seasonal manifestation	30 (78%)	-
Atopic manifestations	32 (64%)	-
Urban residence	41 (82%)	40 (80%)
Family history of asthma	47 (94%)	1 (2%)
Family history of atopy	38 (76%)	2 (4%)
Mean duration of asthma (years)	4.21	-
Total	**50**	**50**

Table 2. Mean total IgE level and PFT in patients compared to controls.

	Cases	**Controls**	**P value**
IgE (IU/ml) mean+-SD	745.12+-137	44.64+-31	P<0.05
FEV1 (% predicted)	70.51+-11.3	98.87+-3.21	P<0.001
FVC (% predicted)	87.40+-4.95	100.5+-3.75	P<0.001
FEV1/FVC (% predicted)	73.59+-11.28	96.12+-2.54	P<0.001
FEF 25-75% (% predicted)	63.12+-9.8	95+-4.12	P<0.001
Total	**50**	**50**	**-**

FEF: Forced Expiratory Flow, *FEV1*: Forced Expiratory Volume in one second, *FVC*: Forced Vital capacity, *IgE:* Immunoglobulin E.

Table 3. Serum KL-6 level in asthmatic patients with acute exacerbation compared to normal controls.

	Cases					**Control**					**P value**
	Mean	**SD**	**Median**	**Minimum**	**Maximum**	**Mean**	**SD**	**Median**	**Minimum**	**Maximum**	
Kl-6 (U/ml)	115.79	86.10	84.10	33.00	320.00	55.64	25.66	57.30	7.09	94.50	<0.001

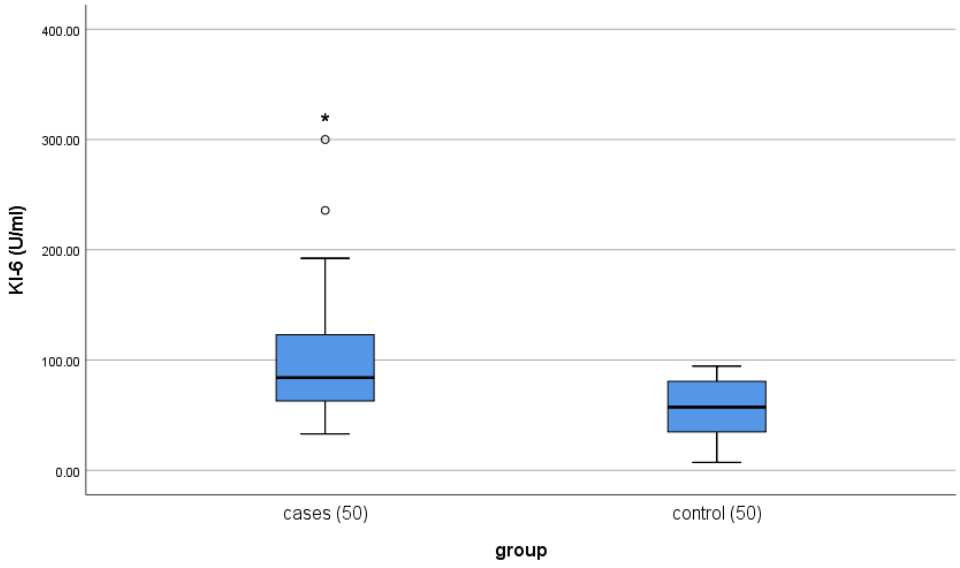

Figure 1. Comparison between serum KL-6 level in asthmatic patients during acute exacerbation and normal controls. P value <0.001.

DISCUSSION

Previously, it was believed that the primary cellular source of KL-6 is type II pneumocytes [11] and that KL-6 concentration is extremely high in epithelial lining fluid.[17,18] Therefore, the increase in circulating KL-6 levels in interstitial pneumonitis has been suggested to be attributed to an increase in KL-6 production by alveolar type II pneumocytes regeneration and/or an enhanced permeability taking place as a result of the air–blood barrier destruction in the affected lungs.[19] Recent studies have presented the KL-6 as a potent pro-proliferative and antiapoptotic agent upon lung fibroblasts.[9,10]

Previous reports on adult asthmatic patients showed no significant increase in serum KL-6 levels.[18,20] However, our study on children revealed a statistically significant elevation of KL-6 serum levels in acute asthma flare cases compared to controls. This may indicate that serum KL-6 is a sensitive marker of lung damage in children.

The elevation of KL-6 serum levels takes place in various interstitial lung diseases; these elevated levels are associated with alveolar epithelial cell damage. There is an association between serum KL-6 concentrations and alveolar-epithelial barrier dysfunction as they were reported to have a correlation with the indices of alveolar-capillary permeability.[21] In the current study, alveolar injury was observed in asthmatic patients with acute exacerbation; this was indicated by higher KL-6 levels compared to normal controls. The increased KL-6 levels in some of our patients, not all of them, indicate the possibility of mild alveolar epithelial damage.

Bronchiolar epithelial cells and bronchial serous gland cells could be other sources of KL-6 in the bronchial lumen. However, the alveolar-capillary barrier has to be highly permeable to allow for increased serum KL-6 levels in asthma.[22] Therefore, a degree of alveolar damage must be present in the cases with elevated serum concentrations of KL-6.[9]

Thus, our results positively suggest that asthmatic children suffer from alveolar damage. Further studies to prove the correlation between KL-6 and various degrees of asthma severity could make KL-6 a reliable simple non-invasive tool for identification of patients in need for hospital admission. In the current work, we studied the degree of alveolar damage associated with the air way obstruction in pediatric asthmatic patients with acute exacerbation by measuring the serum level of KL-6 as a trial for saving the financial and medical burden by differentiating between patients who need intensive medical care by hospital admission and those who can be managed properly in the outpatient clinic.

To our best knowledge, serum KL-6 level in asthmatic children has been investigated in only one previous study.[23] If these findings are extrapolated on younger patients where PFT can be difficult to perform or not available in all health care facilities, KL-6 serum level would be a simple and practical measure to identify asthma severity.

CONCLUSION

Measuring plasma KL-6 levels in pediatric asthma patients may be a useful diagnostic tool for detecting and monitoring the severity of airway inflammation. The use of serum KL-6 alone enabled us to differentiate between asthmatic patients in exacerbation and healthy controls. In this context, screening of asthmatic children for KL-6 may be a practical tool to identify patients at risk for acute exacerbation. The findings are indeed limited by the sample size.

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