

# Assessment of dynamic thiol-disulfide homeostasis in patients with lipoid proteinosis (Urbach-Wiethe syndrome)

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## SUMMARY

**OBJECTIVE:** Lipoid proteinosis is a rare autosomal recessive genetic dermatological disease that occurs due to the accumulation of hyaline material in the skin and mucous membranes. This study aimed to investigate whether dynamic thiol-disulfide homeostasis is a new marker of oxidative stress in patients suffering from lipoid proteinosis.

**METHODS:** The study group involved 17 patients with lipoid proteinosis and 17 healthy controls with same gender and age. Native thiol, total thiol, disulfide levels, and thiol-disulfide indexes were measured with the fully automated spectrophotometric method described by Erel and Neselioglu, and the results of the two groups were statistically analyzed.

**RESULTS:** Serum total thiol and native thiol levels were significantly lower in lipoid proteinosis group compared to the control group ( $p=0.020$  and  $p=0.014$ , respectively). The disulfide levels were found to be higher in lipoid proteinosis group, but there was no significant difference between two groups.

**CONCLUSIONS:** Impaired dynamic thiol-disulfide homeostasis was observed in lipoid proteinosis patients, suggesting that thiol-disulfide homeostasis may have a role in the pathogenesis of this disease.

**KEYWORDS:** Lipoid proteinosis. Mucous membrane. Oxidative stress. Thiol. Disulfides.

## INTRODUCTION

Lipoid proteinosis, also known as Urbach-Wiethe syndrome, is an infrequent genetically inherited disease, characterized by the accumulation of amorphous hyaline substance in various parts of the body, including the skin and mucous membranes<sup>1</sup>. In the first year of life, it usually presents with hoarseness, which occurs as a result of the accumulation of hyaline-like substances in the vocal cords<sup>2</sup>. In addition, it causes typical symptoms such as oral erosions, thickened eyelid papules, skin lesions, and sublingual frenulum in the following years<sup>3</sup>. This disease is a multisystem disease that includes not only dermatological symptoms but also neurological and psychiatric symptoms<sup>4</sup>.

It has been reported that the mutated extracellular matrix protein 1 (*ECM1*) gene plays a role in the pathogenesis of lipoid proteinosis disease<sup>5</sup>. The *ECM1* gene mediates a critical role in several biological activities, such as angiogenesis, cell adhesion, and cell differentiation. It also contributes to the preservation of the structural integrity and functions of the skin<sup>6</sup>. Mutations in this gene cause ECM1 protein not to be produced or loss of function in the produced proteins<sup>5</sup>. Non-functional ECM1

protein causes pathological changes in homeostatic balance. This results in the formation of clinical manifestations typical of lipoid proteinosis disease<sup>7</sup>. Although it is stated that lipoid proteinosis occurs due to genetic reasons, information about the pathophysiology of this disease is quite limited.

Besides genetic and environmental factors, deterioration in oxidant-antioxidant balance has been demonstrated to have an significant role in the onset and progression of several diseases<sup>8,9</sup>. This process, which is reported as oxidative stress, can be assessed using several biochemical factors<sup>10</sup>. Thiol-disulfide homeostasis is a novel and important systemic marker of oxidative stress<sup>11</sup>. Thiols can easily react with free radicals due to their structure, disulfide bonds are formed as a result of this oxidation reaction. These reactions are reversible and reducible. Thus, the dynamic conversion between thiols and disulfide bonds helps maintain the intracellular redox environment<sup>12</sup>. Thiol-disulfide homeostasis has a crucial function in many biological activities, such as oxidative stress, programmed cell death, cellular signal transduction, antioxidant defense, and enzymatic activities<sup>13,14</sup>. In

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this study, we aimed to investigate whether dynamic thiol-disulfide hemostasis acts as a new marker of oxidative stress in lipoid proteinosis patients.

## METHODS

This study included 17 patients who were diagnosed with lipoid proteinosis as a result of clinical and histopathological examinations and 17 gender- and age-matched healthy control. Patient and healthy control who had a history of alcohol consumption / smoking; had an infectious disease, acute or chronic systemic diseases; and took drug or vitamin were excluded from this study. The study was approved by the Clinical Research Ethics Committee of the Harran University (approval number: 21/22/11). It was conducted as per the Declaration of Helsinki and Good Clinical Practice guidelines. The informed consent form was obtained from all participants or their parents.

