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Association of Serum KL-6 Levels with Interstitial Lung Disease in Patients with Systemic Sclerosis Disease

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Abstract

Background: Interstitial lung disease (ILD) affects a large proportion of people with systemic sclerosis (SSc).

Objectives: The aim of this study was to identify the role of KL-6in patients with SSc. For this objective, we studied the association between the KL-6 level, computed tomography (CT) score, and pulmonary function tests in SSc patients.

Methods. 32 SSc patients underwent chest HRCT scans, pulmonary function tests and serum KL-6measurement.We usedWarrick scoresto distinguish the severity and extent of ILD at HRCT.KL-6 were sampled and measured, by enzyme linked immunosorbent assay (ELISA).

Results:A positive correlation was found between serum KL-6 levels and the Warrick score (r 0.871, P < 0.001). KL-6 levels inversely correlated to with forced vital capacity(FVC%) (r-0.72, P < 0.001) , forced expiratory volume in 1second(FEV1%) (r -0.571, P < 0.001) and diffusion capacity for carbon monoxide(DLCO%) (r -0.543, P < 0.001).

Conclusion:KL-6 levels correlate with findings seen in HRCTs and PFTs, indicating that they serve to assess the severity of SSc-ILD.

Keywords: Systemic sclerosis (SSc); interstitial lung disease (ILD); Krebs von den Lungen 6 (KL-6).

Introduction:

Systemic sclerosis (SSc) is a generalized disorder with abnormalities of the microvasculature and the connective tissues, characterized by scarring (fibrosis) and vascular obliteration in the skin, gastrointestinal tract, lungs, heart and kidney. Its pathogenesis is complex and still largely unknown [(1)]. Interstitial lung disease occurs in 50-90% of patients with SSc and constitutes the major cause of death.[(2, 3)]

Monitoring pulmonary symptoms is crucial. Imaging techniques and Pulmonary function tests (PFTs) are the mainstay of the diagnosis of pulmonary involvement.[4]
In the detection and characterization of pulmonary involvement, high-resolution CT (HRCT) is more accurate than chest radiography and is now considered the gold standard for ILD evaluation.[5]However, PFT needs the cooperation of patients, and the substantial radiation exposure of HRCT makes it inconvenient to be frequently pursued. These necessitate a convenient and noninvasive tool to determine existence and progression of ILD earlier in clinical practice.[6]

KL-6, a glycoprotein antigen first described by Kohno, et al[7], is expressed mainly on type II pneumocytes in alveoli and respiratory bronchiolar epithelial cells.[8]and its serum level probably indicates the damage in alveolar type II cells.[9]Therefore, elevated serum KL-6 levels may indicate the onset of ILD in patients with SSc.
The aim of this study was to identify the role of B-lines in patients with SSc.

Patients &Methods:

32 systemic sclerosis patients attending the inpatient and outpatient clinics of Rheumatology & Rehabilitation Department, Egypetian University hospitals, were evaluated prospectively during regular office visits during the period from June 2020 to December 2020. The inclusion criteria were: (I) diagnosis of SSc, according to the American College of Rheumatology/European League Against Rheumatism classification criteria[10] and (II) age >18 years.All patients were subjected tofull medical histories, physical examinations, and laboratory tests were conducted for all patients.
Interstitial lung disease (ILD) was defined as bibasilar interstitial fibrosis on chest radiographs, and ground-glass opacities, reticular opacities, or honeycombing on HRCT. Imaging findings evaluated by a radiologist defined the presence and pattern of ILD.[11] Pulmonary function tests (PFT), including forved vital capacity (FVC), forced expiratory volume in1 second (FEV_1) and diffusion capacity for carbon monoxide (DLCO), were conducted to evaluate the severity of ILD.Activity of ILD was assessed by changes in serial examinations by HRCT and PFT.[12]We used a HRCT scoring based on theWarrick score toidentifiey and scoredpulmonary involvement.[13]
The work has been carried out in accordance World Medical Association (Declaration of Helsinki) for studies involving humans before prospective collection of patient's data and after informed consent was obtained from patients.
The study excluded patients with a history of asthma, chronic obstructive pulmonary disease, lung neoplasm, occupational lung disease, heart failure, renal failure, Chest infection (pneumonia) and other rheumatic diseases.

