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Original Research

Dynamic Thiol/ Disulfide Balance in Children with Acute Malnutrition

Mahmut Demir[1], Özcan Erel[2]

[1]Department of Pediatrics, Harran University School of Medicine, Şanlıurfa, Turkey

[2]Department of Biochemistry, Yıldırım Beyazıt University School of Medicine, Ankara, Turkey

ABSTRACT

Objective: In the human body, a balance exists between the production of free radicals and suppression of increased levels of reactive oxygen species by the antioxidant system. If this balance is disturbed, oxidative stress occurs. Free radicals produced during normal metabolism or pathological processes affect the structure and functions of thiol-dependent enzymes and the thiol/ disulfide balance in the cell environment. The aim of our study was to evaluate the dynamic thiol/disulfide balance in children with acute malnutrition.

Methods: Fifty-two patients diagnosed with acute malnutrition according to Waterlow classification and 40 healthy children were included in the study. The weight, weight Z score, height, height Z score, and BMI of the patients in the study group were measured. The thiol/disulfide balance was measured in both groups using the automatic method developed by Erel and Neselioğlu.

Results: There was no statistically significant difference between the patient and control groups in terms of average native thiol and total thiol levels. The disulfide levels and the disulfide/native thiol and disulfide/total thiol ratios were significantly higher in the patient group than in the control group, whereas the native thiol/total thiol ratio was significantly lower in the patient group than in the control group.

Conclusion: Our findings indicate that the thiol/disulfide balance is weakened in children with acute malnutrition and is shifted toward disulfide.

Keywords: Children, malnutrition, oxidative stress, thiol/ disulfide balance

INTRODUCTION

Malnutrition is a clinical condition that arises due to inadequate intake of one or more nutrients (1). Malnutrition is defined as normal body weight by age, height by age, and/or body weight by height below -2 SD due to lack of protein and/or energy. Malnutrition is a major health problem in underdeveloped and developing countries where a significant proportion of the world's population resides (2). In our country, it continues to be an important health problem affecting the pediatric age group. Although it is easy to diagnose patients with severe malnutrition, it is quite difficult to diagnose patients with mild or moderate malnutrition. It is important to detect mild and moderate malnutrition to prevent their progression to chronic and severe malnutrition. Several methods have been developed or are being developed for earlier diagnosis of malnutrition (3).

Different markers are used to evaluate the oxidant/antioxidant balance in the body (4, 5). The determination of thiol/disulfide balance is a new method developed by Erel et al. (6), which is used in the evaluation of oxidant/antioxidant balance. Thiol balances oxidative stress by reducing the levels of reactive oxygen species or by accelerating their inactivation. The reactive oxygen species and thiol groups present in the environment are oxidized and transformed into reversible disulfide bonds. The disulfide is oxidized by oxidizing molecules in the environment and converted into reversible bond structures. The resulting disulfide bond structures can be reduced back to the thiol groups, thereby maintaining the thiol-disulfide balance. The thiol-disulfide balance plays a critical role in antioxidant defense, detoxification, apoptosis, regulation of enzyme activities, and mechanisms of transcription and cellular signal transduction (7, 8).

The purpose of the study was to investigate the thiol/disulfide balance, a recently discovered indicator of oxidative stress affected by decrease or increase in catabolic processes, in patients affected by acute malnutrition.

METHODS

Children who were admitted to the general pediatric outpatient clinic with the complaint of developmental retardation and whose weight was below 3% percentile were included in this study. Percentage charts developed by Olcay Neyzi et al. (9) were used for weight and height measurements. The weight, weight Z score, height, height Z score, and BMI (Body Mass Index) of the patients were calculated. Waterlow classification was used as malnutrition criterion (10) (Table 1). A detailed medical history of

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Corresponding Author: Mahmut Demir **E-mail:** mahdem81@yahoo.com

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all the children included in the study was received and detailed physical examinations and laboratory examinations were carried out. The approval of ethical committee was received. Informed consent was obtained from the parents of all the children included in the study.