Blood specimen from the volunteers (lipoid proteinosis patients and healthy controls) were collected following at least 8 h of fasting into biochemical tubes and centrifuged at 1500g for 10 min, and then the separated serum specimens were stored in the freezer at -86°C until to analyze thiol-disulfide homeostasis. Serum thiol-disulfide homeostasis parameters were evaluated using the methods described by Erel and Neselioglu<sup>11</sup>. Total thiol and native thiol levels were measured directly. Then, disulfide levels were obtained by calculating the half of difference between the total and native thiol content. The oxidized thiol ratio (disulfide/total thiol), reduced thiol ratio (native thiol/total thiol), and thiol oxidation-reduction (disulfide/native thiol) ratios were also computed. At the end of these measurements and calculations, six parameters were obtained. Native thiol, total thiol, and disulfide levels were represented as  $\mu\text{mol/L}$ .

## Statistical analyses

SPSS program version 25.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Pearson's chi-square analytic method was used to compare categorical variables. Data obtained from this study were investigated using Shapiro-Wilk or Kolmogorov-Smirnov test to determine whether or not they are normally distributed. The Student's t-test was used to compare continuous variables with a normal distribution, whereas the Mann-Whitney U-test was applied to comparisons of non-normally distributed variables. Values are expressed as mean $\pm$ standard deviation for normally variables and median (Q1–Q3) for non-normally variables. A p-value<0.05 was considered statistically significant.

## RESULTS

There were no statistically significant differences between lipoid proteinosis patients and control group in terms of age [17.0 (14–20.5) vs. 18.0 (10.5–21.5), p=0.704], gender distribution [F/M (%); 9/8 (52.9/47.1) vs. 12/5 (70.6/29.4), p=0.290], and body mass index (BMI) (22.25 $\pm$ 1.56 kg/m<sup>2</sup> vs. 21.50 $\pm$ 1.63 kg/m<sup>2</sup>, p=0.186). Characteristics of the study groups are represented in Table 1.

The native thiol and total thiol levels were significantly lower in lipoid proteinosis patients compared to the control group (216.58 $\pm$ 37.43  $\mu\text{mol/L}$  vs. 251.89 $\pm$ 41.83  $\mu\text{mol/L}$  and 324.56 $\pm$ 30.05  $\mu\text{mol/L}$  vs. 356.41 $\pm$ 44.10  $\mu\text{mol/L}$ ; p=0.014 and p=0.020) (Table 2, Figure 1). However, the mean serum level of dynamic disulfide was insignificantly increased in the lipoid proteinosis patients [51.58 (36.04–71.19)  $\mu\text{mol/L}$ ] compared to the control group [50.23 (44.73–62.45)  $\mu\text{mol/L}$ , p=0.931]. Moreover, oxidized thiol, which indicates oxidation, was higher in lipoid proteinosis patients compared to the control group, while the antioxidant indicator, reduced thiol, was found to

**Table 1.** Characteristics of the study groups.

	Lipoid proteinosis (n=17)	Control (n=17)	p-value <sup>a</sup>
Age (years)	17.0 (14-20.5)	18.0 (10.5-21.5)	0.704 <sup>b</sup>
Gender (F/M), n (%)	9/8 (52.9/47.1)	12/5 (70.6/29.4)	0.290
BMI (kg/m <sup>2</sup> )	22.25 $\pm$ 1.56	21.50 $\pm$ 1.63	0.186 <sup>c</sup>

<sup>a</sup>Data are expressed as median (Q1–Q3), numbers (%), and mean $\pm$ standard deviation where appropriate. <sup>b</sup>Obtained from Mann-Whitney U-test. <sup>c</sup>Obtained from independent-samples t-test.

