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Original Research Article

Expressions and significance of 25 (OH) D, KL-6 and IL-32 in serum and alveolar lavage fluid in patients with connective tissue disease-related interstitial lung disease

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Abstract

Purpose: *To investigate the expressions and significance of 25 hydroxyvitamin D [25(OH)D3], Krebs von den Lungen (KL)-6, and interleukin (IL)-32 in serum and alveolar lavage fluid (SALF) in patients with connective tissue disease-related interstitial lung disease (CTD-ILD).*
Methods: *Forty CTD-ILD patients admitted to Affiliated Hospital of Jianghan University (The Sixth Hospital of Wuhan) were retrospectively categorized into a study group (SG), while 45 healthy individuals were categorized into a control group (CG). The SG was grouped based on the imaging performance of the high-resolution computed tomography (HRCT). If the lesions were ground-glass-like changes, featuring predominant exudative changes in spots and patchy shadows, the patients were placed in subgroup A of the study group, but if they were fibrous cord-like, grid-like, and honeycomb-like fibrotic lesions, the patients were placed in subgroup B of study group.*
Results: *Serum 25(OH)D3 in the SG was lower than in the CG, while the serum levels of KL-6 and IL-32 were higher than in CG. Furthermore, in the SG, KL-6 and IL-32 levels were lower in serum than in alveolar lavage fluid ($p < 0.05$). Sub-group A showed higher SALF levels in KL-6 and IL-32 than sub-group B. Forced vital capacity (FVC) and the diffusing capacity of the lungs for carbon monoxide (DLCO) in SG were lower than in CG ($p < 0.05$). Serum 25(OH)D3 had negative correlation with the SALF of KL-6 and IL-32 in CTD-ILD patients ($p < 0.05$), while KL-6 and IL-32 levels in serum were positively correlated with those in alveolar lavage fluid ($p < 0.05$).*
Conclusion: *The results indicate the possible involvement of 25(OH)D3, KL-6, and IL-32 in the development of CTD-ILD. Thus, serum 25(OH)D3, SALF KL-6, and IL-32 levels may affect lung function and therefore, can serve as indicators of the patients' condition.*

Keywords: *Connective tissue disease-related interstitial lung disease (CTD-ILD), Peripheral venous blood, Alveolar lavage fluid, 25 Hydroxyvitamin D, Krebs von den Lungen-6; Interleukin-32*

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INTRODUCTION

Connective tissue disease (CTD) is an autoimmune disease, and is characterized by connective tissue and chronic vascular inflammatory lesions [1]. The specific pathogenesis of CTD is not yet entirely clear. Currently, it is widely believed that the

occurrence and development of CTD is related to a variety of factors, including genetic factors, immune factors, infection, etc. [2]. CTD disease may involve the skin, muscle, lung, and central nervous system, and the lung is a vulnerable site. But CTD-related interstitial lung lesions (LCDs) are more common [3]. CTD-LCD not only causes a decrease in the quality of life, but is also a crucial cause of poor prognosis and death. As the clinical manifestations of CTD-LCD are insidious, the early symptoms are not easily detected, and when patients are diagnosed, they are mostly in the middle and advanced stages of the disease, and thus, the best time for treatment is often missed [4]. The 25 hydroxyvitamin D [25(OH)D3] is a metabolite of vitamin D, while KL-6 is a mucus glycoprotein primarily generated by type II alveolar epithelial cells and bronchial epithelial cells. On the other hand, IL-32 is a pro-inflammatory factor secreted by lymphocytes, epithelial cells, and monocytes.

Previous studies [5] showed that serum 25(OH)D3 expression was down-regulated and serum KL-6 and IL-32 expression up-regulated in CTD-LCD patients. Meanwhile, a related study [6] revealed that the level of KL-6 in alveolar lavage fluid in CTD-LCD patients was markedly higher than that in controls. However, the expression of 25(OH)D3, KL-6, and IL-32 in alveolar lavage fluid and serum in CTD-LCD patients and the relationship between them have rarely been investigated. In current research, the expressions and significance of 25(OH)D3, KL-6, and IL-32 in alveolar lavage fluid and serum in CTD-LCD patients, in order to determine their relationship with lung function.

