Correlation between Serum KL-6 level and Severity of SARD-related ILD

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Abstract

Objective: To understand the correlation between serum KL-6 level and severity of SARD-related ILD in Indonesia.

Methods: This was a cross-sectional study to evaluate the correlation between serum KL-6 level and Interstitial Lung Disease (ILD) severity based on Chest High-Resolution Computed Tomography (Chest HCRT) among patients with Systemic Autoimmune Rheumatic Disorders (SARD) who visited the Rheumatology Clinic of Dr. Hasan Sadikin General Hospital during the period of January 2019 to February 2020. Secondary data were retrieved from a study on the Effects of Ciplukan Herbs on Organ Fibrosis. KL-6 serum concentration was measured from stored biological material and the correlation between the serum KL-6 level and ILD severity was analyzed by Rank Spearman's test.

Results: Thirty-four patients participated in this study with a median age of 37 years-old. Most of the participants were female (94.1%), Sundanese (64.7%), and had systemic sclerosis as an underlying disease (48.5). The median serum KL-6 level was 57.1 U/mL (21.6-444.1). Most participants belonged to severe ILD group (58.8%) with a mean serum KL-6 level of 65.1±48.9 U/mL. The highest mean serum KL-6 level (111.6±121.8) was observed in the moderate ILD group comprising of 32.4% of the participants. The remaining 11.8% participants belonged to mild ILD group with a mean serum KL-6 level of 61.1±24.9 U/mL. Serum KL-6 level was demonstrated to have a weak correlation with ILD severity (r=-0.229, p=0.193).

Conclusion: There is an insignificant weak correlation between serum KL-6 level and ILD severity based on chest HRCT in SARD patients.

Keywords: ILD, KL-6, SARD

Introduction

Systemic autoimmune rheumatic disease (SARD) is a multiorgan systemic inflammatory disease associated with immune system dysregulation and leading to disability, organ failure, and premature death. Systemic autoimmune rheumatic disease, also known as connective tissue disease or collagen vascular disease, consists of antiphospholipid syndrome, microscopic angiitis, granulomatous polyangiitis, cryoglobulinemia, systemic lupus erythematosus, systemic sclerosis, mixed connective tissue disease, and rheumatoid arthritis.[1]

Immune system dysregulation in PRAS leads to intetstital lung disease (ILD).[2] Data showed that 13.9–15% of SARD patients get ILD.[3] The main investigation modalities in establishing the diagnosis of ILD are conventional X-ray and thorax HRCT. Conventional X-rays have relatively low sensitivity.[4] Thorax HRCT is the gold standard of ILD diagnosis with a sensitivity of 95% which can also determine the severity of ILD.[5] HRCT examination is not

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yet widely available, limited to certain health care centers, and relatively expensive.[6] Thorax HRCT also carries a risk of radiation exposure so it is not safe for routine disease monitoring.[7] Krebs von den Lugen-6 (KL-6) serum is a biological marker that has high sensitivity in the diagnosis of ILD, monitoring the course of the disease, assessing therapeutic response, and predicting the prognosis of ILD.[8] Secretion of KL-6 into the bloodstream occurs due to severe damage to the alveolar basement membrane and increased permeability of the blood-air barrier. High serum KL-6 levels were found in more than 70% of SARD associated-ILD cases.[9]. Serum KL-6 level had a strong and significant correlation with the severity of PPI and the area of lung segments involved based on chest HRCT.[10,11]

Lee *et al.* showed that KL-6 levels had a strong and significant correlation with the severity of ILD and the area of the involved lung segment ($r=0.561$, $p<0.001$) based on thorax HRCT.[10,11] Yamakawa *et al.* reported a significant correlation between serum KL-6 levels and ILD severity in SARD ($r=0.418$, $p=0.010$).[8] Another study by Cho *et al.* concluded that KL-6 correlated with ILD severity based on thorax HRCT for interstitial lung disease ($r=0.561$).[11]