**Table 2.** Thiol-disulfide levels and ratios of the lipoid proteinosis patients and control group.

	Lipoid proteinosis (n=17)	Control (n=17)	p-value <sup>a</sup>
Native thiol ( $\mu\text{mol/L}$ )	216.58 $\pm$ 37.43	251.89 $\pm$ 41.83	<b>0.014<sup>b</sup></b>
Total thiol ( $\mu\text{mol/L}$ )	324.56 $\pm$ 30.05	356.41 $\pm$ 44.10	<b>0.020<sup>b</sup></b>
Disulfide ( $\mu\text{mol/L}$ )	51.58 (36.04–71.19)	50.23 (44.73–62.45)	0.931 <sup>c</sup>
Oxidized thiol (%)	8.24 $\pm$ 3.04	7.36 $\pm$ 1.58	0.301 <sup>b</sup>
Reduced thiol (%)	33.51 $\pm$ 6.09	35.27 $\pm$ 3.16	0.299 <sup>b</sup>
Thiol oxidation-reduction (%)	11.85 (7.45–16.27)	9.74 (8.29–13.31)	0.524 <sup>c</sup>

Bold values indicate statistically significant at p<0.05. <sup>a</sup>Data are expressed as mean $\pm$ standard deviation and median (Q1–Q3) where appropriate. <sup>b</sup>Obtained from independent-samples t-test. <sup>c</sup>Obtained from Mann-Whitney U-test.

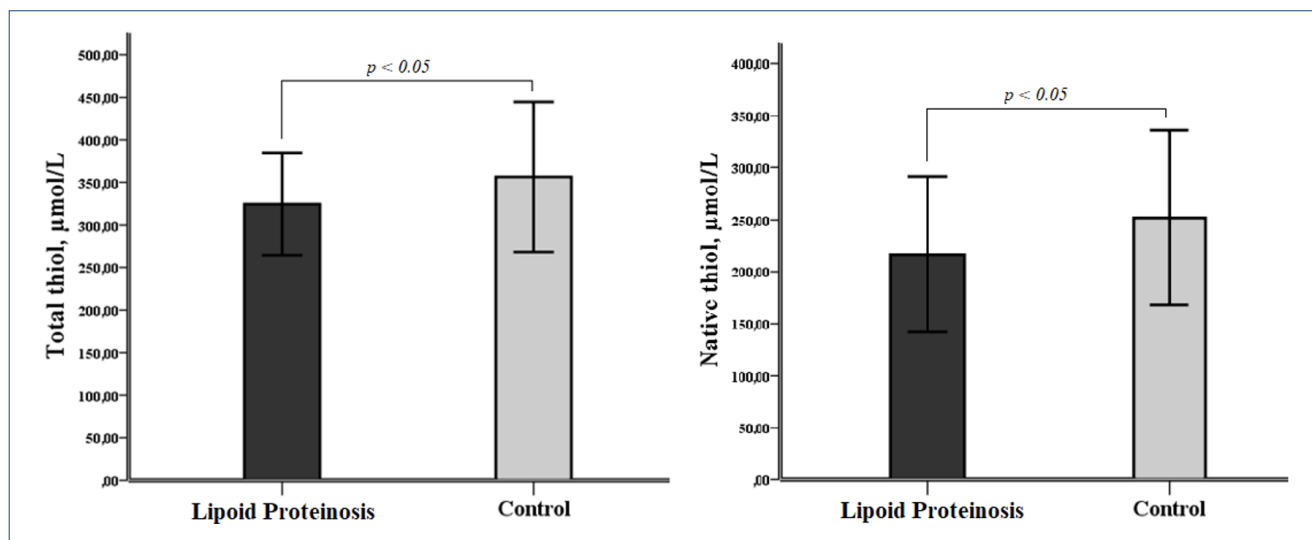


Figure 1. Total thiol and native thiol levels of lipid proteinosis patients and control group.

be lower, but these changes were not statistically significant. Thiol oxidation-reduction ratio was higher in lipid proteinosis patients than in the control group, but it was not statistically significant (Table 2).

