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The evaluation of thiol-disulfide homeostasis in children with Triple-A syndrome

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Abstract. – OBJECTIVE: Triple-A syndrome occurs due to the dysfunction of the ALADIN protein as a result of a mutation in the *AAAS* gene. ALADIN is involved in redox homeostasis in human adrenal cells and steroidogenesis. It has also been shown to have important roles in DNA repair and the protection of cells against oxidative stress. We planned to investigate serum thiol/disulfide homeostasis, which is a part of redox hemostasis in patients with Triple-A syndrome.

PATIENTS AND METHODS: The study included patients with the Triple-A syndrome (26 patients) and healthy children (26 patients). Thiol and disulfide levels of patients and healthy groups were compared. In addition, patients with the Triple-A syndrome were divided into 2 subgroups according to the mutation type, and their thiol and disulfide levels were compared.

RESULTS: Triple-A syndrome patients had increased native thiol (SH), total thiol (SH+SS) concentrations, and native thiol/total thiol (SH/SH+SS) ratios than healthy controls. However, Triple-A syndrome patients had lowered disulfide (SS), disulfide/native thiol (SS/SH), and disulfide/total thiol (SS/SH+SS) ratios than the controls. When the group with the p.R478* mutation and the group with other mutation were compared, disulfide level, disulfide/native thiol ratio, and disulfide/total thiol ratio were statistically higher in the group with the p.R478* mutation, while native thiol/total thiol ratio was found to be lower. However, no statistical difference was found between native thiol and total thiol levels.

CONCLUSIONS: This is the first study in the literature to evaluate thiol-disulfide homeostasis in patients with the Triple-A syndrome. Patients with Triple-A syndrome had an increased level of thiol compared with healthy controls. Comprehensive studies are needed to clarify these thiol levels, which are thought to be compensatory. Also, mutation type affects thiol-disulfide levels.

Key Words:
Triple-A syndrome, Thiol/disulfide homeostasis, Antioxidants.

Introduction

Triple-A syndrome is a rare autosomal recessive syndrome characterized by primary adrenal insufficiency, achalasia, and alacrimia. Although it is a rare syndrome, it is common in societies with a high rate of consanguineous marriage, as in our country. Few large cohorts exist worldwide[1]. Triple-A syndrome occurs due to the dysfunction of the ALADIN protein as a result of a mutation in the *AAAS* gene. ALADIN plays a role in redox homeostasis in human adrenal cells and steroidogenesis. Its deficiency impairs redox homeostasis and inhibits steroidogenesis in human adrenal cells[2-4]. The *AAAS* mutation has been shown to cause decreased nuclear import of DNA ligase 1 and aprataxin, which are involved in DNA repair and protection of cells against oxidative stress[4-6]. These findings led to the idea that thiol-disulfide homeostasis, which is one of the other components of redox hemostasis (oxidant-antioxidant balance), should also be evaluated in patients with the Triple-A syndrome. Dynamic thiol-disulfide homeostasis plays a critical role in antioxidant protection, detoxification, and apoptosis and is of vital importance for the organism[7,8]. Until recently, only one side (thiol) of the thiol/disulfide balance could be measured, while today both sides of the equilibrium can be determined using the latest test methods and the thiol/disulfide balance can be completely evaluated[9,10]. Thiol-Disulfide homeostasis has never

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been investigated in the Triple-A syndrome until now, and our study will be the first in this area. Therefore, we planned this study to investigate the dynamic thiol-disulfide balance of 26 patients with Triple-A syndrome.

Patients and Methods

Subjects were enrolled after the medical Ethics Committee approved the study protocol (approval number 2019/321) and parents provided written informed consent. The study included patients with the Triple-A syndrome (26 patients), and healthy children (26 patients). The diagnosis of all patients with the Triple-A Syndrome was genetically confirmed (3 different mutations in the *AAAS* gene: p.R478* mutation, p.L356Vfs*8, or p.L134Gfs*5). After measuring the native thiol and total thiol levels of the patients, disulfide levels, disulfide/native thiol ratios, disulfide/total thiol ratios, and native thiol/total thiol ratios were calculated. Patients with the Triple-A Syndrome were compared with the healthy control group. Furthermore, patients with the Triple-A syndrome were divided into two subgroups based on the type of mutation (first group: p.R478* mutation, second group: p.L356Vfs*8 or p.L134Gfs*5 mutation), and their thiol and disulfide levels were compared.