Exclusion Criteria

Children with acute infections, tuberculosis, congenital abnormalities, epilepsy, mental motor retardation, diabetes mellitus, hypothyroidism, celiac disease, and acute and chronic diseases of organs such as the kidney, lung, liver, and heart were excluded from the study.

Blood Samples

Before the start of the study, a complete blood count of patients and children in the control group was carried out using automatic blood count device (Abbot Cell dyn 3500 III, USA). Blood samples of the patients selected for the study were centrifuged at 3500 rpm for 10 minutes, following the shaped elements were discarded along with the tube. Some of the serum samples obtained as supernatants were stored at -80°C until used for detection of dynamic kinetic thiol/disulfide levels. On the same day, the remaining serum samples were used for the determination of electrolyte, Thyroid Stimulating Hormone (TSH), free T4, tissue transglutaminase IgA, endomysium IgA, CRP, vitamin D, vitamin B12, and folic acid levels and for kidney and liver function tests.

Measurement of Thiol / Disulfide Balance Parameters

The thiol/ disulfide balance tests were conducted using a novel method developed by Erel & Neselioglu (6). The serum thiol/disulfide balance parameters were measured using an automated clinical chemistry analyzer (Roche, Cobas 501, Mannheim, Germany). The amount of disulfide was calculated using the formula: (total thiol-native thiol)/2. After the native (SH) and total thiol (-SH +-S-S-) levels were determined, the disulfide levels (SS) and reduced thiol (native thiol/total thiol), oxidized thiol (disulfide/total thiol), and thiol oxidation-reduction (disulfide/native thiol) ratios were obtained by mathematical calculation (6).

Main Points:

- Dynamic thiol/ disulfide balance has critical roles in antioxidant protection, detoxification, signal transmission, apoptosis, enzyme activity, and regulation of cellular signal mechanism by transcription factor.
- The determination of thiol/disulfide balance is a new method developed by Erel et al. which is used in the evaluation of oxidant/antioxidant balance.
- Thiol groups function as antioxidants and regulate the redox system. Increased thiol levels indicate antioxidant defense mechanism.
- Increased disulfide levels, disulfide/total thiol, and disulfide/native thiol ratios indicate increased oxidative stress.
- The thiol/disulfide balance is weakened in children with acute malnutrition and is shift toward disulfide. As a result, it was found that total oxidative stress activity increased and total antioxidant levels decreased compared to the healthy group.

Statistical Analysis

Statistical analyzes were performed using Number Cruncher Statistical System (NCSS) 2007 software (Kaysville, Utah, USA). While evaluating the study data, the differences among the groups were examined using the Student's t-test and Pearson's chi square test. Spearman's correlation analysis was used for evaluating correlations the relationships between variables and thiol/disulfide balance . The limit value for statistically significant differences was 0.05.

RESULTS

In our study, 24 of the 52 patients with acute malnutrition were male (46.2%) and 28 were female (53.8%). The mean age in the patient group was 88.54±55.71 months. Of the 40 children included in the study as control group, 21 were male (52.5%) and 19 were female (47.5%). The mean age of the control group was 75.15±49.50 months. No statistically significant difference was detected between the two groups in terms of age and gender ($p>0.05$) (Table 2).

While there was no significant difference between the two groups regarding length measurements ($p>0.05$), there were significant differences with respect to height Z score, weight, weight Z score, and BMI ratios ($p<0.05$) between the two groups (Table 2).

