Dermatology Practical & Conceptual

Evaluation of Thiol Disulfide, Ischemia Modified Albumin, and Prolidase Parameters in Patients with Localized Scleroderma

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ABSTRACT

Introduction: Localized scleroderma is a rare inflammatory skin disease that causes sclerosis in the dermis and subcutaneous tissue. Oxidative stress may play a role in the etiology or be responsible for the chronicity or progression of the disease.

Objectives: We aimed to investigate the presence of oxidative stress in patients with localized scleroderma by examining thiol-disulfide balance, ischemia-modified albumin (IMA), and prolidase parameters.

Methods: Twenty patients over the age of 18 who were diagnosed with localized scleroderma both clinically and histopathologically and 20 control subjects were included in the study. Age, sex, age at disease onset, duration of the disease, and presence of accompanying systemic diseases were questioned and recorded. Lesion type and modified Rodnan and LoSSI scores were calculated through dermatological examination. CRP, sedimentation rate, total thiol, native thiol, and disulfide levels indicated by the Erel profile, IMA level, and prolidase levels were measured and compared in both the patient and control groups.

Results: Levels of native thiol (P=0.958), total thiol (P=0.979), disulfide (P=0.449), (disulfide/native thiol%) (P=0.368), (disulfide/total thiol%) (P=0.361), (native thiol/total thiol%) (P=0.368), and

prolidase (P=0.121) were similar in both patient and control groups. Only IMA was significantly different. No significant relationship was found between the levels of native thiol, total thiol, disulfide, (disulfide/native thiol), (disulfide/total thiol), IMA, prolidase, and Rodnan and LoSSI scores.

Conclusion: According to the data obtained from this study, we can say that the thiol-disulfide balance is not disrupted and that prolidase levels are not affected in localized scleroderma; however, IMA is negatively affected.

Introduction

Localized scleroderma (LS, morphea) is a rare inflammatory skin disease that causes sclerosis in the dermis and subcutaneous tissue, with a pathogenesis that is not fully understood. Inflammatory phase manifests as erythematous purple patches and plaques, which later become white sclerotic lesions surrounded by a violet-colored halo [1]. It is more common in females and occurs in children between the ages of 2 and 14; in adults, it is more prevalent in middle age [2]. Environmental factors such as trauma, radiation, genetics, drugs and chemicals, infections, vascular dysregulation, autoimmunity, and oxidative stress can all play a role in its etiology [3,4].

Oxidative stress (OS) arises from an imbalance between the oxidant system and the antioxidant system in the organism, either due to excessive production of oxidant radicals or to a decrease in antioxidants or to both. This can lead to cell membrane damage, critical enzymes in intermediary metabolism and other proteins, and DNA constituents, resulting in tissue damage and necrosis in cells [5] . Consequently, they contribute to the onset or progression of many chronic diseases, including skin diseases [6]. Various parameters have been evaluated in studies aimed at understanding the impact of oxidative stress on skin diseases, including thiol-disulfide balance, ischemia-modified albumin (IMA), and prolidase [7-10]. Oxidant substances cause decarboxylation in intracellular proteins, hydrolysis of peptide bonds, and formation of disulfide cross-links [11].

Plasma thiols are potent antioxidants that physiologically neutralize free radicals [12]. Thiol groups are oxidized by reactive oxygen species (ROS), resulting in the reversible formation of disulfide bonds. Total thiol groups are highly sensitive to oxidations and are the first to be consumed during oxidative stress, thus neutralizing free radicals and preventing oxidative stress. These can be reduced back to thiol groups, depending on the situation [5,6,11]. This dynamic balance of thiol-disulfide is essential for critical cellular functions such as the antioxidant defense system, cellular transcription, cellular signal transduction, detoxification, enzymatic pathways, and apoptotic pathways [13]. Ischemia-modified albumin is a modified type of serum albumin that forms under oxidative stress conditions. This protein arises due to the reduced binding capacity of albumin's N-terminal region for cobalt, copper, and nickel following cellular events caused by ischemia [9,14]. Although primarily a marker for cardiac ischemia, it has been reported in various studies that IMA levels can also increase in various inflammatory conditions such as psoriasis and rheumatoid arthritis and in disorders related to oxidative stress [14-17]. Prolidase is an exopeptidase important in the collagen cycle abundantly found in human and animal tissues, playing a significant role in collagen turnover and cell growth [10,18]. It is involved in the synthesis of collagen within cells and the catabolism of proteins containing proline or hydroxyproline. It has been suggested for the detection of disorders in collagen metabolism. Due to its widespread tissue distribution, abnormalities in prolidase enzyme activity could be associated with many clinical conditions and play a role in disease progression, particularly in fibrotic diseases and chronic injuries, where serum prolidase activity varies and is affected by oxidative stress [10,18-21].

