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Dilek Ergün *, Reca Ergün *, Esin Özkan , Merve Bacanlı , Muslu Kazım Körez

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Serum Prolidase Activity in Silicosis and Its Relationship with Oxidative Stress and Inflammation Markers

Dilek Ergün [1,*], Recai Ergün [1,*], Esin Özkan [2], Merve Bacanlı [3] and Muslu Kazım Körez [4]

[1] Selcuk University, Faculty of Medicine, Department of Pulmonary Medicine

[2] Rush University Medical Center; ozkanesin@yahoo.com

[3] University of Health Sciences Turkey, Gulhane Faculty of Pharmacy, Department of Pharmaceutical Toxicology, mervebacanli@gmail.com

[4] Selcuk University Faculty of Medicine, Department of Biostatistics, mkkorez@gmail.com,

* Correspondence: dilekkirbiyik@gmail.com (D.E.); recaiergun@gmail.com (R.E.); Tel.: +905056873000 (D.E.); +905053700078 (R.E.)

Abstract: (1) Background:This study aimed to evaluate the prognostic significance of serum prolidase activity in silicosis and to examine its relationship with oxidative stress and inflammatory parameters. (2) Methods: One hundred thirteen patients with silicosis, 70 workers with a history of exposure who did not develop the disease and 53 healthy volunteers of similar age and gender were included in the study. Serum levels of prolidase, superoxide dismutase, catalase, 8-hydroxy-2-deoxyguanosine, glutathione peroxidase, glutathione reductase, tumor necrosis factor α, fibroblast growth factor, interferon-gamma, and interleukins (IL) 1α and 1β were measured. Genetic damage was assessed by measuring 8-hydroxy-2′-deoxyguanosine in serum. (3) Results: The difference was not statistically significant because serum prolidase activity was higher in the silicosis and silica-exposed group than in healthy controls.Serum superoxide dismutase and glutathione peroxidase levels were significantly lower in the exposure and silicosis groups compared to the control group. 8-hydroxy-2′-deoxyguanosine and tumor necrosis factor α, fibroblast growth factor, interferon-gamma, and IL-1α were significantly higher in the exposure and silicosis groups than in the control group. In individuals with high prolidase activity, both oxidative stress and inflammation parameters were high. (4) Conclusion:The fact that serum prolidase activity is closely associated with inflammation, fibrosis, and oxidative stress in silicosis suggests that prolidase can be used in the early diagnosis and follow-up of silicosis. Early disease diagnosis is important for stopping exposure and taking preventive measures.

Keywords: serum prolidase activity; silicosis; oxidative stress; inflammation

1. Introduction

Silicosis is an interstitial lung disease resulting from fibrosis of the lungs caused by inhalation of crystalline silicon dioxide or silica. It is still one of the most important occupational diseases worldwide. Even when silica exposure is eliminated, the disease can progress to progressive massive fibrosis and lead to impaired lung function [1]. In silicosis, direct cytotoxicity, oxidant production, proinflammatory cytokine and reactive oxygen species (ROS) release and growth factor release in the pathological process stimulate scar formation and lung fibrosis [2].

The enzyme prolidase catalyzes the hydrolysis of dipeptides containing proline or hydroxyproline in the carboxyl-terminal position. Proline is recycled and used in new protein synthesis, while hydroxyproline is excreted in the urine. Since proline and hydroxyproline constitute approximately 25% of the amino acids in collagen structure, prolidase plays an important role in collagen degradation [3].

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Prolidase is involved in collagen metabolism, new matrix formation, and cell growth. Prolidase, therefore, plays an important role in various physiological processes such as wound healing, inflammation, angiogenesis, cell proliferation, and carcinogenesis [3,4]. Deviations in prolidase activity may be a useful marker in cancers and many conditions characterized by fibrosis [4]. In people with liver disease, serum prolidase activity is increased, especially in the early stage of fibrosis, and it has been suggested that plasma prolidase activity may be useful in assessing fibrotic processes in chronic liver disease in humans [5]. The relationship between lung fibrosis and prolidase in silicosis patients is unclear and has never been studied. It is thought that prolidase enzyme may be a useful marker in silicosis as in other diseases with fibrosis.