## DISCUSSION

Lipoid proteinosis is a very rare disease worldwide. It is autosomal recessive genodermatosis that occurs due to the accumulation of hyaline-like material in the skin and mucous membranes due to mutations in ECM1<sup>5</sup>. Although the mutated gene causing lipid proteinosis has been identified, the exact mechanism underlying this inherited disorder is still unknown. It is known that oxidative stress is effective in the pathophysiology of many diseases<sup>15</sup>. To the best of our knowledge, there is no study evaluating the oxidative stress from the perspective of thiol-disulfide homeostasis in patients with lipid proteinosis. Our results show that thiol-disulfide homeostasis shifts toward the oxidative side in lipid proteinosis patients.

The shift of changes in the cellular redox state toward the oxidation direction is defined as oxidative stress. And this condition is an important risk factor for the development of various pathologies in the structure and function of many organs<sup>15</sup>. Dynamic thiol-disulfide homeostasis is frequently preferred as a new and important marker of oxidative stress<sup>11</sup>. This technique can be used as a reliable, practical technique to evaluate oxidative stress<sup>16</sup>.

There are studies reporting that dermatological diseases cause changes in thiol-disulfide homeostasis. Sener et al. reported that disulfide levels were increased in patients with rosacea<sup>17</sup>. Akdag et al., in a study with chronic urticaria patients, found

that native thiol and total thiol levels significantly decreased<sup>18</sup>. Another study revealed higher levels of native thiol and total thiol and lower levels of disulfide in atopic dermatitis<sup>8</sup>. Kilic et al., in a study with psoriasis patients, found that plasma disulfide levels significantly decreased and native thiol levels increased<sup>19</sup>. It has been reported that oxidative stress has a role in the pathophysiology of these dermatological diseases, whose clinical features, laboratory findings, and treatments are different from each other.

While there are studies on the role of inflammatory parameters, genetic tests, histopathological examinations, and radiological findings in the pathophysiology of lipid proteinosis disease, there is only one study reporting its relationship with oxidative stress<sup>10,20-23</sup>. Total oxidant status, lipid hydroperoxide, and advanced oxidation protein products are important oxidant parameters. Celik et al. stated that these oxidant parameters increased in the lipid proteinosis group compared to the control group. In the same study, it was stated that antioxidant parameters (total antioxidant status and ferric-reducing antioxidant power) decreased and oxidant antioxidant balance is impaired in patients with lipid proteinosis<sup>10</sup>.

Almost all of the physiological and biochemical reactions take place in a sensitive redox homeostasis. Thiol-disulfide level has a critical importance in providing and maintaining this homeostasis<sup>11,24</sup>. In our research, it was found that the total thiol and native thiol levels were decreased and disulfide levels were increased in the lipid proteinosis patients when compared to the control group. Thiols, which act as free radical scavengers, are primarily consumed as antioxidants in case of oxidative stress<sup>25</sup>.

Therefore, the decrease in the total thiol pool paves the way for an increase in oxidative stress. Thiol-disulfide balance in patients with lipoid proteinosis was found to be impaired and shifted to disulfide direction. These data indicate the presence of oxidative stress. Increased disulfide formation may cause structural and functional disorders at the cellular level. Thiol-disulfide balance is likely to have a role in etiopathogenesis of lipoid proteinosis.

## CONCLUSIONS

This is the first study to examine the relationship between lipoid proteinosis and oxidative stress from the perspective of a new measurement method, thiol-disulfide homeostasis. Our results show that thiol-disulfide homeostasis shifts toward the oxidative side in lipoid proteinosis patients. This study may contribute to the understanding of the etiopathogenesis of lipoid proteinosis. Further research studies with larger numbers of patients

are needed to define the role of thiol-disulfide homeostasis in the process of lipoid proteinosis.

## Limitation

The limitations of this study include the small number of patients. However, it should be considered that this disease is a very rare dermatological disease worldwide.

## AUTHORS' CONTRIBUTIONS

**ST:** Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. **HC:** Conceptualization, Formal Analysis, Writing – review & editing. **AT:** Formal Analysis, Writing – review & editing. **MA:** Conceptualization, Data curation, Formal Analysis, Writing – review & editing. **IA:** Conceptualization, Data curation, Formal Analysis, Writing – review & editing. **YY:** Data curation, Formal Analysis, Writing – review & editing.

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