The thiol/disulfide balance parameters of both groups are shown in Table 3. The average native thiol levels in patients

Table 1. Waterlow classification used in malnutrition

Degree of malnutrition	Waterlow Classification	
	Wasting (%) Weight for height	Stunting (%) Height for age
Normal: Grade 0	90–110	>95
Mild: Grade 1	80–89	90–94
Moderate: Grade 2	70–79	85–89
Severe: Grade 3	<70	<85

Table 2. Demographic characteristics of the study population

	Acute malnutrition (n=52)	Control (n=40)	p
Age (month)	88.54±55.71	75.15±49.50	0.234
Gender; n (%) Male	24 (46.2)	21 (52.5)	0.546
Female	28 (53.8)	19 (47.5)	
Weight (kg)	18.07±9.27	24.30±12.96	0.012*
Weight Z score	–1.98±0.51	0.35±0.48	0.001*
Height (cm)	110.40±24.89	115.65±23.99	0.340
Height Z score	–1.95±0.69	0.68±0.73	0.001*
BMI (kg/m^2)	13.91±1.57	17.00±1.95	0.001*

Parameters are expressed as means ± standard deviation; BMI: Body mass index;
*$p<0.05$ was considered statistically significant.

and control groups were determined as 388.48±59.74 µmol/L and 407.03±61.20 µmol/L, respectively. There was no significant difference in the average native thiol levels between the two groups (p=0.140; p>0.05). Total thiol levels in patient and control groups were determined as 461.42±60.21 µmol/L and 444.05±61.74 µmol/L, respectively. There was no significant difference between the two groups with respect to the total thiol levels (p=0.190; p>0.05). The disulfide levels were significantly higher in the patient group than in the control group (p=0.001; p<0.05). The disulfide/native thiol and disulfide/total thiol ratios were found to be significantly higher in the patient group than in the control group (p=0.001; p<0.05) (p=0.049; p<0.05). Conversely, the native thiol/total thiol ratio was found to be significantly lower in the patient group than in the control group (p=0.001; p<0.01). The results for correlation analysis of thiol/disulfide balance parameters and other parameters of the patient group are shown in Table 4.

Table 3. Thiol/disulfide balance parameter levels among groups

	Acute malnutrition (n=52)	Control (n=40)	p
Native thiol (µmol/L)	388.48±59.74	407.03±61.20	0.140
Total thiol (µmol/L)	461.42±60.21	444.05±61.74	0.190
Disulfide (µmol/L)	36.47±18.08	18.51±7.31	0.001*
Disulfide/ Native thiol (%)	9.92±5.89	4.66±2.01	0.001*
Disulfide/ Total thiol (%)	7.93±3.67	4.20±1.66	0.001*
Native thiol/ Total thiol (%)	84.15±7.33	91.59±3.33	0.001*

Parameters are expressed as means ± standard deviation.
*p<0.05 was considered statistically significant

DISCUSSION

Malnutrition is a pathological condition that arises due to deficient or unbalanced intake of one or more nutrients, which leads to protein deficiency or energy deficiency or both (11).

Free oxygen radicals are synthesized in the body in small amounts during normal metabolism without causing any harm to the body. However, when the body is affected by viral diseases or exposure to ionizing radiation and environmental pollution, then free oxygen radicals are produced in excessive quantities, resulting in oxidative stress (12).

There is a balance between production of free radicals and suppression of the increase in reactive oxygen species levels in the body by the antioxidant system. If this balance is disturbed, then oxidative stress occurs. In previous studies on oxidative stress, levels of antioxidant enzymes such as malondialdehyde, glutathione peroxidase, and catalase were measured (12). In these studies, it was found that serum oxidative stress level is higher in patients with malnutrition than in the control group and that the antioxidant capacity is low in patients with malnutrition (13). In another study, both total serum oxidative capacity and total antioxidant capacity were lower in malnourished patients than in the control group (14). It was thought that this might be related to the decrease in endogenous free radical production due to body metabolic processes and to slowing of energy consumption in malnutrition (14). Recently, the plasma thiol/disulfide balance, measured using a new method developed by Erel and Neselioglu in 2014 (6), was used to obtain information about oxidative stress. We also used this method to examine the thiol/disulfide homeostasis in our study.