Prolidase activity in bronchial asthma [6] and chronic obstructive pulmonary disease COPD [7,8] is lower in patients with dilated cardiomyopathy, pancreatitis and pancreatic cancer. Prolidase activity has been reported to be different in each chronic disease; prolidase activity is decreased in patients with chronic uremic type 2 diabetes mellitus [9] and is increased in chronic liver diseases [10].

Although there are studies evaluating oxidative stress and inflammatory markers in silicosis, there is no study evaluating serum prolidase activity. In this study, we aimed to determine whether serum prolidase activity is a new marker in the diagnosis and follow-up of silicosis. We also aimed to contribute to understanding pathophysiologic mechanisms in silicosis by determining oxidative stress parameters, inflammatory markers and serum prolidase activity. With the help of the identified markers, we aimed to contribute to the prevention of the disease by terminating the exposure at an early stage and taking preventive measures.

2. Methods

This study was approved by the Clinical Research Ethics Committee of Diskapi Yildirim Beyazit Training and Research Hospital (Date and Number: March 27, 2017, 36-13). All participants signed informed consent.

2.1. Study Population and Study Procedures

The study population consisted of silica-exposed workers referred to the Ankara Hospital for Occupational and Environmental Diseases, one of the three reference centers in Turkey, by an occupational physician or pulmonologist with suspicion of silicosis. Subjects who did not have a chronic disease had a history of exposure, did not have silicosis, and worked in the same workplace were included in the exposure group. Age- and gender-matched healthy subjects without dust exposure were selected as the control group. Our study consisted of 113 silicosis patients, 70 workers with silica exposure who did not develop the disease and 53 healthy volunteers, totaling 236 male participants.

Those with chronic diseases characterized by fibrosis in the lung or any organ (interstitial lung disease, scleroderma, liver cirrhosis, ankylosing spondylitis, rheumatoid arthritis, cardiomyopathy), those taking medications that affect prolidase levels, active infections and malignancies were excluded. All participants completed a detailed clinical questionnaire including age, gender, height, weight, smoking habit, chronic disease, pulmonary symptoms, use of personal protective equipment, total exposure time, and occupational history.

Pulmonary function tests were performed with appropriate techniques in all participants. Chest radiographs of all participants were evaluated by two pulmonologists with International Labor Office (ILO) radiography reading training certificates. Any disagreements were resolved by consensus. We defined silicosis as associated radiological signs (ILO classification of ≥1/0) and radiological findings on high-resolution computed tomography (HRCT) in a worker exposed to silica [11].

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2.2. Biological Sampling

Venous blood was collected from each volunteer as a biological sample. Blood was collected in silicone-coated tubes and protected from light. Blood samples were centrifuged at 2000 rpm for 15 minutes. The serum samples obtained were stored at -80°C until the analysis day. Serum samples stored at room temperature on the day of the experiment were used to study biomarkers.

2.3. Determination of Serum Prolidase Activity and Other Parameters

All kits (Prolidase, catalase (CAT), 8-hydroxy-2-deoxyguanosine (8-OH-dG), glutathione peroxidase (GPx), fibroblast growth factor (FGF), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin (IL) 1α, 1β) were obtained from the Cloud-Clone Corp. (Wuhan, China); other additional reagents were purchased from Cayman Chemical (Michigan, USA). Biotek Instruments (Vermont, USA) was employed to make spectrophotometric measurements.

The levels of prolidase, inflammatory and oxidative stress parameters were measured using commercial kits using the ELISA technique. The absorbance of each well was measured at 450 nm.

