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Role of circulating serum YKL-40 level in assessment of severity in hypersensitivity pneumonitis patients

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ABSTRACT

BACKGROUND: Hypersensitivity pneumonitis (HP) is a diffuse parenchymal lung disease characterized by immune-mediated inflammation of lung parenchyma triggered by exposure to a wide variety of inhaled antigens. YKL-40 plays a fundamental role in protecting against pathogens, antigen-induced and oxidant-induced injury responses, inflammation, and tissue repair and remodeling. This is achieved by regulating many biological processes.**OBJECTIVE:** The aim of this study was to evaluate the YKL-40 serum level as a possible biomarker for disease activity in hypersensitivity pneumonitis. **METHODS**: The study included 25 patients who were diagnosed primarily as hypersensitivity pneumonitis in Chest Department, Faculty of Medicine, Cairo University from April 2021 to August 2021. Moreover, 25 healthy age- and sex-matched controls were enrolled.**RESULTS:** YKL-40 was significantly higher among HP group, in fibrotic more that non-fibrotic subtype. YKL-40 had a negative correlation with oxygen saturation (SaO2) on room air, minimum SO2 in 6MWT and distance in 6MWT in HP patients. In addition, YKL-40 has a positive correlation with dyspnea score in HP patients. The fibrotic subgroup, compared to non-fibrotic subgroup had significantly higher serum YKL-40 level. A cut-off value of 629.4 pg/ml for serum YKL-40 level can distinguish between the HP patients and normal individuals (sensitivity =96%, and specificity =96%). Moreover, a cut-off value of 1181.5 pg/ml for serum YKL-40 level can distinguish between the fibrotic and non-fibrotic HP sub-types (sensitivity =85.7%, and specificity =81.8%).**CONCLUSION:** YKL-40 is a useful prognostic marker for hypersensitivity pneumonitis and can help in discriminating between fibrotic and non-fibrotic subtypes.

Introduction

Interstitial lung diseases (ILDs) include a wide variety of diffuse pulmonary disorders, characterized by a spectrum of inflammatory and fibrotic changes of alveolar walls and eventually the distal bronchiolar airspaces as well as the lung interstitium. ILDs may occur in isolation or associated with systemic

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diseases, including idiopathic interstitial pneumonias, collagen vascular disease-associated interstitial pneumonia, hypersensitivity pneumonitis, radiation pneumonitis, drug-induced ILDs, acute respiratory distress syndrome, and sarcoidosis [1]. Hypersensitivity pneumonitis is a form of diffuse parenchymal lung disease that is increasingly encountered [2]. It is an immunologically mediated inflammation of lung parenchyma that occurs in susceptible individuals that is triggered by a variety of antigens [3]. High-resolution computed tomography (HRCT), bronchoscopy and surgical lung biopsy are crucial steps in making a definite diagnosis of different ILDs [4]. Moreover, serial lung function tests are generally helpful in monitoring disease activity and/or predicting the prognosis in ILDs patients [5]. However, these examinations require specialized medical facilities and often cause considerable discomfort to patients. Identification of serum biomarkers for ILDs would greatly improve our current diagnostic tools. Potential advantages of serum biomarkers include being easy to perform, reproducible, and less expensive and less invasive [6].YKL-40 (chitinase-3-like-protein-1) is one of the important biomarkers for ILD's. It is a member of the mammalian chitinase-like protein family, which is coded by a gene located on chromosome 1q32.1. It is produced by a wide variety of cells, including airway epithelial cells, macrophages, neutrophils, monocytes, vascular smooth muscle cells. YKL-40 has been found to be involved in many normal and pathological conditions, including cell proliferation and survival, migration, recombination, and tissue remodeling. Serum YKL-40 level is increased in patients with ILD and is closely related to the deterioration of lung function and prognosis of such category of patients [7]. The purpose of the present study was to determine the role of circulating serum YKL-40 as a prognostic marker and in assessment of severity in hypersensitivity pneumonitis patients.