Measurement of Thiol/Disulfide Homeostasis Parameters

Blood samples were obtained between 8 a.m. and 10 a.m. after 8-10 hours of fasting. The samples were then centrifuged at 1500 rpm for 10 minutes. Separated serum samples were immediately frozen and stored at -80°C until analyzed. All thiol/disulfide parameters were studied in the same samples. Serum concentrations of native and total thiol and ratios of disulfide, and native and total thiol were determined by a spectrophotometric method using an automatic clinical chemical analyzer (Roche, Cobas 501, Mannheim, Germany) as previously described by Erel and Neselioglu[10].

Statistical Analysis

In the statistical evaluation of the data, the statistics package for social sciences (SPSS) computer package program v. 26 was used (IBM, Corp., Armonk, NY, USA). The distribution of variables was evaluated by using the Kolmogorov-Smirnov/Shapiro-Wilk tests. Normally distributed data variables were compared with Student's *t*-test. Nonnormally distributed data variables were compared using the Wilcoxon rank test or the Mann-Whitney U test. The results were presented as mean ± SD. *p*-values less than 0.05 were accepted as the significance level.

Results

The distribution of boys and girls in the Triple-A syndrome group and healthy group was similar (Triple-A syndrome: 14 boys, 12 girls; healthy group: 14 boys, 12 girls). Triple-A syndrome patients had increased native thiol (SH), native thiol (SH+SS) concentrations, and native thiol/native thiol (SH/SH+SS) ratios than healthy controls ($p<0.001$, $p<0.001$ and $p<0.001$, respectively). However, the Triple-A syndrome patients had lowered disulfide, disulfide/ native thiol (SS/SH), and disulfide/native thiol (SS/SH+SS) ratios than the healthy controls ($p=0.037$ and $p<0.001$, $p<0.001$, respectively) (Table I). When the group with the p.R478* mutation and the group with the other mutation were compared, disulfide level, disulfide/native thiol and disulfide/total thiol ratio were statistically higher in the group with the p.R478* mutation, while native thiol/total thiol ratio was found to be lower. However, no statistical difference was found between native thiol and

Table I. Comparison of laboratory findings of the healthy group and Triple A syndrome group.

	Triple A group (n = 26) mean ± SD	Healthy group (n = 26) mean ± SD	p
Age	11.22 ± 4.74	11.26 ± 4.01	0.956
Native Thiol (µmol/l)	459.87 ± 59.98	382.52 ± 38.35	**< 0.001**
Total Thiol (µmol/l)	497.18 ± 59.59	423.15 ± 40.51	**< 0.001**
Disulfide (µmol/l)	18.65 ± 4.88	20.31 ± 2.25	**0.037**
Disulfide/Native Thiol (%)	4.14 ± 1.27	5.33 ± 0.60	**< 0.001**
Disulfide/Total Thiol (%)	3.80 ± 1.06	4.81 ± 0.49	**< 0.001**
Native Thiol/Total Thiol (%)	92.39 ± 2.13	90.36 ± 0.98	**< 0.001**

Table II. Comparison of laboratory findings according to the type of genetic variant.

	Group with p.R478* mutation (n = 19) mean ± SD	Group with other mutation[a] (n = 7) mean ± SD	p
Age	11.28 ± 4.74	11.06 ± 5.12	0.995
Native Thiol (µmol/L)	446.84 ± 56.66	495.21 ± 57.99	0.120
Total Thiol (µmol/L)	485.94 ± 56.58	527.69 ± 60.90	0.135
Disulfide (µmol/L)	19.54 ± 5.02	16.24 ± 3.77	**0.048**
Disulfide/Native Thiol (%)	4.45 ± 1.29	3.29 ± 0.74	**0.015**
Disulfide/Total Thiol (%)	4.06 ± 1.07	3.08 ± 0.64	**0.015**
Native Thiol/Total Thiol (%)	91.86 ± 2.15	93.83 ± 1.29	**0.015**

[a]Group with other mutation: p.L356Vfs*8 or p.L134Gfs*5.

total thiol levels (Table II). Comparison of thiol and disulfide levels of patients (Group with the p.R478* mutation and group with the other mutation) and healthy controls is shown in Figure 1.