Measurement of KL-6 Patients' serum was stored at -80° before analysis. ELISA Kits were used to determine KL-6 (human KL-6, SUNRED, Shangahi, China) according to the manufacturer's instruction.

Statistical Analysis:

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20 (IBM SPSS statistics for windows version 20.0. Armonk, NY: IBM Corp.).

Results:

Thirty two patients with SSc (28 women and 4 male; median age, 36.5 years [range 22 - 64 years]) satisfied the inclusion criteria. The median duration of disease was 3 years. The diffuse skin subset was present in 75.06% .

The majority of SSc patients had pulmonary, GIT and cardiac manifestations with percent (84.3%, 47.2%, 41.7%)respectively

Our result revealed non-significant correlation between KL-6 and either age, diesease duration or laboratory parameters(CBC, ESR, CRP).**Table (1).**

The mean of serum kl-6 level are 606.8 and the mean HRCT score is 4.34, PFTs mean values are: DLCO 77%, FVC 70% and FEV1 67%.

Table (1) Correlation between ultrasound KL-6 and the studied parameters:

	R	P
Age	**0.186**	**0.307**
Disease duration	**0.174**	**0.302**
TLC	**-0.125**	**0.463**
Hb	**-0.289**	**0.173**
Plt count	**0.198**	**0.251**
ESR	**0.235**	**0.089**
CRP	**0.145**	**0.546**

r Spearman rank correlation coefficient *p<0.05 is statistically significant **p≤0.001 highly statistic significant.

Abbreviation:

CBC:*complete blood count,* ***TLC:*** *Total Leukocytes Count,* ***Hb****:Hemoglobin,****Plt****: Platelet,* ***ESR****:Erythrocyte sedimentation rate,****CRP****: C-reactive protein.*

A positive correlation was found between serum KL-6 levels and the Warrick score (r 0.871, P < 0.001).**Fig. (1)**

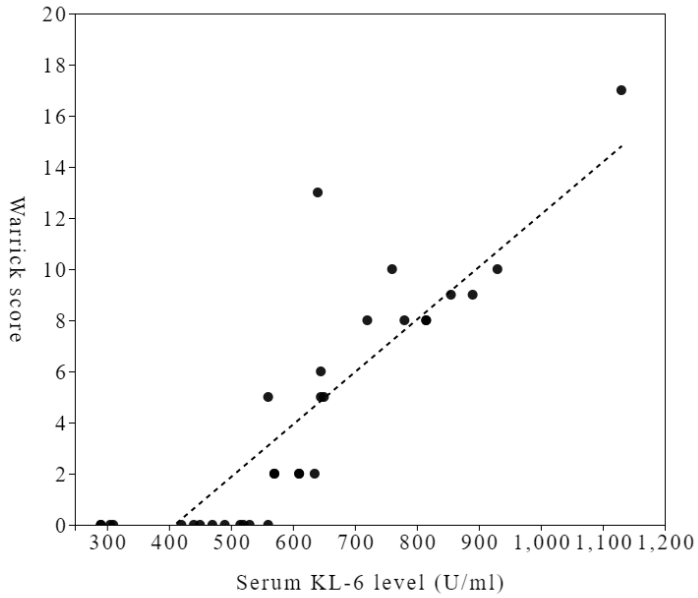
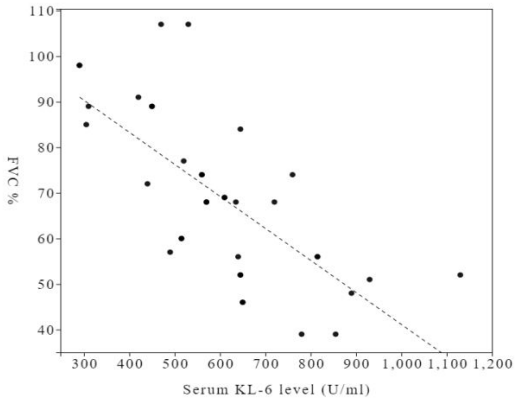
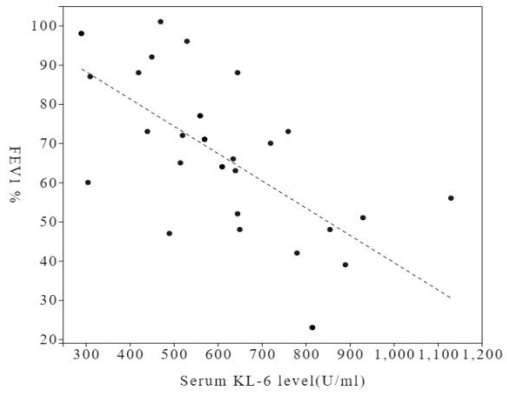