METHODS

Patients

The clinical information for 45 CTD-ILD patients admitted to Affiliated Hospital of Jianghan University (The Sixth Hospital of Wuhan) between January 2019 and December 2021 were retrospectively analyzed and categorized as study group (SG). The study was approved by the institutional ethical and followed international guidelines for human studies.

Inclusion criteria

The inclusion criteria were (1) meeting the diagnostic and classification criteria for CTD-ILD as revised by the American College of Rheumatology in 1987 and 1997; (2) first diagnosis of connective tissue; (2) first diagnosis of interstitial lung disease (ILD); (3) age ≥ 18 years; (4) not taking hormonal drugs or antibiotics in the past 3 months; and (5) no other pulmonary diseases were combined.

Exclusion criteria

These include (1) combined with systemic acute and chronic infections; (2) co-existing with malignant diseases; (3) history of thoracic and abdominal surgery in the last 6 months; (4) those with chronic respiratory diseases.

There were 10 cases of rheumatoid arthritis-related ILD (RA-ILD), 3 cases of systemic lupus erythematosus-related ILD (SLE-ILD), 14 cases of systemic sclerosis associated ILD (SSc-ILD), 11 cases of polymyositis/dermatomyositis ILD (PM/DM-ILD), and 7 cases of Sjogren syndrome-related ILD (SS-ILD).

Another 45 healthy adults who underwent health check-up in Affiliated Hospital of Jianghan University (The Sixth Hospital of Wuhan) in the corresponding period served as the control group (CG). The SG further was classified as two groups based on the imaging parameters of high-resolution computed tomography (HRCT); among them, 17 patients with the lesions showing exudative changes such as ground glass-like changes and speckled patchy shadows were assigned to group A, and 28 patients with the lesions of fibrous cord-like, grid-like, and honeycomb-like fibrotic lesions were assigned to group B.

Evaluation of parameters

Levels of 25(OH)D, KL-6 and IL-32

Serum 25(OH)D, KL-6 and IL-32 levels were determined by collecting 2 mL of peripheral blood and centrifuging at 3000 rpm, Enzyme-linked immunosorbent assay (ELISA) was utilized to determine the 25(OH)D, KL-6, and IL-32 levels, with kits purchased from R&D Systems (Minneapolis, MN, USA).

To determine the 25(OH)D, KL-6, and IL-32 levels in the bronchoalveolar lavage fluid, 100 mL of normal saline was instilled through a mechanical ventilation tracheal tube, oxygen was given, and the lavage fluid was recovered via suction under negative pressure. The samples were concentrated 10 times with 50 % polyethylene glycol, left to stand for 24 h, and then 10 % of the recovered amount was diluted with 0.9 % NaCl and frozen at -80 °C. 25(OH)D, IL-32 and KL-6 were determined by ELISA.

Pulmonary function was measured using a German Jäger lung function instrument, and the

forced vital capacity (FVC) as well as the diffusing capacity of the lungs for carbon monoxide (DLCO) were examined in both groups.

Statistical methods

SPSS19.0 statistical software was employed for result analysis, with measurement data expressed as mean ± SD; t-test was performed to compare the mean data between two groups, while the relationship among the indicators was analyzed using Pearson correlation analysis.

RESULTS

Patients' baseline data

No statistically difference was observed in two groups in terms of baseline data such as gender, age, and body mass index ($p > 0.05$; Table 1).

25(OH)D3, KL-6 and IL-32 levels

The SG had lower of 25(OH)D3 level and higher KL-6 and IL-32 levels in serum than the CG ($p < 0.05$); in SG, KL-6 and IL-32 levels were lower in serum than in alveolar lavage fluid ($p < 0.05$), suggesting that CTD-ILD patients had markedly lower 25(OH)D3 levels and higher KL-6 and IL-32 levels in serum than the healthy controls (Table 2).