Table 4. Correlation analysis of thiol/disulfide balance parameters and other risk factors in acute malnutrition

		Native thiol (µmol/L)	Total thiol (µmol/L)	Disulfide (µmol/L)	Disulfide/ Native thiol (%)	Disulfide/ Total thiol (%)	Native thiol/ Total thiol (%)
Age (months)	r	0.169	0.115	–0.040	–0.075	–0.075	0.075
	p	0.230	0.419	0.779	0.598	0.598	0.598
Weight (kg)	r	0.161	0.099	–0.045	–0.076	–0.076	0.076
	p	0.254	0.484	0.752	0.590	0.590	0.590
Weight Z score	r	–0.151	–0.162	0.039	0.069	0.069	–0.069
	p	0.284	0.252	0.786	0.628	0.628	0.628
Height (cm)	r	0.148	0.095	–0.030	–0.061	–0.061	0.061
	p	0.294	0.505	0.832	0.665	0.665	0.665
Height Z score	r	–0.006	0.098	0.141	0.115	0.115	–0.115
	p	0.966	0.491	0.317	0.418	0.418	0.418
BMI (kg/m^2)	r	–0.091	–0.107	0.000	0.028	0.028	–0.028
	p	0.521	0.452	0.998	0.844	0.844	0.844

BMI: body mass index; *p<0.05 was considered statistically significant.

Thiol groups function as antioxidants and regulate the redox system. Dynamic thiol/ disulfide balance has critical roles in antioxidant protection, detoxification, signal transmission, apoptosis, enzyme activity, and regulation of cellular signal mechanism by transcription factor (6-8, 15). Abnormal thiol/disulfide balance leads to deterioration of these vital cellular functions. Due to oxidative stress, pathologies occur in organelles such as mitochondria, vesicles, and cell membrane, resulting in impaired functioning of various physiological processes such as signal transduction, enzyme activation, regulation of the immune system, gene expression, folding of newly synthesized proteins. In addition, during inflammation, chemical and oxygen radicals generated by factors such as radiation may damage the thiol/ disulfide balance (6, 16). In both cases described above, thiol/ disulfide balance is expected to be weakened compared to the control group.

Until present day, several studies have been conducted on the role of dynamic thiol/ disulfide balance in the etiopathogenesis of many diseases. In a study conducted on patients with vitiligo by Üstüner (17), total thiol levels were determined to be higher in the patient group than in the control group. It was stated that disulfide levels and disulfide/native thiol and disulfide/total thiol ratios of were higher in patients with vitiligo than in the control group. It was also stated that the serum total thiol and disulfide levels correlated with disease activity in patients with vitiligo and that this can be used as a new inflammatory indicator in determining the prognosis of the disease. In another study conducted on patients with autoimmune subclinical hypothyroid by Ateş et al. (18), native thiol levels were found to be lower in the patient group, although the result was not statistically significant. Additionally, total thiol levels were also found to be low in the patient group. Disulfide levels and disulfide/native thiol and disulfide/ total thiol ratios were reported to be higher in the patient group and the thiol/disulfide balance was reported to shift in favor of disulfide.

In the literature, there are very few studies conducted on the role of thiol/ disulfide balance. there are studies demonstrating that an abnormal thiol disulphide balance state is involved in the pathogenesis of a variety of diseases. In a study conducted by Durmuş et al. (19) in children with type 1 diabetes mellitus (T1DM), disulfide/native thiol and disulfide/total thiol ratios were significantly higher whereas the native thiol levels and native thiol/total thiol ratio were lower in the patient group. Disulfide/ native thiol and disulfide/total thiol ratios were reported to be higher and the thiol/disulfide balance was reported to shift in favor of disulfide. In another study conducted on patients with pediatric adenoid hypertrophy by Ozdamaret al. (20), disulfide levels and disulfide/native thiol and disulfide/total thiol ratios were found to be higher in the patient group than in the control group. It was found that the native thiol and total thiol levels in these were lower in the patients than in the healthy group. Disulfide and disulfide/native thiol and disulfide/total thiol ratios were reported to be higher and the thiol/disulfide balance was reported to shift in favor of disulfide. In yet another study conducted on children with duchenne muscular dystrophy patients by Incecik et al. (21), total thiol and native thiol levels were found to be lower in the patient group than in the control group, although the difference was not statistically significant. Additionally, the disulfide levels, and disulfide/native thiol and disulfide/ total thiol ratios were also found to be low in the patient group.