Recent studies have continued to investigate the role of oxidative stress in the pathogenesis of skin diseases, including scleroderma, which is associated with the fibrosis of connective tissue [8-10]. Various oxidant and antioxidant parameter levels have been measured in systemic scleroderma, and numerous hypotheses have been proposed [22-31]. However, these parameters have not been sufficiently studied in localized scleroderma, which has a similar pathogenesis [32,33]. Therefore, in this study, we aimed to investigate the presence of oxidative stress in LS by simultaneously measuring the levels of oxidative markers such as thiol-disulfide, IMA, and prolidase, and comparing them with a control group.

Methods

This a single-center prospective case-control study was approved by the local ethics committee, conducted in accordance with the principles of the Declaration of Helsinki (approval number: E2-21-476 /02.06.2021) and was carried out ethically. Written informed consent forms were obtained from patients and volunteers.

Twenty patients over the age of 18, diagnosed with LS clinically and histopathologically and followed up at our clinic, and 20 control subjects were included in the study. Age, sex, age at disease onset, duration of the disease, and presence of accompanying systemic diseases were questioned and recorded. Lesion type, modified Rodnan and LoSSI scores were calculated through dermatological examination [34,35]. (In the modified Rodnan score, the skin area is divided into a total of 17 different regions, and the score is calculated by manual palpation. Scoring is done according to the degree of thickness of the skin between 0 and 3; 0=Normal, 1=Mild thickening, 2=Moderate thickening, and 3=Severe thickening, and the maximum score is 51. The modified LoSSI score evaluates the skin erythema, thickness, and development of lesions or lesion enlargement in 18 anatomical skin areas of the body. It is calculated by adding scores between 0 and 3. The total score is between 0 and 162.) Individuals with chronic systemic diseases, neoplastic diseases, pregnant women, and those who had received systemic or topical drug treatment in the preceding three months were excluded from the study. The control group was selected from healthy individuals coming in for general check-ups. Laboratory tests included CRP sedimentation rate, total thiol, native thiol, and disulfide levels indicated by the Erel profile, IMA level, and prolidase levels.

Participants provided venous blood samples after 12 hours of fasting, which were then centrifuged at 1500 rpm for 10 minutes. The separated serum samples were frozen and stored at -80 °C until analysis. All oxidative parameters were studied in the same samples.

Serum native and total thiol concentrations and disulfide to native and total thiol ratios were determined by the spectrophotometric Erel method using automatic clinical chemical analyzer (Roche, Cobas 501, Mannheim, Germany) [13]. Functional thiol groups were exposed using sodium borohydride. Unused reduced sodium borohydride was inhibited by the reduction of 5,5'-dithiobis-(2-nitrobenzoic) acid (DTNB) with formaldehyde. The total thiol groups formed by reduced and native thiol groups were determined after reaction with DTNB. The dynamic disulfide amount was found by dividing the difference between total thiol and native thiol by two. Ratios of disulfide/total thiol, disulfide/native thiol, and native thiol/total thiol were calculated, and the results were reported in absorbance units (ABSU) [36]. The Bar-Or method measures the IMA content with a 0.1% cobalt binding. After 10 minutes of incubation with cobalt, dithiothreitol was added. Finally, a 0.9% sodium chloride solution was added, and the absorbance was measured using a spectrophotometer.