2.3. Statistical Analysis

All statistical analyses were performed in R Statistical Software version 4.1.2. (The R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org). Before the analyses, the normality of the data was checked by Shapiro-Wilk's normality test and Q-Q graphs, and Levene's homogeneity test checked the homogeneity of group variances. The findings of the numerical variables in the study were presented as mean ± standard deviation or median and quartiles (first quartile - third quartile), and categorical variables were presented as frequency (n) and percentage. The presence or absence of statistically significant differences in the demographic, clinical, functional and radiological findings of the silicosis, exposure and healthy control groups were evaluated by one-way ANOVA followed by Tukey HSD multiple comparisons; Welch's F test followed by Games-Howell multiple comparisons; Kruskal Wallis H test in groups that did not show normal distribution, followed by multiple comparisons with Dunn's test with Bonferroni correction for parameters found significant. Independent sample t-test or Mann-Whitney U test was used to evaluate whether there was a statistically significant difference in the prolidase, oxidative stress and inflammatory findings of silicosis, exposure and healthy control groups and simple and complicated silicosis groups. The groups ' working times and mask use rates were compared with the Pearson chi-square test. In addition, Spearman's rho correlation coefficient was used to determine whether there was a statistically significant relationship between serum prolidase activity and demographic, clinical, functional, and radiologic findings.

3. Results

3.1. Demographic and Clinical Characteristics

Table 1 presents the characteristics of the study population. Although silicosis patients were older than the silica-exposed group, there was no statistical difference in age between both groups and healthy controls ($p > 0.005$). Although smoking rates in the control group were similar to those in the general population, smoking rates were significantly higher in the silicosis and silica-exposed groups ($p < 0.001$). There was a statistical difference between the groups in the duration of study exposure ($p = 0.004$). Patients with silicosis had longer working hours than the silica-exposed group. While 43.4% of patients with silicosis had worked for 15 years or more, this rate was 27.1% in the group with exposure. In the silicosis group, 39.8% always wore masks, while this rate was 51.4% in the group with exposure.

According to the lung function test findings of the subjects, forced expired volume (FEV1) and forced vital capacity (FVC) values were significantly lower in both the silicosis and exposure groups compared to healthy controls ($p<0.001$). Peak expiratory flow (PEF) and FEV1/FVC values were not

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statistically different between the groups. The diffusion capacity of carbon monoxide (DLCO) was found to be lower in the silicosis group compared to exposed individuals, but no statistical significance was found (p >0.005).

Table 1. Demographic, clinical, functional and radiological findings of all groups.

	Silicosis (n=113)	Silica exposed workers (n=70)	Healthy controls (n=53)	*p*-value
Age (yr)	41.61 ± 7.14a	38.04 ± 7.12b	41.66 ± 9.90	**.004**[3]
BMI (kg/m^2)	26.06 ± 3.66	26.01 ± 3.19	27.11 ± 3.68	.155[2]
Smoking status				**<.001**[4]
Nonsmoker	19 (16.8)a	11 (15.7)a	24 (45.3)b	
Smoker	66 (58.4)a	50 (71.4)a	20 (37.7)b	
Exsmoker	28 (24.8)	9 (12.9)	9 (17)	
Cigarette Pack-years	13.50 (9.25 – 20)	12 (8 – 19)	12 (10 – 16)	.610[1]
Total of work time (yr)	15 (10 – 19)	9 (5 – 16.75)	-	**.004**[6]
Total of work time				**.003**[4]
1–5 yr	13 (11.5)a	19 (27.1)b	-	
6–10 yr	18 (15.9)	19 (27.1)	-	
11–15 yr	33 (29.2)	13 (18.6)	-	
>15 yr	49 (43.4)a	19 (27.1)b	-	
Working hours per day (hours/day)	8.14 ± 1.18	8.02 ± 0.48	-	.124[6]
Use of masks				.173[4]
Never	18 (15.9)	6 (8.6)	-	
Rarely	8 (7.1)	8 (11.4)	-	
Often	42 (37.2)	20 (28.6)	-	
Always	45 (39.8)	36 (51.4)	-	
FEV1 (% pred)	90.41 ± 17.70 a	92.36 ± 13.47a	102.60 ± 10.55b	**<.001**[3]
FVC (% pred)	90.74 ± 15.92a	92 ± 11.11a	100.68 ± 12.21b	**<.001**[2]
FEV1/FVC	81.83 ± 6.70	83.36 ± 5.71	83.90 ± 4.72	.094[2]
PEF (% pred)	84.18 ± 21.21	80.77 ± 20.95	88.15 ± 15.55	.460[2]
DLCO (% pred)	99.01 ± 19.81	105.18 ± 19.34	-	.703[5]
ILO profusion score	6 (5 – 9)a	1 (1 – 1)b	1 (1 – 1)b	**<.001**[1]
ILO classification				
Category 0	-	70(100)	53(100)	
Category 1	61 (54)	-	-	
Category 2	31 (27.4)	-	-	
Category 3	21 (18.6)	-	-	
Large opacity		-	-	
A	13 (11.5)	-	-	
B	3 (2.7)	-	-	
C	3 (2.7)	-	-	