Discussion

Antioxidants can be classified as endogenous antioxidants (glutathione, bilirubin, NADPH/NADP, enzymes such as glutathione peroxidase, catalase, superoxide dismutase, etc.), diet-derived antioxidants (Vit C, Vit E, Vit B carotene, polyphenols, etc.) and metal-binding proteins (albumin, ceruloplasmin, ferritin, transferrin, etc.). Antioxidants are further classified as enzymatic or non-enzymatic[11]. Organic compounds containing a sulfhydryl group are called thiols (-SH) and thiols are a part of the non-enzymatic antioxidant system. Thiols are highly susceptible to oxidation due to -SH groups, and thiol protein groups are important antioxidants that constitute 52.9% of total serum antioxidant capacity in healthy individuals[12]. Disruption of oxidant and antioxidant balance is called oxidative stress and it has been implicated in the etiopathogenesis of some diseases[11,13].

There is evidence of increased oxidative stress and impaired redox homeostasis in the Triple-A syndrome[2-4,14-16]. Therefore, in our study, we investigated the state of thiol-disulfide hemostasis, which is an important component of redox hemostasis. In previous studies[3,6,16], a mutation in the *AAAS* gene has been shown to cause an increase in reactive oxygen species, a decrease in sensitivity to oxidative stress, a decrease in cell viability, a decrease in reduced glutathione/oxidized glutathione ratio, and disorders in antioxidant enzymes (superoxide dismutase, catalase and, glutathione reductase). These studies reflect changes at the cytoplasmic or mitochondrial level in fibroblast cell cultures or tumoral cell lines. Unlike these studies, we evaluated thiols, which are an important part of plasma antioxidant levels. Albumin thiols and low molecular weight thiols such as cysteine, cysteinyl glycine, homocysteine, glutathione, γ-glutamylcysteine, and hydrogen sulfide make up the plasma thiol pool (the thiol pool in human plasma: the central contribution of albumin to redox processes). In our study, we showed an increase in both total native thiol and native thiol/total thiol (SH/SH+SS) levels, suggesting a compensatory increase in favor of antioxidants in plasma, in contrast to the deterioration in favor of increased oxidants at the cellular level shown in previous studies[3,6,16]. This increase in thiol levels (native thiol, total thiol, and native thiol/total thiol ratio) was interpreted as a compensatory increase in response to increased oxidative stress in patients with the Triple-A syndrome. In addition, this thiol increase can be explained by the compensated endogenous antioxidant/thiol increase in patients with the Triple-A syndrome due to malnutrition, achalasia and chronic disease, and to insufficient exogenous antioxidant intake (i.e., vitamin E, vitamin C, carotene, B12, and folate). However, studies evaluating all exogenous and endogenous antioxidant systems together are needed to confirm these relationships.

There was no difference in native thiol and total thiol levels between the group with the p.L356Vfs*8 or p.L134Gfs*5 mutation and the group with the p.R478* mutation. However, a significant difference was found between the disulfide level, disulfide/native thiol ratio, disulfide/total thiol ratio, and native thiol/total thiol ratio (p=0.048, p=0.015, p=0.015, and p=0.015, respectively). Although the clinics of patients with the p.R478* mutation and other mutations (p.L356Vfs*8 or p.L134Gfs*5) were similar, sufficient data were not found to explain this differ-