Prolidase activities were measured by Myara method [37]. A commercial kit (Human Xaa-Pro Dipeptidase/Prolidase Enzyme-linked Immunosorbent Assay kit (Cusabio Biotech Co Ltd) was used with ELISA method. (Myara method: The enzyme was activated by preincubation with MnCl2 for 2 hours (step 1), incubated with Gly-Pro substrate (Km = 2.9 mM) for 30 minutes (step 2), enzyme activity was calculated by measuring the amount of proline released (Ninhidrin rxn) (step 3).)

Statistics

Statistical analysis presented data as counts, arithmetic means, and standard deviations. The differences in average values between two groups were investigated using the Student's t-test for parametric values and the Mann-Whitney U test for non-parametric values. The relationship between patients' parameters and their demographic and clinical features was evaluated using Pearson correlation analysis. A p-value of <0.05 was considered statistically significant in all tests. Data were analyzed using the SPSS 20.0 software package.

Results

The average age was 40.24 ± 14.77 in patients and 37.86 ± 10.98 in controls, and 85% of both the patient and the control groups were female (n=17). The patient and control groups were similar in terms of age and sex (P=0.706). Comparison of serum parameter levels between patient and control group. Levels of native thiol (P=0.958), total thiol (P=0.979), disulfide (P=0.449), disulfide/native thiol% (P=0.368), disulfide/total thiol % (P=0.361), native thiol/total thiol % (P=0.368), and prolidase (P=0.121) were similar in both patient and control groups. Only IMA was significantly different (Table 1). While a negative correlation was observed between native thiol, total thiol, and age, no significant correlation was observed between disulfide, disulfide/native thiol rates, disulfide/total thiol rates, IMA, and prolidase levels and age. While a negative correlation was observed between native thiol, total thiol, and disease duration, no significant correlation was observed with disulfide, disulfide/native thiol rates, disulfide/total thiol rates, IMA, and prolidase levels. No significant relationship was found between the levels of native thiol, total thiol, disulfide, disulfide/native thiol rates, disulfide/total thiol rates, IMA, prolidase, and Rodnan and LoSSI scores. Native thiol (P<0.001, r= -0.538) and total thiol (P<0.001, r= -0.553) were negatively correlated with age. Native thiol and total thiol levels decreased with increasing age. Native thiol (P=0.008, r= -0.574) and total thiol (P=0.007, r= -0.583) were negatively correlated with disease duration. Native thiol and total thiol levels decreased with increasing disease duration. Native thiol and total thiol levels decreased with increasing disease duration (Table 2).

Table 1. Native Thiol, Total Thiol, Disulfide, Disulfide/Native Thiol (%), Disulfide/Total Thiol (%), IMA, Investigation of the Relationship between Prolidase Levels and Demographic and Clinical Characteristics of Patients.

Comparison of mean parameters between patient and control groups			
Parameters	Patient Mean ± sd	Control Mean ± sd	p-value (Mann-Whitney U Test)
Native thiol (µmol/)	424.61 ± 71.57	427.88 ± 62.84	0.958
Total thiol (µmol/L)	468.99 ± 77.19	473.55 ± 66.46	0.979
Disulfide (µmol/L/)	22.19 ± 8.72	.	0.449
Disulfide/Native thiol (%)	5.28 ± 2.15	5.39 ± 1.18	0.368
Disulfide/Total thiol (%)	4.71 ± 1.71	4.84 ± 0.98	0.361
Native Thiol/Total thiol (%)	90.56 ± 3.43	90.30 ± 1.97	0.368
IMA(ABSU)	0.63 ± 0.18	0.88 ± 0.22	**0.001**
Prolidase(U/L)	1015.87 ± 31.34	994.91 ± 50.78	0.121

Table 2. The Correlation between Parameters and Age, Disease Duration, and Scoring.

