RESEARCH ARTICLE

# Evaluation of serum thiol/disulfide homeostasis in pediatric patients diagnosed with vitamin B12 deficiency

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**Tekgündüz S, Solgun HA, Gulseren S, Ozcan E. Evaluation of serum thiol/disulfide homeostasis in pediatric patients diagnosed with vitamin B12 deficiency. J Pedia Health Care Med. 2022;5(1):01-04.**

## ABSTRACT

**Background:** It may not be easy to recognize vitamin B12 deficiency by both clinical and laboratory tests. In its deficiency, it may be asymptomatic or cause life-threatening symptoms such as severe myelopathy and pancytopenia. The first examination required for the diagnosis of vitamin B12 deficiency is usually the measurement of serum vitamin B12 level. Due to the limitations of serum vitamin B12 measurement in detecting vitamin B12 deficiency, methyl malonic acid, homocysteine, or both will be measured in patients with clinical findings consistent with deficiency.

**Materials and Methods:** Our study was planned prospectively as a case-control study. Forty-five (Female: 15, Male: 30) patients who had Vitamin B12 deficiency between May 2019 and August 2019, and 40 healthy (Female: 25, Male: 15) who had normal vitamin B12 levels for control purposes were included in the study (p: 0.019), with the permission of the volunteer, native thiol, total thiol, disulfide ((total thiol-native thiol)/2), implication (ischemia modified albumin), which were increased from serum, were studied.

**Results:** The Average ages were 14.2 ± 2.15 years in the group with VITB12 deficiency (min 10 max 18) and 14.8 ± 2.77 years in the group without VITB12 deficiency (min 10 max 18) respectively. While the mean hemoglobin, MCV, WBC, platelet, ferritin, folate levels, native thiol levels, Disulfide (total thiol-native thiol)/2 ratio, IMA (Ischemia Modified Albumin) levels were similiar in range; Vitamin B12 levels, homocysteine levels, MMA (Methyl Malonic Acid) levels were significantly different in both groups. There was no significant difference for Index 1 (disulfide/native thiol*100), Index 2 (disulfide/total thiol*100) and Index 3 (native thiol/total thiol*100) values in both groups.

**Conclusion:** Thiols are compounds that react with oxidants to form disulfide bonds. There are studies showing that thiol-disulfide homeostasis has changed in cases such as type-1 diabetes mellitus, myocardial infarction, atherosclerosis, idiopathic recurrent abortions, attention deficit, and hyperactivity disorder. In this study, vitamin B12 deficiency and serum thiol-disulfide correlation were evaluated for the first time in the literature.

**Key Words:** *Childhood; Vitamin B12 defficency; Thiol- disulfide levels*

## INTRODUCTION

In vitamin B12 deficiency, there may be an increase in Mean Erythrocyte Volume (MCV) or Erythrocyte Distribution Width (RDW). Rather than an absolute value above the reference range given for MCV, a different MCV finding than expected for the patient's age, the status of iron parameters (high or low serum iron values), and a possible thalassemia association is important in deciding the presence of macrocytosis [1].

In peripheral blood smear, macrocyte erythrocytes, macroovalocites, immature, megaloblastic, nucleated erythrocytes, anisocytosis, hyper segmented neutrophils (at least 1% of neutrophils have 6 lobes or at least 5% have 5 lobes) and immature leukocytes. As an evidence of infective erythropoiesis (intramedullary hemolysis), Lactate Dehydrogenase (LDH), indirect bilirubin, Aspartate Aminotransferase (AST) may be found high and haptoglobin low [2]. Leukopenia may be accompanied by thrombocytopenia or pancytopenia. Serum Methyl Malonic Acid (MMA) and serum (or plasma) Homocysteine (HCY) values can be measured high.

The first examination required for the diagnosis of vitamin B12 deficiency is usually the measurement of serum vitamin B12 level. Although clinical signs of deficiency at an extremely low level (<100 pg/ml (<73.8 pmol/L)) are often accompanied, such low levels are rarely encountered [3]. Based on the reference range given by the laboratories, the ratio of both false negative and false positive values is quite high. And the remaining part is located on haptochorin (TC-I), a protein of unknown function [4]. Due to the limitations of serum vitamin B12 measurement in diagnosing vitamin B12 deficiency, it will be appropriate to measure methyl malonic acid, homocysteine or both in patients with clinical findings consistent with the deficiency [5].