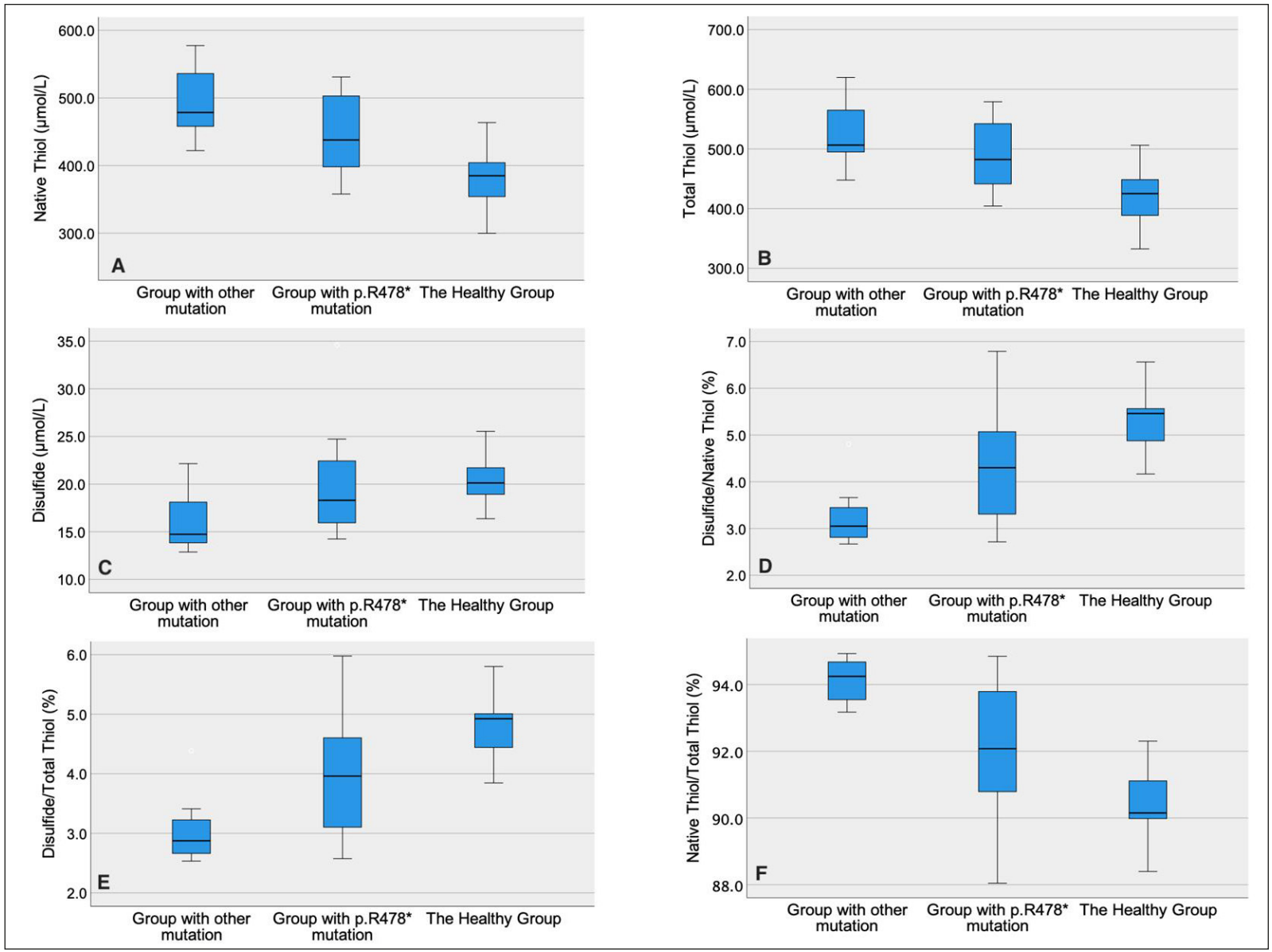

Figure 1. Native Thiol levels (**A**) and Total Thiol levels (**B**) were the lowest in the healthy group. Disulfide levels (**C**) were highest in the healthy group. Disulfide/Native Thiol ratio (**D**) and Disulfide/Total Thiol ratio (**E**) were lower in the patient group (lowest in those with p.L356Vfs*8 or p.L134Gfs*5 mutations). Native Thiol/Total Thiol (%) (**F**) was higher in patients with Triple A syndrome.

ence in thiol level. The type of mutation (termination or frameshift), the length of the mutant protein, or the location of the mutation, on the other hand, may be effective in this. Further studies are needed in this regard.

Limitations

The current study has two limitations: a small study population (due to the rarity of Triple-A syndrome) and no comparison of thiol/disulfide homeostasis parameters with other enzymatic and non-enzymatic measures of oxidative stress.

Conclusions

This is the first study in the literature to evaluate thiol-disulfide homeostasis in patients with Triple-A syndrome. Patients with the Triple-A syndrome had an increased level of thiol compared with healthy controls. Comprehensive studies are needed to clarify these thiol levels, which are thought to be compensatory. Also, mutation type affects thiol-disulfide levels.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Informed Consent

Informed consent was obtained from all participants or their first-degree relatives in this study.

Availability of Data and Materials

The datasets are available from the corresponding author on reasonable request.

Authors' Contribution

RP, AU, and ÖE conceived the idea. RP, IT, and RY conducted the experimental work. RP, RY, IT, ÖE, and AU participated in writing, discussion, and data analysis. All authors approved the final version of the publication.

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Ethics Approval

The study was conducted according to the principles of the Declaration of Helsinki and approved by the Institutional Ethical Review Board (Ethics Committee of the Faculty of Medicine, approval number: 2019/321).

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