	Age r value p-value	Duration of disease r value p-value	Rodnan r value p-value	LoSSI r value p-value
Native thiol (µmol/L)	-0.538 **<0.001**	-0.574 **0.008**	-0.051 0.833	0.056 0.814
Total thiol (µmol/L)	-0.553 **<0.001**	-0.583 **0.007**	-0.011 0.963	0.110 0.645
Disulfide (µmol/L)	-0.252 0.112	-0.222 0.346	0.158 0.506	0.255 0.278
Disulfide/native thiol (%)	-0.007 0.966	0.011 0.963	0.136 0.567	0.193 0.414
Disulfide/total thiol (%)	0.001 0.993	-0.014 0.954	0.135 0.571	0.198 0.403
Native thiol/total thiol (%)	-0.001 0.995	0.014 0.954	-0.135 0.571	-0.198 0.403
IMA (ABSU)	-0.188 0.240	0.074 0.756	0.219 0.354	0.338 0.145
Prolidase (U/L)	-0.265 0.094	-0.089 0.709	0.053 0.823	0.013 0.958
Rodnan	0.231 0.313	-0.065 0.778		
LoSSI	0.100 0.666	-0.198 0.390		

Abbreviations: R: correlation coefficient.

Discussion

In morphea, the pathological process starts in the vascular endothelium. Excessive release of various adhesion molecules on the endothelial cell surface triggers an inflammatory cascade involving proinflammatory cytokines and chemokines as well as growth factors with fibrogenetic potential. Transforming growth factor β (TGF-β) stimulates fibroblast hyperactivation that exhibits abnormal profibrotic activity, increases collagen synthesis, and decreases collagenase synthesis [1,3,23,38]. ROS in the environment directly or indirectly mediate extracellular and intracellular oxidative processes and stimulate the accumulation of matrix proteins by affecting endothelial cells and fibroblasts [24,28,29].

Murrel revealed the effect of OS in the pathogenesis of scleroderma for the first time [39]. Several subsequent studies

have supported this hypothesis for both localized and systemic scleroderma, but the specific role of OS in the pathogenesis of vascular injury and fibrosis has yet to be elucidated [23,27,31,40-42]. ROS are considered to play a background role in the pathology of this disease and are considered to be instrumental in the manifestation of clinical symptoms. ROS generated by various reactions can stimulate the production of proinflammatory cytokines. Fibrotic cytokines induce proliferation and activation of fibroblasts, increase type I collagen synthesis, and cause vascular dysfunction. Skin changes typical for scleroderma have been demonstrated in vivo by intradermal injection of ROS-producing compounds in several animal studies [23]. There are studies showing that antioxidant capacity decreases in patients with systemic scleroderma [27,30,42]. Bozkurt et al. found that serum total oxidant capacity and oxidative stress index (OSI) were high in patients with systemic scleroderma. They also reported that prolidase activity is also high in fibrotic diseases, and this may be an important marker for OS [18].

LS, which is a localized form of scleroderma, is thought to have a common pathogenesis; therefore, similar studies on OS have also been conducted in LS, albeit to a lesser extent. The researchers investigated OS parameters in both tissue and plasma [32,33]. In these studies, it was attempted to prove the presence of OS in LS, with significant changes in OS parameters such as serum S glutathione transferase and total oxidant capacity compared to controls.

Based on the same hypothesis, we investigated thiol/disulfide, IMA, and prolidase enzymes, which are among the OS parameters thought to play a role in the pathogenesis of LS. Unlike other studies, these parameters were measured simultaneously in patient sera and compared with the control group. Thiols are antioxidants that react quickly in oxidative environments. In various studies, it has been reported that disulfide levels increase in inflammatory diseases and decrease in malignant diseases [6,8,26]. Therefore, it has been stated that the increase in disulfide concentrations is associated with OS, and the increase in native thiol levels may be an indicator of a reaction against the oxidative environment [6].

Kılınç et al. investigated total oxidant capacity (TOC), OSI, and arylesterase as OS markers in LS patients in a previous study using a control group; they found a significant increase in the patient group [32]. Uzun Çakmak et al. found that glutathione S-transferase isoenzymes were significantly higher in tissue biopsies obtained from patients with LS compared to control tissues [33].

In this study, when we compared LS patients with the control group, we found that the native thiol, total thiol, disulfide values, and their ratios to each other did not change, i.e., the thiol-disulfide balance was not impaired. Prolidase activity levels were similar to controls. IMA levels were lower in the patient group ($P<0.05$).