BMI: body mass index, FEV1: forced expired volume, FVC: forced vital capacity, PEF: peak expiratory flow, DLCO: diffusion capacity of carbon monoxide, ILO: International Labour Organization. 1 Kruskal Wallis H test,2 One-way Analysis of Variance, 3 Welch F test 4 Pearson's chi-square test, 5 Independent sample t-test, 6 Mann-Whitney U test. Results with statistically significant differences are indicated in bold.

According to the ILO classification, 61 (54%) of 113 silicosis patients were classified as Category 1, 31 (27.4%) as Category 2, and 21 (18.6%) as Category 3. The predominant opacity type was p opacity, with 57.5%, and Category 1 was the most common type of profusion. Large opacities were detected in 19 (17%) silicosis patients. Thirteen large opacities were A, 3 were B, and 3 were C. The mean ILO profusion score of silicosis was 6 (5-9).

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3.2. Prolidase, Oxidative Stress, and Inflammatory Parameters

Although serum prolidase activity was higher in the silicosis and silica-exposed group than in healthy controls, the difference was not statistically significant. Serum prolidase levels were highest in silicosis, while prolidase levels tended to increase after silica exposure (Table 2).

Table 2. Prolidase, oxidative stress, and inflammatory parameters across all groups.

	Silicosis (n=113)	Silica exposed workers (n=70)	Healthy controls (n=53)	p-value
Prolidase (ng/mL)	0.38 (0.28 – 0.46)	0.36 (0.30 – 0.43)	0.35 (0.28 – 0.43)	.558[1]
SOD (U/mL)	0.04 (0.03 – 0.04)a	0.04 (0.03 – 0.04)a	2.02 (2.01 – 2.03)b	**<.001[1]**
GPx (nmol/min/mL)	4.52 (3.65 – 5.65)a	4.34 (3.49 – 4.78)a	6.22 (5.44 – 6.43)b	**<.001[1]**
GR(nmol/mL/min) (×103)	0.004 (0.003 – 0.010)	0.004 (0.003 – 0.010)	0.004 (0.002 – 0.005)	.151[1]
CAT (U/mL)	1.09 (0.53 – 1.61)	0.94 (0.56 – 1.41)	1.27 (0.65 – 1.51)	.218[1]
8-OH-dG (pg/mL)	2640 (1780 – 3805)a	2322.50 (1820 – 3037.50)a	1575 (1255 – 1805)b	**<.001[1]**
TNF-α (pg/mL)	119.70 (109.70 – 219.70)a	119.70 (111.95 – 216.90)a	101.70 (90.70 – 108.70)b	**<.001[1]**
FGF (pg/mL)	350 (275.56 – 445.56)a	405.56 (291.11 – 551.94)a	271.11 (165.56 – 462.22)b	**<.001[1]**
IFN-γ (pg/mL)	776.63 (704.54 – 830.79)a	772.04 (667.35 – 854.13)a	617.46 (513.71 – 687.88)b	**<.001[1]**
IL-1β (pg/mL)	261.42 ± 82.88	259.42 ± 92.48	250.56 ± 97.35	.760[2]
IL-1α (pg/mL)	71.48 ± 6.23a	75.67 ± 5.87b	63.65 ± 6.43c	**<.001[2]**