In our study, we evaluated thiol/disulfide balance in patients with acute malnutrition. There was no statistically significant difference between the two groups with respect to the native thiol and total thiol levels. Conversely, disulfide levels, disulfide/native thiol, and disulfide/total thiol ratios were found to be significantly higher in the patient group than in the control group, while native thiol/total thiol ratio was found to be significantly lower in the patient group than in the healthy group. In our study, we found that in children with acute malnutrition, the thiol/ disulfide balance tended to shift to the disulfide, i.e., to the right, indicating increased oxidative stress. Increased disulfide levels, disulfide/total thiol (oxidized thiol ratio), and disulfide/native thiol (thiol oxidation-reduction ratio) ratios indicate increased oxidative stress, whereas low native thiol/total thiol ratio in children with acute malnutrition compared to healthy children also indicates low antioxidant defense mechanism. This is due to the increase in free radicals and oxidative stress caused by the suppression of immune systems in patients with malnutrition and the low intake of nutrients such as protein, glucose, and vitamins. To our knowledge, our study is the first to examine the dynamic thiol / disulfide homeostasis in children with acute malnutrition.

As stated above, there is evidence that abnormal thiol/disulfide balance plays a role in the pathogenesis of some diseases. Therefore, detection of dynamic thiol/disulfide balance can provide valuable information about a variety of normal or abnormal biochemical processes.

In our study, there was no correlation was found among the age, gender, weight, weight Z score, height, height Z score, and BMI measurements and thiol/disulfide balance tests of affected children and thiol/ disulfide balance tests. However, in the study carried out by Ateş et al. (18), there was negative correlation was found among age of patients with autoimmune subclinical hypothyroid patients and native thiol levels, total thiol levels, disulfide/native thiol ratio, and disulfide/total thiol ratio.; They also reported a positive correlation between the ages of patients and with disulfide level and the ratio of natural thiol/total thiol ratio (18). They also reported a positive correlation between the ages of patients and disulfide level and natural thiol / total thiol ratio. It was also stated that the level of disulfide increases with the oxidation of thiols as the level of obesity increases (6). In addition, they reported that as age increases, disulfide levels increase, and thiol levels decrease. In addition, in previous studies it has been reported that oxidative stress increases with age, leading to thiol/ disulfide homeostatic imbalance (6).

Limitations and Strengths

As the number of patients in this study was low, it is difficult to generalize the study results to all the patients with acute malnutrition. Furthermore, we did not undertake the evaluation of thiol/disulfide balance parameters according to the degree of acute malnutrition in the patients. In the study, dynamic thiol/

disulfide balance, which is only one of the complex mechanisms of oxidative stress, was evaluated, while other oxidative stress parameters were not evaluated. We could not determine the relationship among dynamic thiol/disulfide balance, other oxidative stress parameters, and acute malnutrition. In future studies, thiol disulfide balance, other oxidative stress parameters, or new biomarkers should be examined in large patient groups according to the degree of acute malnutrition to further validate our findings. The main strength of our study is that all the thiol/disulfide parameters were measured using a new and fully automated method. In addition, strict exclusion criteria were applied to minimize the confounding variables.

CONCLUSION

In this study, we found that the thiol/disulfide balance is weakened in children with acute malnutrition and is shift toward disulfide. It was observed that children with acute malnutrition had increased total oxidative stress activity and decreased total antioxidant levels compared to the healthy group. To the best of our knowledge, this is the first study on evaluation of thiol/disulfide balance in children with acute malnutrition. Based on the results of this study, we believe that there is a need for further studies with more patients studies in a larger number of patient groups, which are classified according to acute stages of malnutrition.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Harran University Faculty of Medicine Ethics Committee (11.05.2017/05).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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