Serum and alveolar lavage fluid levels of 25(OH)D3, KL-6, and IL-32 in CTD-ILD patients

The HRCT images of study subgroups A and B were characterized based on the manifestations of the lesions. If the lesion were mainly exudative with ground glass-like changes and speckled patchy shadows, they were assigned to group A, but if the lesions were mainly fibrotic with fibrous cords, lattices, and honeycomb-like fibrosis, they were placed in group B. If the lesions were mainly fibrous streaks, latticework or honeycomb-like fibrosis, they were also assigned to group B. It suggested that group A exhibited markedly higher KL-6 and IL-32 levels in serum and alveolar lavage fluid than group B ($p < 0.05$; Figure 1).

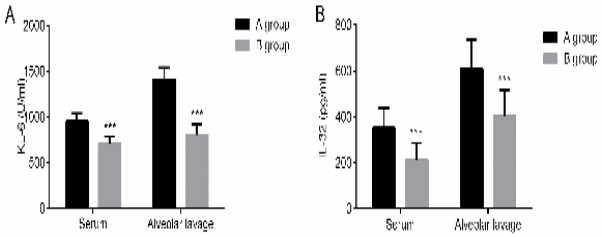

Figure 1: Comparison of KL-6 and IL-32 levels in different HRCT (exudation-based and fibrosis-based) manifestations in the study groups. (A) KL-6 levels in serum and alveolar lavage fluid were significantly higher in group A than in group B; (B) IL-32 levels in serum and alveolar lavage fluid were significantly higher in group A than in group B. Compared with group A, ***$p < 0.001$

Table 1: Comparison of baseline data (mean ± SD)

Group	Disease type	N	Sex		Mean age (years)	Body mass index (kg/m^2)
			Male	*Female*		
Study	*RA-ILD*	10	2	8	46.79 ± 11.46	21.38 ± 2.91
	SLE-ILD	3	0	3	47.34 ± 9.38	20.98 ± 2.86
	SSc-ILD	14	3	11	46.92 ± 12.07	22.15 ± 2.88
	PM/DM-ILD	11	3	8	47.15 ± 10.86	21.99 ± 2.85
	SS-ILD	7	2	5	48.32 ± 9.94	21.38 ± 2.76
Control	-	-	12	33	48.23 ± 10.72	21.74 ± 2.83

Table 2: Comparison of serum 25(OH)D, KL-6 and IL-32 levels (mean ± SD, n = 45)

Group		25(OH)D3 (ng/mL)	KL-6 (U/mL)	IL-32 (pg/mL)
Study	*Serum*	12.12 ± 5.69	936.42 ± 70.71	315.27 ± 93.14
	Alveolar lavage fluid	-	1397.68 ± 112.35	526.33 ± 124.68
Control	*Serum*	18.35 ± 6.27	61.35 ± 12.86	152.39 ± 20.37
t1/t2		4.936	81.677/23.309	11.460/9.097
P1/P2		0.000	0.000/0.000	0.000/0.000

Note: t1 is the t value of comparison between the study group and control group, and t2 is the t value of comparison between serum and alveolar lavage fluid indices in the study group. P1 is the *p*-value for comparison between the study group and control group, and P2 is the *p*-value for comparison between serum and alveolar lavage fluid indices in the study group

25(OH)D3, KL-6, and IL-32 levels in CTD-ILD patients

Patients with RA-ILD, SLE-ILD, SSc-ILD, PM/DM-ILD and SS-ILD did not exhibit statistical differences in serum levels of 25(OH)D3, KL-6, and IL-32 ($p > 0.05$); they did not also exhibit differences in KL-6 and IL-32 levels in the alveolar lavage fluid ($p > 0.05$), suggesting no marked differences in the expression of serum 25(OH)D3, KL-6, and IL-32 levels in patients with RA-ILD, SLE-ILD, SSc-ILD, PM/DM-ILD and SS-ILD (Figure 2).