SOD: superoxide dismutase, GPx: glutathione peroxidase, GR: glutathione reductase, 8-OH-dG: 8-hydroxy-2 deoxyguanosine, CAT: Catalase, TNF-α: tumor necrosis factor α, FGF: fibroblast growth factor; IFN-γ: interferon gamma, IL-1β: interleukin 1β, IL-1α: interleukin 1α. 1 Kruskal Wallis H test, 2 One-way Analysis of Variance. Results with statistically significant differences are indicated in bold.

Serum superoxide dismutase (SOD) and GPx levels were significantly lower in the exposure and silicosis groups compared to the control group. Although CAT was lower than the control, it was not statistically significant. Similarly, 8-OH-dG, an indicator of silica-induced DNA damage, and inflammatory parameters TNF-α, FGF, IFN-γ, and IL-1α were found to be significantly higher in the exposure and silicosis groups compared to the control group (Table 2).

A comparison of prolidase, oxidative stress and inflammatory parameters in simple silicosis and complicated silicosis groups is given in Table 3. Prolidase levels were higher in subjects with complicated silicosis than those with simple silicosis. However, the difference between the groups was not statistically significant. Except for GPx, there was no statistically significant difference between the two groups in all other parameters.

Table 3. Prolidase, oxidative stress, and inflammatory parameters across all groups.

	Simple Silicosis (n= 94)	Complicated Silicosis (n=19)	p-value
Prolidase (ng/mL)	0.38 (0.30 – 0.45)	0.40 (0.22 – 0.51)	.957[1]
SOD (U/mL)	0.04 (0.02 – 0.04)	0.04 (0.03 – 0.04)	.149[1]
GPx (nmol/min/mL)	4.51 (3.63 – 5.60)	5.65 (4.40 – 6.03)	**.043[1]**
GR(nmol/mL/min) (×103)	0.004 (0.003 – 0.010)	0.005 (0.003 – 0.010)	.384[1]
CAT (U/mL)	1.09 (0.84 – 1.60)	0.82 (0.26 – 1.50)	.280[1]
8-OH-dG (pg/mL)	2627.50 (1800 – 4295)	2680 (1422.50 – 3245)	.291[1]
TNF-α (pg/mL)	120.70 (110.70 – 232.70)	118.70 (108.70 – 142.20)	.222[1]
FGF (pg/mL)	350 (264.44 – 442.49)	403.33 (324.44 – 466.11)	.327[1]
IFN-γ (pg/mL)	783.71 (700.79 – 830.79)	737.04 (707.46 – 828.71)	.349[1]
IL-1β (pg/mL)	263.80 ± 84.16	249.65 ± 77.23	.500[2]
IL-1α (pg/mL)	71.46 ± 6.42	71.55 ± 5.35	.954[2]

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SOD: superoxide dismutase, GPx: glutathione peroxidase, GR: glutathione reductase, 8-OH-dG: 8-hydroxy-2 deoxyguanosine, CAT: Catalase, TNF-α: tumor necrosis factor α, FGF: fibroblast growth factor; IFN-γ: interferon gamma, IL-1β: interleukin 1β, IL-1α: interleukin 1α. [1] Mann-Whitney U test, 2. Independent samples t-test. Results with statistically significant differences are indicated in bold. Data are presented as mean ± standard deviation, median (quartiles) or frequency (n) and percentage (%).

A positive significant correlation was found between serum prolidase activity and IFN-γ only (Spearman's rho=0.140, p=.032). On the other hand, no statistically significant correlation was found between serum prolidase activity and other biochemical parameters (all p>0.05).