SUBJECTS & METHODS

Study plan and population: This is a retrospective cohort case-control study conducted in Chest Department (Kasr El-Aini Hospital, Faculty of Medicine, Cairo University) in collaboration with Chemical Pathology Department (Faculty of Medicine, Cairo University) during the period between April 2021 and August 2021. The study population was divided into two groups:

- Group I (HP group): 25 hypersensitivity pneumonitis patients who were recruited from the interstitial lung diseases clinic, chest department, faculty of medicine, Cairo university. - Group II (Controls): 25 healthy age- and sex-matched control subjects.The research ethical committee of the Faculty of Medicine, Cairo University, approved the study. Informed consent was taken from all subjects of this study. Inclusion criteria: Patients who were diagnosed primarily as HP based on typical clinical presentations, HRCT, spirometry, six-minute walk test (6MWT). Exclusion criteria: • Patients with other forms of interstitial lung diseases. • Patients with hemodynamic instability and/or cardiac failure. • Patients with any history of malignancy, renal or hepatic failure.

Methods:

All patients were subjected for the following

1-Thorough history taking with special concern on history of breeding birds, patients'

symptoms especially dyspnea & its duration and grading using the modified medical Research Council (mMRC) scale. The mMRC scale is a self-rating tool to measure the degree of disability that breathlessness poses on daily activities on a scale from 0 to 4 [8, 9]. 2-Full clinical examination with special emphasis on auscultation of the chest that may show fine crackles throughout the lungs. Oxygen saturation was measured using pulse oximeter.

3-YKL-40 serum level measurement by Enzyme-Linked Immunosorbent Assay (ELISA): Three to five milliliters whole blood were collected in a sterile empty vacutainer which were centrifuged to collect serum. Sera of patients were kept frozen at -20ºC till time of YKL-40 measurement using ELISA. The used kit was an Enzyme-Linked Immunosorbent Assay (ELISA) based on that the plate had been pre-coated with human YKL-40 antibody (Thermo Fisher Scientific 168 Third Avenue. Waltham, MA USA 02451). 4-HRCT chest scan by Siemens 16-channel MDCT: Scans were obtained at end of inspiration and in supine position from the lung apices to the bases. Contrast medium was not injected in any patient. Data were reconstructed with 1.0mm section thickness and at 10-mm intervals for transverse images. The HRCT patterns typical for nonfibrotic HP include: centrilobular diffuse micronodular pattern, ground-glass opacification and mosaic attenuation (reflecting coexistent small airways disease) mainly in upper and middle lung lobes [10]. The HRCT findings specifically in fibrotic HP are those of fibrosis: reticulation, architectural distortion and traction bronchiectasis with or without honeycomb changes. The fibrosis may be patchy, peri-broncho vascular, or subpleural (mimicking usual interstitial pneumonia), and may be observed in any zonal distribution [11,12]. 5-Pulmonary function test was done in the form of spirometry by Master Screen PFT 2012, CareFusion 234 GmbH, Germany (V-781267-057 version 03.00). 6-Six-minute walk test (6MWT): The 6MWT is a marker of exercise tolerance with assessment of desaturation difference between the baseline SO2 and post-test SO2. The 6MWT was performed indoors, along a flat, straight, enclosed corridor with a hard surface (according to ATS guidelines, 2002) [13].

Statistical Analysis:

Descriptive statistics are presented in the form of mean and standard deviation for normally distributed numerical variables, while median and interquartile range were used for the non-normally distributed numerical variables. Independent samples t-test and Mann-Whitney tests were used to compare the different variables in the HP and control groups and in the fibrotic and non-fibrotic groups. The correlation between YKL and other parameters was tested using Pearson's correlation. ROC curve was used to get the cut-off points for the YKL level to distinguish between normal and HP patients and between the fibrotic and non-fibrotic HP subgroups. IBM® SPSS version 26 for windows software was used for the analysis, and a p-value <0.05 is considered statistically significant.