Serum KL-6 levels were influenced by ethnic differences. Evidence from previous studies in Asia and Europe showed different levels of KL-6. Most of the information regarding the role of serum KL-6 as a biological marker of ILD were obtained from studies in Japan and western countries. The effect of ethnic differences on ILD progression has been reported by Ishikawa *et al.*, Cao *et al.*, and Horimasu *et al.* The difference in KL-6 levels was caused by the polymorphism of the MUC1 gene.[12]

Research regarding the relationship between serum KL-6 levels and ILD severity is important to be carried out to serve as the basis for evaluating the use of serum KL-6 as a modality for regular monitoring of ILD in SARD population. Research regarding SARD-related ILD is still limited and to date there have been no studies regarding the use of serum KL-6 in the diagnosis or monitoring of ILD in Indonesian patient population. In this study, we evaluated the correlation between serum KL-6 levels and ILD severity based on chest HRCT in SARD patients.

Methods

Participants of this study was SARD patients in Rheumatology Clinic of Hasan Sadikin General Hospital Bandung, Indonesia in 2019-2020. Inclusion criteria were aged 18-60 years, diagnosed with SARD whose data was recorded in the research "Effects of *Ciplukan* Herbs on Organ Fibrosis", had been scored and staged for ILD severity based on HRCT thorax, and had stored biological material (less than 2 years) for serum KL-6 examination. Exclusion criteria were pneumonia, tuberculosis, history of tuberculosis, malignancy or history of malignancy, and functional class III-IV cardiac decompensation.

Data collection was initiated by looking for SARD patients with ILD who participated in the research "Effects of Ciplukan Herbs on Organ Fibrosis".[13] Serum KL-6 levels were collected as primary data. Blood samples from all the patients were collected in the same day or in maximum interval of 7 days from thorax HRCT examination. Serum were stored in the refrigerator at -80°C. Serum KL-6 concentrations were measured using an enzyme-linked immunosorbent assay kit (Bioassay Technology Laboratory, China), according to the manufacturer's instructions. The ILD score and severity data were taken from the registry confirmed by two thoracic radiology consultants at the Radiology Department. The scoring system used is the Warrick *et al.* method which combines the severity and extent of the lesion.[14] The severity of the ILD was determined by the method of Mohammadi *et al.* which classifies the scoring results into mild, moderate, or severe degrees.[15]

This was a cross-sectional study with correlation analysis. Sample measurement was determined using the correlation test formula, with a minimum total sample of 32 participants was required. Secondary data for baseline characteristic was obtained from medical records, including age, sex, and medications.

The data that had been collected from research participants who met the inclusion and exclusion criteria were analyzed using the Statistical Package for the Social Science (SPSS) program. The numerical scale was presented with the mean and standard deviation if the distribution is normal or median and the range if the distribution was not normal. Categorical scale data was presented in the form of frequency and percentage.[16] Bivariate analysis was performed to examine the correlation of KL-6 levels with ILD severity based on chest HRCT. The correlation test was carried out using the Spearman's Rank correlation test.

This study had been approved by the Ethics

Committee of Hasan Sadikin General Hospital Bandung with ethical approval number LB.02.01/X.6.5/132/2021. For all patients who were enrolled in this study, the informed consent form was signed by the patient herself/ himself.

Result

A total of 60 medical record of SARD patients with ILD were obtained during the study period. There were 26 patients excluded, consisting of 25 patients with incomplete data and 1 patient with active pulmonary tuberculosis infection. Data analysis was performed on 34 patients who met the inclusion and exclusion criteria. Almost all of this study participants were women. The most common underlying disease is systemic sclerosis. The most common types of therapy are methylprednisolone and methotrexate. Complete baseline characteristics can be seen in Table 1.

The median KL-6 level in this study was 57.1 U/mL (21.6-444.1) and the mean ILD score based on the Warrick *et al.* was 16.8±6.4. The largest proportion of participants were in severe ILD group (Table 2.).