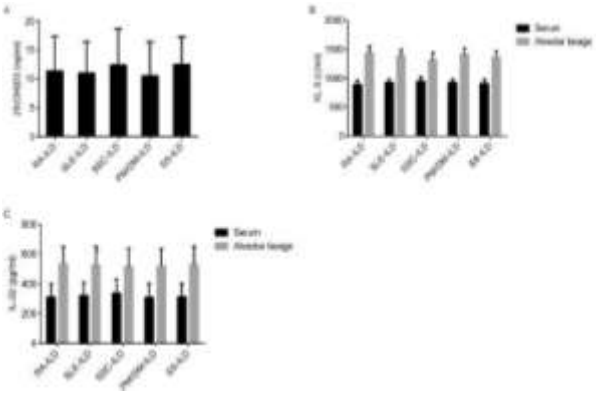

Figure 2: Comparison of 25(OH)D3, KL-6, and IL-32 levels amongst different disease types in the study group. RA-ILD: rheumatoid arthritis interstitial lung disease; SLE-ILD: systemic lupus erythematosus interstitial lung disease; SSc-ILD: systemic sclerosis interstitial lung disease; PM/DM-ILD: polymyositis/dermatomyositis interstitial lung disease; SS-ILD: Sjögren's syndrome interstitial lung disease.

Lung functions

The SG exhibited lower forced vital capacity (FVC) and DLCO than CG ($p < 0.05$). This suggests that patients with CTD-ILD had poorer lung function indicators than healthy controls (Figure 3).

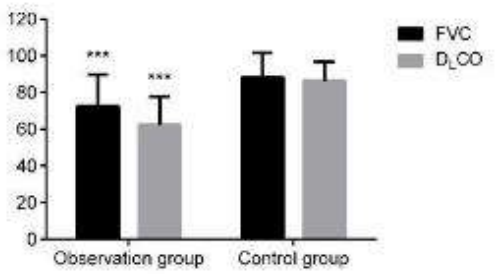

Figure 3: Comparison of lung function. ***Note:*** FVC: Forced vital capacity; DLCO: Diffusing capacity of the lungs for carbon monoxide. Compared with control group, ***$p < 0.001$

Relationship between 25(OH)D3 in serum and KL-6 and IL-32 in serum and alveolar lavage fluid of CTD-ILD patients

In patients with CTD-ILD, 25(OH)D3 in serum had negative correlation with KL-6 and IL-32 in serum and alveolar lavage fluid ($p < 0.05$), and KL-6 and IL-32 in serum were positively correlated with those in alveolar lavage fluid ($p < 0.05$; Table 3).

Relationship between 25(OH)D3, KL-6, IL-32 and lung function indicators in CTD-ILD patients

In patients with CTD-ILD, 25(OH)D3 in serum had positive correlation with FVC and DLCO ($p < 0.05$), and KL-6 and IL-32 levels in serum and alveolar lavage fluid had negative correlation with FVC and DLCO ($p < 0.05$; Table 4).

Table 3: Relationship between serum 25(OH)D3 and serum and alveolar lavage fluid of KL-6 and IL-32 in patients with CTD-ILD

Item	Alveolar lavage fluid KL-6		Alveolar lavage fluid IL-32		Serum KL-6		Serum IL-32	
	r	*P*	r	*P*	r	*P*	r	*P*
Serum 25(OH)D3	0.347	0.0	-0.399	0.0	-0.475	0.0	0.621	0.0
Serum KL-6	0.495	0.0	0.543	0.0	-	-	-	-
Serum IL-32	0.612	0.0	0.538	0.0	-	-	-	-

Table 4: Relationship between 25(OH)D3, KL-6, IL-32 and lung function indicators in CTD-ILD patients

Item	FVC (%)		DLCO (%)	
	r	*P*-value	r	*P*-value
Serum 25(OH)D3	0.365	0.002	0.428	0.006
Serum KL-6	-0.439	0.000	-0.582	0.000
Serum IL-32	-0.476	0.000	-0.513	0.000
Alveolar lavage fluid KL-6	-0.677	0.000	-0.896	0.000
Alveolar lavage fluid IL-32	-0.525	0.000	-0.739	0.000

DISCUSSION

CTD-ILD is a diffuse lung disease that involves the interstitium, alveoli, and bronchioles. The pathological changes of CTD-ILD are characterized by fibroblast proliferation and massive extracellular matrix accumulation [7]. CTD-ILD predominates in women, accounting for about 78 % of cases, with a high incidence rate among the 40 - 60 years age group [8], which causes damage and the distortion of normal lung tissues and the decrease in lung function. Early CTD-ILD lacks typical symptomatic manifestations, and as it progresses, dry cough, absence of sputum, dyspnea, and respiratory failure may occur [9]. Among CTD-ILD patients, RA-ILD, SLE-ILD, and SSc-ILD are common, and the incidence varies among different disease types, featuring SSc-ILD with the highest incidence (59.3 %) and SLE-ILD (4.5 %). Compared to CTD alone, patients with CTD-ILD have significantly lower survival rates and poorer patient prognosis [10].