3. Discussion

It has been reported that prolidase may be a useful marker in detecting early and monitoring fibrosis-related diseases. In this study, we analyzed prolidase activity; oxidative stress enzyme activities such as SOD, CAT, GPx, glutathione reductase (GR); 8-OH-dG, an indicator of silica-induced DNA damage; and inflammatory markers such as FGF, TNF-α, IFN-γ, IL-1α and IL-1β in serum samples of silicosis patients, silica-exposed workers and healthy individuals. To our knowledge, this is the first study evaluating serum prolidase activity in patients with silicosis. We also evaluated the relationship between serum prolidase activity, oxidative stress, and inflammatory parameters. The findings of this research may clarify the pathogenesis of diseases characterized by fibrosis, such as silicosis, and guide early diagnosis and prevention measures.

An increase or decrease in prolidase activity may indicate the presence of a disease state and disease progression. Studies have reported altered prolidase activities in some fibrotic diseases with excessive collagen turnover. A decrease in prolidase enzyme activity can occur when collagen deposition progresses to the point of fibrosis or when tissue hardens due to scar formation, resulting in decreased fluid flow in the tissue. After chronic inflammation in the airways, the tissues gradually become fibrotic, during which collagen turnover slows down, and prolidase activity decreases [12].

Low serum prolidase activity indicates ineffective collagen turnover in rheumatic diseases, possibly leading to low prolidase activity [13]. Scleroderma is characterized by excessive collagen deposition and tissue fibrosis due to reduced collagen degradation. Serum prolidase activity was found to be decreased in patients with scleroderma, especially in the diffuse form [14]. In systemic sclerosis (SSc) in the early stages of CKD, high amounts of proline may be released by the breakdown of proline-containing iminodipeptides or hydroxyproline due to increased collagen turnover. High proline levels have an inhibitory effect on SPA [15,16]. Although prolidase was lower in scleroderma patients with lung involvement compared to healthy controls, it was not statistically significant. It was emphasized that prolidase may be a marker for SSc in diagnosis and follow-up [17]. Serum prolidase levels were significantly lower in active ankylosing spondylitis (AS) patients compared to inactive patients and healthy controls. Low serum prolidase levels are probably associated with fibrosis due to decreased collagen turnover and decreased physical function in AS patients [18].

In hepatic fibrosis induced in experimental animal studies [5,19,20] and in patients with chronic liver disease [21–23] studies, higher plasma and liver prolidase activity was found, and it was claimed that prolidase may be an index of liver fibrosis [19–23]. Higher serum prolidase activity was found in patients with chronic liver disease compared to healthy subjects; however, only a few biopsy-positive cirrhotic patients had increased serum prolidase activity. A rat model study of cirrhotic liver fibrosis suggested that serum prolidase activity levels may be higher in the early fibrosis stages and then decline as the disease progresses [19]. Prolidase may increase in the early stages of fibrosis due to sudden collagen deposition and decrease in later stages as the liver may not sustain prolidase production [4].

Prolidase activity was measured as a diagnostic marker in an experimental animal model of lung fibrosis. Bleomycin-induced fibrosis in rat lungs. Fibrosis scores and prolidase activity were significantly increased by bleomycin stimulation. There was a positive correlation between prolidase activity and fibrosis scores. It was emphasized that prolidase activity can be used to diagnose and

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follow up fibrotic lung diseases. However, it was emphasized that more clinical and experimental studies are needed to confirm these results [24].

In our study, post-covid fibrosis was evaluated in 68 patients with moderate and severe COVID-19. However, the prolidase value was higher in the fibrotic group at admission compared to the group without fibrosis. The difference between the groups was not statistically significant. We found no correlation between total fibrosis score and prolidase calculated on thoracic CT of post-covid patients at month 3, which could be explained by the fact that it is early in the fibrosis process for changes in prolidase activity. Different results could have been obtained if patients had been evaluated in long-term follow-up [25].