Table 1 Baseline Characteristics

Variable	n=34
Age (years)	
Median (minimum-maximum)	37 (21-64)
Sex, (n (%))	
Female	32(94,1)
Male	2(5,9)
Underlying Disease, (n(%))	
Systemic Sclerosis	16(48,5)
SLE	14(40)
MCTD	3(8,6)
Rheumatoid Arthritis	1(2,9)
Medications, (n(%))*	
Methylprednisolone	33(97,1)
Metothrexate	32(94,1)
Aspilet	26(62,9)
Nifedipin	19(55,9)
Ciplukan herbs	17(50,0)
Diltiazem	13(38,2)
Mycophenolate mofetil	11(32,4)
PPI/ H2 receptor antagonist/ antasida	9(26,5)
Azatioprin	9(26,5)
Cyclophosphamid	9(26,5)
Cyclosporin	6(17,6)

Note: *One patient could get multiple medications; PPI: proton pump inhibitor; MCTD: mixed connective tissue disease; SLE: systemic lupus erythematous

Table 2 Concentration of KL-6, ILD Score, and Severity of ILD

Variable	n=34
KL-6 concentration (U/ml) Median (range)	57.1 (21.6–444.1)
ILD Score Mean ± standard deviation	16.8±6.4
ILD Severity (n(%))	
Mild	3 (11.8)
Moderate	11 (32.4)
Severe	20 (58.8)

Table 3 and Figure showed that most of the participants had a severe ILD while the highest mean serum KL-6 level was found in the moderate ILD severity group. Dosage and duration of therapy could affected the severity of ILD and serum KL-6 levels but the secondary data source in this study did not record the dose and duration of therapy.

Correlation analysis using Rank-Spearman test at 95% confidence level showed that there was a weak correlation in a negative direction (r=-0,229) that was not statistically significant (p=0.193).

Discussion

This was the first study in Indonesia to assess the value of KL-6 in diagnosing and rmonitoring SARD related-ILD in Indonesia. The results of this study showed a weak correlation which is statistically insignificant between KL-6 levels and disease severity

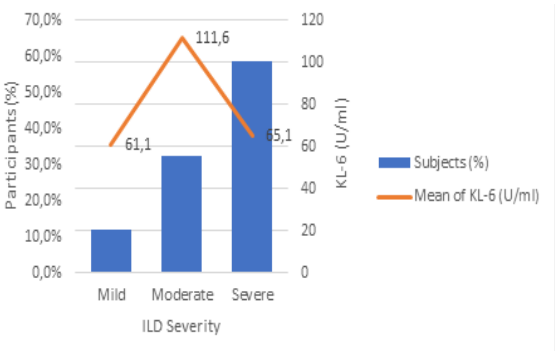

Figure Frequency of ILD Severity and Serum KL-6

Table 3 Serum KL-6 and Medications according to ILD Severity

	Mild	Moderate	Severity	Total
N (%)	3 (11.8)	11 (32.4)	20 (58.8)	34 (100)
Serum KL-6 (U/ml) Mean±Standard deviation	61.1±24.9	111.6±121.8	65.1±48.9	
Medications, n(%)*				
Methylprednisolone	3 (100)	11 (100)	19 (95.0)	33 (97.1)
Methothrexate	3 (100)	11 (100)	18 (90.0)#	32 (94.1)
Ciplukan herbs	1 (33.3)	6 (54.5)	10 (50.0)	17 (50)
MMF	1 (33.3)	2 (18.2)	8 (40.0)	11 (32.4)
Azatioprin	2 (66.7)	4 (36.4)	3 (15.0)	9 (26.5)
Siklofosfamid	3 (100)	3 (27.3)	3 (15.0)	9 (26.5)
Siklosporin	1 (33.3)	1 (9.1)	4 (20.0)	6 (17.6)

Note: *) One patient could get multiple medications; #) Methotrexate was topped once patient received cyclophosphamide or MMF. MMF: mycophenolate mofetil

although based on a literature review, KL-6 was the biological marker with the best diagnostic value in the identification of ILD, followed by surfactant protein-D (SPD) and matrix metalloproteinases 12 (MMP 12).).[17] Spearman's Rank correlation test with a 95% confidence level between serum KL-6 levels and ILD severity showed a weak correlation with a negative direction (r=-0.229) which was not statistically significant (p>0.05). This weak correlation was thought because KL-6 was influenced by many factors apart from the severity of the ILD including genetic factors medications consumed by the patient.