Results

A total of 50 participants were included in this study (age range 20-65 years, mean±SD 45.9±11 years). No statistically significant difference in age was found between HP group and controls (Table 1). All individuals included in this study are females. In the HP

group, 18 patients (72%) showed a history of bird exposure while no individuals from the control group showed a history of bird exposure. HP group had statistically significant lower values compared to controls as regards FVC% (54.7±19.3% versus 85.6±3.7% respectively, p<0.001), FEV1% (57.5±19.7% versus 85.5±4.7% respectively, p<0.001), and distance in 6MWT (209.6±70.4 m versus 315.8±29.9 m respectively, p<0.001). On the other hand, FEV1/FVC showed non-significant difference between the two groups. (Table 1). The HP group, compared to controls had significantly higher dyspnea score (median 3, IQR 1 versus median 2, IQR 2 respectively, p<0.001) as well as higher serum YKL-40 level (1398±910 pg/ml versus 304.9±180.4 pg/ml respectively, p<0.001). On the other hand, SO2 on room air and minimum SO2 in 6MWT was significantly lower in HP group compared to controls (92±7.5% and 82±17.5% versus 97±2% and 97±2% respectively, with p<0.001 in both). (Table 1)

Serum YKL-40 level and age had a statistically significant positive correlation in HP group (r=0.545, p=0.005) and negative correlation in controls (r=-0.422, p=0.035). Moreover, there was a statistically negative correlation between YKL-40 and both SO2 on room air and minimum SO2 in 6MWT in the HP group (r=-0.431, p=0.031 and r=0.484, p=0.014 respectively), while such correlations were lacking in the control group. (Table 2). In addition, YKL-40 and distance in 6MWT had a statistically significant negative correlation in the HP group (r=-0.611, p=0.001) while the correlation of YKL-40 with dyspnea score was a statistically significant positive correlation (r=0.478, p=0.016). Again, such correlations were lacking in the control group. (Table 2)

The HP group was further sub-divided into “fibrotic” (n=14) and “non-fibrotic” (n=11) subgroups. No statistically significant difference in age was found between the two subgroups. Fibrotic subgroup had statistically significant lower values compared to non-fibrotic subgroup as regards FVC% (43±10% versus 70±18% respectively, p=0.001), FEV1% (48±13% versus 70±20% respectively, p=0.003), and distance in 6MWT (157.8±37 m versus 275.4±40 m respectively, p<0.001). On the other hand, FEV1/FVC showed non-significant difference between the two subgroups. (Table 3). The fibrotic subgroup, compared to non-fibrotic subgroup had significantly higher disease duration (3±1 years versus 2±1 years, p=0.047), higher dyspnea score (median 4, IQR 1 versus median 3, IQR 1 respectively, p=0.001) as well as higher serum YKL-40 level (1599±407.3 pg/ml versus 796.7±356.3 pg/ml respectively, p=0.007). On the other hand, SO2 on room air and minimum SO2 in 6MWT was significantly lower in fibrotic subgroup compared to non-fibrotic subgroup (90±8.25% and 78±11.7% versus 96±2% and 93±6% respectively, with p<0.001 in both). (Table 3)

In the non-fibrotic subgroup, YKL-40 had statistically significant negative correlation with SO2 on room air (r=-0.773, p=0.005), FEV1/FVC (r=-0.709, p=0.015) and distance in 6MWT (r=-0.658, p=0.028), in addition to a statistically significant positive correlation with dyspnea score (r=0.666, p=0.025). On the other hand, in the fibrotic subgroup, YKL-40 only had statistically significant positive

correlation with age (r=0.666, p=0.009).

(Table 4)

Table 1. Comparison of studied variables in HP and control groups.