It was found that the progression of CTD-ILD is closely associated with a variety of cytokines and proteins [11]. The 25(OH)D3 is one of the intermediates of vitamin D and has important physiological roles, including regulation of calcium or phosphorus, involvement in bone growth, and immune regulation [12]. A decrease in 25(OH)D3 causes an abnormal expression of metalloproteinases and the massive degradation of the extracellular matrix, which causes pulmonary fibrosis [13]. However, a study has shown that CTD-ILD patients exhibit lower serum 25(OH)D3 than healthy subjects [14]. In this research, it was also reported that CTD-ILD patients had down-regulated serum 25(OH)D3 levels than normal controls, but the difference in patients with different types of CTD-ILD was not significant. KL-6 is a pulmonary surface active protein secreted by bronchial epithelial cells and type II alveolar epithelial cells, and mainly in the alveoli and blood, and it is involved in the normal physiological function of the lung [15].

The IL-32 is an inflammatory factor involved in the development of several inflammatory disorders, including autoimmune diseases [16]. Alveolar cells expresses IL-32 in large quantities when they are stimulated by infection and cytokine interferon-γ [17]. In this study, the KL-6 and IL-32 levels in the alveolar lavage fluid were significantly higher than those in the serum in patients with CTD-ILD, while the KL-6 and IL-32 levels in the serum in CTD-ILD patients were higher than in healthy subjects. This mechanism may be due to CTD-ILD autoimmune dysfunction leading to lung tissue damage and elevated expression of the lung surface active substance KL-6 and the pro-inflammatory factor IL-32 in the alveoli.

From the results of Pearson correlation analysis in CTD-ILD patients, 25(OH)D3 in serum had negative correlation with KL-6 and IL-32 levels in serum and alveolar lavage fluid, and KL-6 and IL-32 in serum had positive correlation with those in alveolar lavage fluid. It indicates that the lower the serum 25(OH)D3 level, the higher the expression of KL-6 and IL-32 upregulated in serum and alveolar lavage fluid. The mechanism may be that the lower 25(OH)D3 aggravated the CTD-ILD immune dysfunction and increased lung cellular immune activity, which aggravated the damage to interstitial and alveolar lung, and increased KL-6 and IL-32 expression levels in serum and alveolar lavage fluid.

CTD-ILD patients have decreased lung compliance due to damage to the interstitium and alveoli, which affects the patients' lung volume and diffusion function [18]. In this research, the results demonstrated that CTD-ILD patients had lower FVC and DLCO than healthy controls. Further correlation analysis revealed that in CTD-ILD patients, 25(OH)D3 in serum had positive correlation with FVC and DLCO, and KL-6 and IL-32 levels in serum and alveolar lavage fluid showed negative correlation with FVC and DLCO. It was shown that the lower the serum 25(OH)D3 and the higher the serum and alveolar lavage fluid levels of KL-6 and IL-32 levels, the more significant the decline in lung function.

The mechanism may be that lower 25(OH)D3 leads to insufficient supply of 25(OH)D3, increased immune disorder and inflammatory response, increased damage to the interstitium and alveoli of the lungs, and causes pulmonary ventilation dysfunction and diffusion dysfunction. Based on the above results, it maybe speculated that serum 25(OH)D3, and KL-6 and IL-32 in serum and alveolar lavage fluid participate in the progression of CTD-ILD and may serve as indicators of the condition of patients.

CONCLUSION

The levels of 25(OH)D3, KL-6, and IL-32 probably participate in the development of CTD-ILD, and serum 25(OH)D3, serum and alveolar lavage fluid KL-6, and IL-32 may be linked to lung function and thus, can be used as indicators of the patients' conditions. Due to the limitation of sample size, the results of this study may have a large bias, and therefore, a larger sample size is needed to obtain more objective data.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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