Fig. (1) Scatter dot graph showing significant positive correlationcorrelation between Serum Krebs von den Lungen-6 (KL-6) and Warrick score

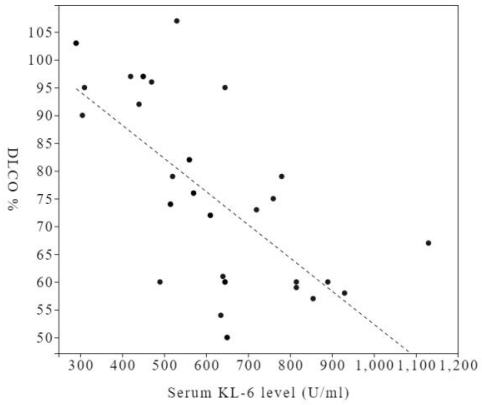
A negative correlation between KL-6 levels with forced vital capacity FVC (r-0.72, P < 0.001) , forced expiratory volume in 1 s FEV1(r -0.571, P < 0.001) and diffusion capacity for carbon monoxide DLCO (r -0.543, P < 0.001).**Fig. (2)**



(A)



(B)



(C)

Fig. (2) Scatter dot graph showing significant negative correlation between Serum Krebs von den Lungen-6 (KL-6) with: (A) FVC%, **(B)** FEV1%,(**C**)DLCO%.

Elevated serum KL-6 levels in patients with respiratory symptoms among the studied patients(r 0.609, P < 0.001)..

Our resultshowed that the best cutoff of KL-6 in prediction of mild disease is ≥525 with area under curve 0.881 with sensitivity 91.67%, specificity 95.65%, positive predictive value (PPV) 95.6% and negative predictive value (NPV) 84.6% with accuracy 91.67% (p<0.001)**Fig. (3)**

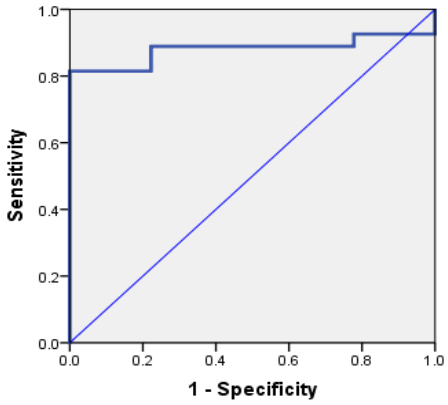


Fig. (3) ROC curve showing performance of KL-6 in prediction of mild disease among the studied patients.