	HP (n=25)		**Controls (n=25)**		**p**
	Mean	***SD***	***Mean***	***SD***	
Age (years)	47.1	11.6	44.7	10.5	0.440
FVC%	54.7	19.3	85.6	3.7	<0.001*****
FEV1%	57.5	19.7	85.5	4.7	<0.001*****
FEV1/FVC%	84.8	8.2	86.8	4.7	0.297
Distance in 6MWD (meters)	209.6	70.4	315.8	29.9	<0.001*****
	Median	***IQR***	***Median***	***IQR***	
Dyspnea score	3	1	2	2	<0.001*****
SO2 on room air (%)	92	7.5	97	2	<0.001*****
Minimum SO2 in 6MWT (%)	82	17.5	97	2	<0.001*****
YKL-40 level (×10² pg/ml)	1398	910	304.9	180.4	<0.001*****

***** Statistically significant

SD *Standard deviation,* ***FVC*** Forced vital capacity, ***FEV1*** Forced expiratory volume in the first second, ***6MWT*** Six-minute walk test, ***IQR*** *Interquartile range*, ***SO2*** Oxygen saturation

Table 2. Correlation between serum YKL-40 level and other variables in HP and control groups.

	HP (n=25)		**Controls (n=25)**	
	r	**p**	**r**	**p**
Age	0.545	0.005*****	-0.422	0.035*****
SO2 on room air	-0.431	0.031*****	0.425	0.034*****
FVC%	-0.387	0.056	0.311	0.131
FEV1%	-0.312	0.128	0.037	0.859
FEV1/FVC%	-0.245	0.238	0.117	0.577
Minimum SO2 in 6MWT	-0.484	0.014*****	0.392	0.053
Distance in 6MWD	-0.611	0.001*****	0.349	0.087
Dyspnea score	0.478	0.016*****	0.135	0.130

***** Statistically significant

SO2 Oxygen saturation, ***FVC*** Forced vital capacity, ***FEV1*** Forced expiratory volume in the first second, ***6MWT*** Six-minute walk test

Table 3. Comparison of studied variables in fibrotic and non-fibrotic HP subgroups.

	Non fibrotic HP (n=11, 44%)		**Fibrotic HP (n=14, 56%)**		**p**
	Mean	***SD***	***Mean***	***SD***	
Age (years)	44.7	10.6	49	12.3	0.364
FVC%	70	18	43	10	0.001*****
FEV1%	70	20	48	13	0.003*****
FEV1/FVC%	83	8	86	9	0.447
Distance in 6MWD (meters)	275.4	40	157.8	37	<0.001*****
	Median	***IQR***	***Median***	***IQR***	
Disease duration (years)	2	1	3	1	0.047*****
Dyspnea score	3	1	4	1	0.001*****
SO2 on room air (%)	96	2	90	8.2	<0.001*****
Minimum SO2 in 6MWT (%)	93	6	78	11.7	<0.001*****
YKL-40 level (×10² pg/ml)	796.7	356.3	1599	407.3	0.007*****

***** Statistically significant
SD *Standard deviation,* ***FVC*** Forced vital capacity, ***FEV1*** Forced expiratory volume in the first second, ***6MWT*** Six-minute walk test, ***IQR*** *Interquartile range,* ***SO2*** Oxygen saturation

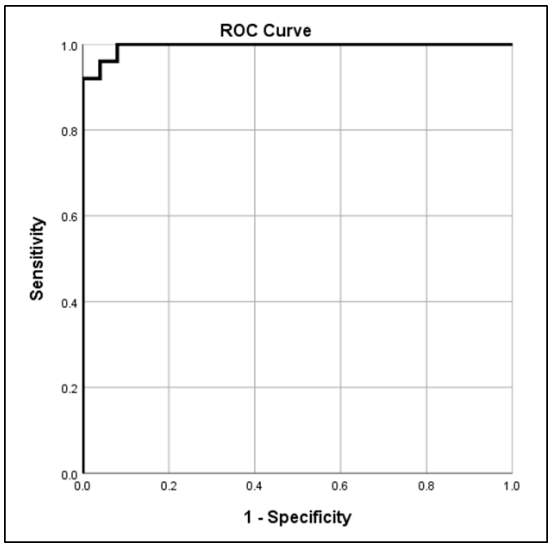
Table 4. Correlation between serum YKL-40 level and other variables in non-fibrotic and fibrotic HP subgroups.