Riccieri et al. suggested that the levels of carbonyl groups, which are biomarkers of protein oxidation, are inversely correlated with the modified Rodnan skin score used for skin symptoms in systemic scleroderma [43]. In this study, no significant correlation was found between the LoSSI and Rodnan scales showing lesion severity in LS and thiol, IMA, and prolidase levels. This may be influenced by the low patient scores in our study. In addition, we think that the low IMA levels in the study may be due to the absence of ischemia in the LS. In conclusion, this evaluation shows that LS is not affected by oxidative stress as much as systemic scleroderma due to its limited involvement or it can limit itself with other antioxidant systems.

It could be argued that the aging process potentially affects OS levels. For example, several studies have shown that aging cells accumulate increasing levels of oxidant-damaged DNA, which can further increase the level of OS. The mean age of the patients in this study was 40.24 years. It was observed that native and total thiol values decreased with increasing age. The decrease in these values with age supports that while the production of free radicals increases with aging, endogenous defense mechanisms decrease and the balance shifts to the OS side [44]. There was no significant difference between IMA and prolidase levels and age. We can say that these enzymes are not affected by age.

In this study, a negative correlation was observed between native thiol and total thiol and disease duration. Native thiol and total thiol levels decreased with increasing disease. This may indicate that OS increases as the disease duration increases, or that the disease becomes chronic as OS increases. This negative correlation suggests that the decrease in native and total thiols may be associated with the chronicization of the disease and that these parameters may be a marker. However, there was no significant difference between IMA and prolidase levels and disease duration.

IMA is related to OS caused by free radicals during ischemia and/or reperfusion. Although most biomarkers are negative in ischemia, IMA is highly sensitive and can be detected at an early stage [14,45]. Therefore, it has been suggested that serum IMA levels may be used as a marker of systemic oxidative stress. Elevated IMA levels have been reported in various clinical pathologic conditions including vascular endothelial cell disorder, diabetes mellitus, liver and kidney diseases, some cancers, and systemic sclerosis in which oxidative status is affected [9,46,47]. Some investigators have shown that vasculopathy in scleroderma causes tissue hypoxia, resulting in increased OS, fibrotic changes, and skin thickening [24]. It has been suggested that it may be distinctive especially during the active period of the disease. In our study, IMA levels were significantly lower compared to controls. This may suggest that LS is not affected by ischemia-induced OS.

Prolidase is an enzyme involved in collagen metabolism. It is widely distributed in many tissues such as bone, connective tissue, and blood cells. Various studies suggest that prolidase could play a significant role in many physiological and pathophysiological processes, including wound healing, inflammation, and angiogenesis through the regulation of growth factors and transcription factors [10,38]. Therefore, it has been proposed that abnormalities in prolidase enzyme activity could play a role in the progression of many diseases [10]. Yıldız et al. have indicated that serum prolidase activity is directly related to oxidative stress (OS) and can be used as an indicator of oxidative stress [20]. Oxidative stress leads to the degradation of collagen, a process regulated by prolidase [19]. Additionally, the severity of OS is directly linked to the inhibition of collagen structure, and prolidase is considered the main enzyme in this process [10]. Various studies to date have shown that collagen, being widely found in many tissues, undergoes changes during the course of various diseases [18]. Particularly in scleroderma with organ involvement and fibrosis, prolidase levels have been found to be high [38]. However, Çelik et al. reported no difference in serum prolidase activity between patients with scleroderma and controls [40]. Savaş et al., in their study, found low serum prolidase levels, suggesting this could be due to a decrease in collagen turnover, and possibly a result of increased synthesis and decreased degradation. They also proposed that this could affect the reduced physical functions in patients [48].

We also investigated prolidase levels in LS, a localized form of systemic scleroderma, and found no change. This could be due to the localized nature of the disease, not involving systemic spread, or possibly due to the small number of patients.

Conclusion

Localized scleroderma is a multifactorial disease with an unknown cause. Based on the data obtained from our study related to OS, we can say that the thiol-disulfide balance is not disrupted and prolidase levels are not affected in LS. However, IMA is negatively affected.

Despite factors such as age and duration, the oxidative balance is not disturbed, suggesting that the organism can correct itself with other antioxidant systems. Further studies are needed to support these findings.

We believe that new and comprehensive studies in the future may elucidate the pathogenesis of LS.

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