Considering all these studies, it is possible to say that prolidase may be an important marker in fibrotic diseases. However, in literature searches, no human studies investigate the relationship between silicosis, a fibrotic lung disease, and prolidase. In our study, although serum prolidase activity was higher in the silicosis and silica-exposed group than in healthy controls, the difference was not statistically significant. Serum prolidase levels were highest in silicosis, while prolidase levels tended to increase after silica exposure. We also found higher levels of prolidase activity in complicated silicosis patients with irreversible massive fibrosis compared to simple silicosis patients. We emphasize that changes in prolidase levels in silica exposure may indicate changes in collagen metabolism in the pathogenesis of silicosis and may also be helpful in the diagnosis and monitoring of disease progression.

In asthma, increased collagen deposition due to chronic inflammation narrows the airways [26]. Patients with prolidase deficiency (PD) have asthma-like symptoms, emphasizing the important role of prolidase activity in lung health and pathologies. Prolidase activity was increased in blood sera collected from adults with bronchial asthma [27]. However, another study found reduced serum prolidase activity in children with bronchial asthma. The fact that prolidase enzyme activity, which plays a role in collagen turnover, is low in asthma patients emphasizes that collagen metabolism is altered and may affect collagen accumulation in the reticular basement membrane [6]. These conflicting studies suggest that age may have different effects on prolidase activity [28]. Although they cause similar pathology, there is no explanation as to why prolidase is decreased in PD and increased in asthma. In our study, we found no correlation between age and prolidase. Elevated prolidase activity in silicosis compared to healthy individuals may indicate chronic inflammation in the pathogenesis of the disease.

Plasma prolidase activity was significantly lower in COPD patients than in healthy controls. It has been suggested that this altered prolidase activity may be due to disorders of collagen metabolism in COPD [7,8]. It was emphasized that prolidase activity may be associated with systemic inflammation and severity of airflow obstruction (FEV1, FEV1/FVC) in stable COPD patients [8,29]. In our study, FEV1 and FVC values were significantly lower in the silicosis and exposure group than in healthy controls. DLCO, which is an indicator of early functional impairment in interstitial lung diseases, was found to be decreased in silicosis patients. No significant correlation was found between prolidase and functional parameters.

Although increased oxidative damage and an imbalance between the oxidant/antioxidant defense system have been reported in silicosis [30–32], the relationship between prolidase and oxidative stress in silica exposure has not been evaluated. In various diseases, prolidase activity is directly related to oxidative stress, and it has been suggested that prolidase is a useful biochemical marker of oxidative stress [17,33–36]. Numerous studies prove that oxidative stress stimulates collagen breakdown by induction of matrix metalloproteinases (MMPs) and induction of an inflammatory microenvironment [37]. These reactive oxygen species (ROS)-induced changes in the extracellular matrix (ECM) microenvironment have been proposed to be responsible for modulating prolidase activity by making the substrate available through collagen degradation [4].

In patients with scleroderma with lung involvement, a weak correlation was found between prolidase and oxidative stress index, while a decrease in antioxidant activity was found. No correlation was found between prolidase, total antioxidant activity, and total oxidative stress levels.

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It was emphasized that decreased antioxidant activity may be responsible for pulmonary damage in patients with scleroderma [17].

In asthma patients, a positive correlation was found between prolidase activity and total antioxidant capacity (TAC) but not with oxidative stress level, suggesting that prolidase is not affected by oxidative stress [6]. In a study conducted in patients with COPD, no relationship was found between prolidase activity and oxidative and antioxidative parameters [8]. In another study, significant correlations were found between plasma prolidase activity and total antioxidant capacity (TAC) and lipid peroxidation (LPO) levels in COPD patients. They suggested that COPD causes oxidative stress in the lungs and that the oxidant-antioxidant balance and collagen turnover are altered in the lungs of COPD patients. Therefore, altered prolidase activity in COPD patients may be due to collagen metabolism disorders [7].