	Non-fibrotic HP (n=11)		**Fibrotic HP (n=14)**	
	r	**p**	**r**	**p**
Age	0.403	0.220	0.666	0.009*****
SO2 on room air	-0.773	0.005*****	0.238	0.413
FVC%	-0.363	0.272	-0.307	0.234
FEV1%	-0.464	0.151	-0.287	0.178
FEV1/FVC%	-0.709	0.015*****	-0.264	0.362
Minimum SO2 in 6MWT	-0.586	0.058	0.297	0.302
Distance in 6MWD	-0.658	0.028*****	0.204	0.484
Dyspnea score	0.666	0.025*****	-0.328	0.252
Disease duration	-0.376	0.254	0.075	0.800

***** Statistically significant
SO2 Oxygen saturation, ***FVC*** Forced vital capacity, ***FEV1*** Forced expiratory volume in the first second, ***6MWT*** Six-minute walk test

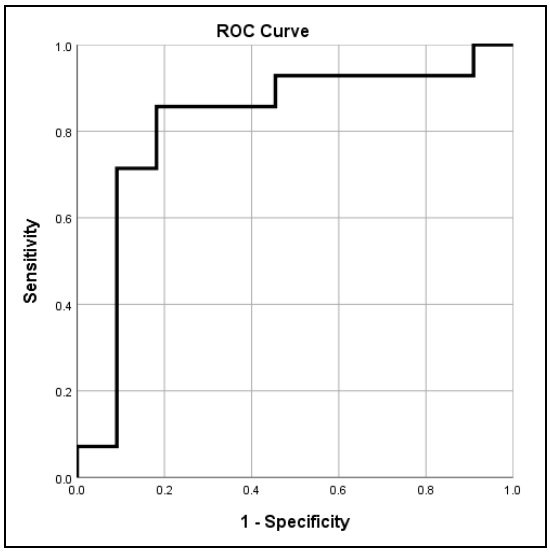
ROC curve was used to choose a cut-off value for serum YKL-40 to distinguish between the HP patients and normal individuals (represented by controls). Calculated area under the curve (AUC) was 0.995. The chosen cut-off value was 629.4 pg/ml giving sensitivity 96%, and specificity 96%. (Figure 1)

Figure 1. ROC curve for the YKL as a diagnostic tool for the HP as compared to the control group.



Moreover, ROC curve was used to choose a cut-off value for serum YKL-40 to distinguish between the fibrotic and non-fibrotic HP groups. Calculated AUC was 0.818. The chosen cut-off value was 1181.5 pg/ml giving sensitivity 85.7%, and specificity 81.8%. (Figure 2)

Figure 2. ROC curve for the YKL as a diagnostic tool for the fibrotic as compared to the non-fibrotic in HP patients.



Discussion

YKL-40 protein is an emerging biomarker in a wide spectrum of diseases involving fibrosis, inflammation and tissue remodeling. It has been proved to stimulate fibroblast growth. In addition, YKL-40 rises in inflammatory conditions, and is integrated in tissue remodeling [15]. Our study included 50 participants with mean age is 45.9± 11 years old ranged between 20-65 years. Included participants were divided into 2 groups: HP group (n=25) and control group (n=25). HP patients' age was 47.1±11.6 years. It is to be mentioned that all included participants were females. These results are matched with the study conducted by Akl et al [16] who studied the demographics of hypersensitivity pneumonitis in Egypt and reported that, out of 118 HP patients, females were ten times more affected than males with ratio 10.8:1 and the mean age was 42.7±12.5 years. On the reverse, Baqir et al [17] found significant male predominance. Most of our HP patients (n=18, 72%) have exposure history to birds while 7 patients (28%) didn't have history of specific antigen exposure. On the other hand, the controls had no history of antigen exposure. Exposure to aerosolized avian proteins, especially from feathers or bird droppings, may result in hypersensitivity pneumonitis (HP),which is known as "bird fancier's lung" (BFL). This had been previously reported in various studies [18, 19] and matches the results of our study. Akl et al [16] found that most of patients with known exposure, were raising birds (78.12%). Similarly, Adams et al [20] stated that 64% of their patients were bird breeders.