Most of the study participants had a severe ILD while the highest mean KL-6 levels were found in the moderate ILD severity group. The low KL-6 levels in the severe group were thought to be due to medications, hook effect, radiological features that persisted regardless of clinical improvement or discontinued disease progression. The correlation value of KL-6 levels with ILD severity was expected to be better and has a positive direction if KL-6 levels also increase with increasing disease severity.

The ILD treatment strategy is based on stopping or suppressing the inflammatory process. Corticosteroids, immunosuppressants, cytotoxic agents, and antifibrotic agents, either alone or in combination, are used in this disorder.[18] Almost all research participants received methylprednisolone and methotrexate therapy. Mycophenolate mofetil and cyclophosphamide are the treatment of choice for severe ILD according to clinical guidelines our hospital. Mycophenolate mofetil is a drug with tablet preparations that are more practical than cyclophosphamide which given intravenously. This convenience may be the reason that more numbers of severe ILD patients received mycophenolate mofetil therapy (40%) than cyclophosphamide therapy (15%). Dose and duration of therapy were not recorded on the registry so that the precise influence of these factor could not be determined on this study.

KL-6 levels in the moderate-severity group (111.6±121.8 U/mL) were higher than those in the mild-grade group (61.1±24.9 U/mL). These findings were consistent with the relationship between KL-6 levels and the degree of lung tissue damage.[9] The small proportion (18.2%) of moderate ILD participants receiving mycophenolate mofetil therapy was thought to be the reason of the high levels of KL-6 in this group because this drug could inhibit the proliferation of fibroblasts which associated with the secretion of KL-6 into the circulation.[19] Most of the participants were in severe ILD group but the average KL-6 level in this group tended to be lower than the moderate ILD group (65.1±48.9 U/mL vs. 111.6±121.8 U/mL). mL). The combination of the effect of mycophenolate mofetil, the drug most often given in this group, with other therapies (cyclophosphamide, methotrexate, and adjuvant therapy of *ciplukan* herbs extract) could be the reason of low KL-6 levels. Mycophenolate mofetil was most often given to the severe ILD group because this drug is the

treatment of choice for severe ILD according to clinical guidelines at the RSHS Rheumatology Clinic along with cyclophosphamide.

Serum KL-6 levels could be influenced by several other factors, including genetic differences and hook effect. Horimasu *et al.*'s obtained higher KL-6 levels in the patient group in Germany than in the group in Japan. This difference in levels was caused by the polymorphism of the MUC1 gene.[20] There are currently no data regarding the genetic profile of the Indonesian population.

The hook phenomenon that causes lower than expected results had been widely reported. Hook effect has been reported to affect various tests using the ELISA method, including the examination of -HCG, prolactin, calcitonin, aldosterone, and tumor markers (CA 125 and PSA).[21] The sample in this study was not re-examined by dilution when a low KL-6 result was obtained at the initial examination. Dilution would result in higher KL-6 levels if there was a hook effect that interferes with the test results. Duration of sample storage was thought did not influence the concentration of KL-6 since most of the samples for examination of serum KL-6 levels were taken on the same day of examination of HRCT of the thorax, while some examinations were carried out with a maximum length of 1 week.

There is a weak correlation between serum KL-6 levels and ILD severity based on chest HRCT in SARD patients. Serum KL-6 levels in this study is in the low range presumably due to drugs, ethnicity, and hook effects. There is tendency of higher serum KL-6 level on moderate ILD group than mild ILD group while the level tends to lower in severe ILD group compared to moderate ILD group. In conclusion, there is a weak correlation which is statistically insignificant between KL-6 serum and ILD severity found in this study.

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