In our study, SOD and GPx levels were significantly lower in the exposure and silicosis groups compared to the control group. 8-OH-dG, an indicator of silica-induced DNA damage, was 1.6 times higher in patients with silicosis and 1.4 times higher in the exposed group than in healthy subjects. Although CAT was lower than the control, it was not statistically significant. Under healthy conditions, when ROS production is low, 8-OH-dG is inhibited by the combined activities of several antioxidants present in plasma. However, in the case of excessive ROS production, as hypothesized by the increase of 8-OH-dG in our study, this protection may be insufficient as a defense mechanism of the organism against the ongoing oxidative load. 8-OH-dG, which indicates oxidative stress, increased, while SOD and GPx, which indicate antioxidant defense, decreased. Although there is no direct relationship between serum prolidase activity and oxidative stress, we have shown that oxidative burden was increased in groups with increased prolidase activity (both silicosis and exposure). This result may suggest that prolidase activity may indicate increased oxidative stress.

Some cytokines produced after phagocytosis of silica by alveolar macrophages have regulatory effects on fibroblast growth and/or collagen synthesis. Pulmonary fibrosis can develop when the balance between fibrotic and anti-fibrotic mediators is altered. IL-1, TNF-α, FGF, platelet-derived growth factor (PDGF), fibronectin, alveolar macrophage-derived growth factor, and type 1 insulin-like growth factor have been reported to increase fibroblast proliferation [1,2]. The critical role of TNF- α in pulmonary fibrosis is evidenced by the fact that anti-TNF- α significantly reduced silica-induced pulmonary fibrosis in a mouse model [38]. Although exposure has been reported to stimulate both fibrogenic and antifibrogenic factors, the balance seems to shift towards fibrotic stimuli. Our study found that inflammatory parameters (TNF-α, FGF, IFN-γ, IL-1α), which play an important role in collagen production and fibrosis, increased in silicosis patients compared to healthy controls. At the same time, elevated levels of these markers in individuals with silica exposure may indicate early pulmonary system involvement. The fact that prolidase activity tends to increase in individuals with high inflammatory parameters may indicate the role of prolidase in the mechanisms of inflammation and fibrosis in silicosis.

The sample size of our study is one of our limitations. In particular, our group of patients with complicated silicosis (n:19) was a very small population. More informative findings may be obtained when studies are conducted on larger groups. We also did not have detailed data on the dust measurement concentrations of the environments in which the individuals worked.

In conclusion, our study showed that serum prolidase activity is closely associated with inflammation, fibrosis and oxidative stress in silicosis. This suggests that prolidase may be used in the early diagnosis and follow-up of silicosis in clinical practice. Early disease diagnosis is important for ending exposure and taking preventive measures. In the follow-up of the disease, it can guide the referral of patients to medical treatment and lung transplantation in case of progression to the fibrotic process. However, more clinical studies are needed to confirm these results.

Data are presented as mean ± standard deviation, median (quartiles) or frequency (n) and percentage (%).

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Abbreviations

The following abbreviations are used in this manuscript:

SPA	Serum prolidase activity
COPD	Chronic obstructive pulmonary diseases
SSc	Systemic sclerosis
AS	Ankylosing spondylitis
SSc – ILD	Scleroderma-associated interstitial lung disease
FEV1	Forced expired volume
FVC	Forced vital capacity
PEF	Peak expiratory flow
PFT	Pulmonary function test
ROS	Reactive oxygen species
SOD	Superoxide dismutase
CAT	Catalase
GSH	Glutathione
GPx	Glutathione peroxidase
GR	Glutathione reductase
8-OH-dG	8-hydroxy-2-deoxyguanosine
TAC	Total antioxidant capacity
TOS	Total oxidant status
TAS	Total antioxidant status
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
FGF	Fibroblast growth factor
TNF-α	Tumor necrosis factor alpha
IFN-γ	Interferon-gamma
PDGF	Trombosit kaynaklı büyüme faktörü
DLCO	Diffusion capacity of carbon monoxide
GTP	Guanosine triphosphate
ECM	Extracellular matrix

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