In our study, YKL-40 level was statistically significantly higher in HP patients compared

to healthy control (p>0.001), as YKL40 median level in HP group was 1398 pg/ml with minimum of 572 pg/ml and maximum of 1841 pg/ml, while YKL-40 median level in control group was 304 pg/ml with minimum of 170 pg/ml and maximum of 634 pg/ml. These results are consistent with a retrospective study [21] conducted on 175 HP patients versus 65 controls, in which the results showed that serum YKL-40 level in HP patients was 1270±90 pg/ml while it was 390±40 pg/ml in controls (p<0.001). Similar results were found in a pilot study [15] that included 26 patients and revealed that YKL-40 level was significantly higher among HP group compared to control group. Our study showed a significant positive correlation between YKL level and dyspnea score in the HP group. This agrees with a prospective study [22] that concluded that the grade of dyspnea was significantly associated with YKL-40 serum level. In the present study, ROC curve is used to choose a cut-off value to distinguish between HP patients and healthy individuals. Calculated area under the curve (AUC) =0.995. The chosen cut-off value was 629.4 pg/ml with sensitivity 96%, and specificity 96%. Long et al [21] used a cut-off of 470 pg/ml of the serum YKL-40 levels which showed sensitivity 88% and specificity 77% to discriminate HP from healthy controls (area under the curve (AUC) =0.904; p<0.001); which agrees with our results. Moreover, in the current study, ROC curve is used to choose a cut-off value to distinguish between the fibrotic and non-fibrotic HP groups. Calculated area under the curve (AUC) =0.818. The chosen cut-off value was 1181.5 pg/ml giving sensitivity 85.7%, and specificity 81.8% for patients above 1181.5 pg/ml as fibrotic HP group. Again, closely matching with our results, Long et al [22] also proposed a cut-off value for disease progression as they stated that at a cut-off level of 1190 pg/ml of serum YKL-40 could predict disease progression with sensitivity 81% and specificity 77%. They also proposed the addition of LDH to serum YKL-40 as a predictor for HP progression. However, this was beyond our objectives in the current study. To our knowledge the present study represents the first of its kind to define a cut-off of YKL-40 between fibrotic and non-fibrotic subtypes of HP in Egypt. This indicates the need for further studies to be conducted to identify properly the correlation between YKL-40 level and extent of fibrosis in HP.

limitations of the study

The present study is limited by the small number of HP patients and controls. The number of participants in our study was affected by the COVI-19 pandemic and relevant restrictions. Moreover, YKL-40 is impacted with many genetic factors that was not assessed in the current study. Also, some studies correlated LDH and YKL-40 in diagnosing HP, which was not investigated in the present study

Conclusions:

YKL-40 level can be used as a biomarker for assessing disease severity in hypersensitivity pneumonitis, as well as for discrimination between fibrotic and non-fibrotic hypersensitivity pneumonitis subtypes. In addition, YKL-40 is significantly associated with disease duration, grade of dyspnea among HP patients.

YKL-40 may be of help as a predictor for respiratory function deterioration in HP patients being negatively associated with SO2

on room air, minimum SO2 in 6MWT and distance in 6MWT.

ETHICAL APPROVAL & CONSENT

The study was approved by the Institutional Review Board of Faculty of Medicine, Cairo University (IRB No. MS-166-2021) and was performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained.

AVAILABILITY OF DATA & MATERIAL

The datasets generated during the current study are not publicly available due to the hospital policy and because the data will be used in future multi-center research to generate nationwide statistics. However, these datasets are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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