Also, our results showing the best cutoff of KL-6 in prediction of moderate to severe disease is ≥685 with area under curve 0.964 with sensitivity 90.0%, specificity 100.0%, positive predictive value (PPV) 100.0% and negative predictive value (NPV) 93.3% with accuracy 95.83% ($p<0.001$). **Fig. (4)**

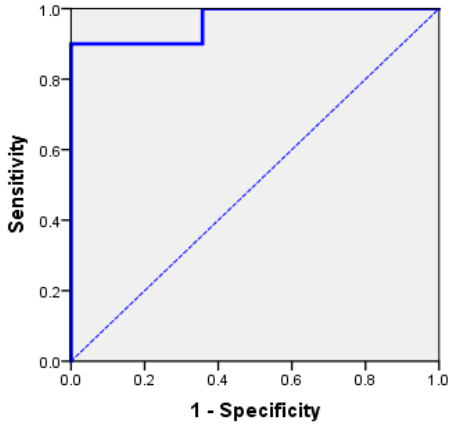


Fig. (4) ROC curve showing performance of KL-6 in prediction of moderate to severe disease .

Discussion:

KL-6 is a mucin-like, high-molecular weight glycoprotein expressed on the surface membrane of alveolar epithelial cells II and bronchiolar epithelial cells, and increases following cellular injury and/or regeneration.[14] Due to its chemotactic, pro-fibrotic and anti-apoptotic roles on lung fibroblasts, serum KL-6 level has been studied in ILD of various etiologies and revealed to be an important serum marker for ILD whatever its cause.[15]

Based on our findings,we observed serum KL-6 concentrations were elevated in patients with SSc-ILD.Increased KL-6 levels were associated with the presence of

pulmonary fibrosismay serve as an important serologic marker for detectinginterstitial lung disease in systemic sclerosispatients (SSc-ILD).Regarding laboratory data, we found that there was non-significant correlation between KL-6and laboratory parameters. Our results came in agreement with **Kumánovicset al.**[16] who reported that there was no correlation was found between KL-6 and laboratory parameters.

In this study showed that there was significant positive correlation between KL-6 and respiratory symptoms ($p < 0.001$).

Regarding KL-6level, we found that there was highly negative significant correlation with all of FVC%, FEV1%, and DLCO%. This is consistent with the **Lee et al.**[17] reported that KL-6 levels also had negative correlation with both FVC% ($p < 0.001$) and DLCO% ($p < 0.001$) .

Furthermore, KL-6 level are correlated with HRCT scores and PFTs **.**we found that there was highly negative significant correlation with FVC%, FEV1%, and DLCO%. This is consistent with the . **Bonellaet al.**[18] reported that KL-6 levels also had negative correlation with both FVC% and DLCO%.We also found that significant positive correlation between KL-6and Warrick score. This is agreement with the**Wang et al.**[19] reported that a significant correlation was found between the serum Warrick score and serum KL-6 levels ($r = 0.45$, $P < 0.01$).Also **Fotoh et al.**[20](2021)in a rheumatoid arthritis patients with ILD demonstrate that there was a strong positive association between the KL-6 serum and the Warrick total score ($r = 0.93$, $p > 0.001$).

showed that the best cutoff of KL-6 in prediction of mild disease was $\geq$ 525 U/mL with area under curve 1 with sensitivity 100%, specificity 85.7%, positive predictive value (PPV) 84.6% and negative predictive value (NPV) 100% with accuracy 92% ($p<0.001$).And the best cutoff of KL-6 in prediction of moderate to severe disease is $\geq$685.Some of the previous studies showed results that are close to ourfindings. **Hamai et al.** [21]showed the KL-6 cut-off values for diagnosis differ among the different types of ILDs, a cut-off value of 500 U/mL is commonly used as the clinical diagnostic standard . And,**Nakashita et al.**[22] reported that normal range of serum KL-6 is less than 500 U/ml and serum level of more than 1,000 U/ml is a predictor of poor prognosis.

Even now HRCT is still considered the "gold standard" for diagnosing and monitoring ILD morphological changes. But even so, the radiological expousre and large, non-portable equipment limit HRCT clinical application to closely monitor the lung[19].

In this clinical context, KL-6useful to evaluate the activity and severity of pulmonary fibrosis.

Conclusion:

The present study confirms that KL-6 is a serological marker which is associated with the presence of ILD, and well correlated with the HRCT score and PFTs, making it not only a marker of presence but also severity.

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