Thiols are compounds that react with oxidants to form disulfide bonds. Thiol-disulfide homeostasis and ischemia modified albumin antioxidation, detoxification, apoptosis, enzymatic activity regulation, transcription factors and cellular signaling mechanisms it has a big role. There are studies showing that thiol-disulfide homeostasis has changed in cases such as type-1 diabetes mellitus, myocardial infarction, atherosclerosis, idiopathic recurrent abortions, attention deficit, and hyperactivity disorder. In this study, vitamin B12 deficiency will be evaluated for the first time in the literature.

## MATERIALS AND METHODS

The study was carried out in the Pediatric Hematology and Oncology Outpatient Clinic of our Training and Research Hospital. Our study was planned prospectively as a case-control study. Forty-five (Female: 15, Male: 30) patients who had Vitamin B12 deficiency between May 2019 and August 2019, and 40 healthy (Female: 25, Male: 15) who had normal vitamin B12 levels for control purposes were included in the study (p: 0.019), with the permission of the volunteer, native thiol, total thiol, disulfide ((total thiol-native thiol)/2), implication (Ischemia Modified Albumin), which were increased from serum, were studied. Blood samples were centrifuged at 3600 cycles for 10 minutes in the biochemistry laboratory, and 1 cc serum was obtained and stored at -80 degrees.

After collecting all samples, all of them are dissolved at the same time and serum thiol-disulfide parameters are developed by Erel & Neselioglu with the new automatic measurement method worked on the Roche Hitachi Cobas c501 automatic analyzer at Ankara City Hospital Biochemistry Laboratory [6]. The terms for Index 1= disulfide/native thiol*100, Index 2: disulfide/total thiol*100, Index 3: native thiol/total thiol*100, ((total thiol-native thiol)/2) ratios were calculated.

## STATISTICAL ANALYSIS AND RESULTS

The average ages were 14.2 ± 2.15 years in the group with VITB12

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TABLE 1
Index values for both groups

| Index values | VitB12 deficiency+ group | VitB12 deficiency- group | Ratio of difference |
|---|---|---|---|
| Index 1 (disulfide/native thiol* 100) | 4.94 ± 1.66 | 5.17 ± 1.78 | p:0.937 (no difference) |
| Index 2 (disulfide/total thiol *100) | 4.45 ± 1.36 | 5.17 ± 1.78 | p:0.932 (no difference) |
| Index 3 (native thiol/ total thiol* 100) | 91.08 ± 2.72 | 90.71 ± 2.93 | p:0.932 (no difference) |

*These index values are effective to compare both the groups (VitB12 deficiency+group and VitB12 deficiency-group) for each mentioned criteria

deficiency (min 10 max 18) and 14.8 ± 2.77 years in the group without VITB12 deficiency (min 10 max 18) respectively. Pearson Chi-Square test was used for ages between the two groups and there was no difference between both groups (p: 0.301). The differences between groups after age values were examined with the ındependent samples test (Table 1).

The average hemoglobin were 14.39 g/L ± 1.2 g/L in the group with VITB12 deficiency and 14.01 g/L ± 1.39 g/L years in the group without VITB12 deficiency respectively. There was no difference between both groups (p: 0.702).

The average MCV were 85 fL ± 4.1 fL in the group with VITB12 and 84.7 fL ± 4.8 fL in the group without VITB12 deficiency respectively. There was no difference between both groups (p: 0.299).

The average WBC were 7024/µl ± 1774/µl in the group with VITB12 and 7093/µl ± 1909/µl in the group without VITB12 deficiency respectively. There was no difference between both groups (p: 0.657).

The average platelet were 285520/$mm^3$ ± 64562/$mm^3$ in the group with VITB12 and 293875/$mm^3$ ± 57171/$mm^3$ in the group without VITB12 deficiency respectively. There was no difference between both groups (p: 0.558).

The average ferritin were 41 ng/mL ± 19 ng/mL in the group with VITB12 and 37 ng/mL ± 25.6 ng/mL in the group without VITB12 deficiency respectively. There was no difference between both groups (p: 0.157).

The average vitamin B12 levels was 146 pg/ml ± 26.52 pg/ml in the group with VITB12 and 424 pg/ml ± 146.9 pg/ml in the group without VITB12 deficiency respectively. There was statistically significant difference between both groups (p: 0.00).

The average folate levels were 7.28 ng/ml ± 2.5 ng/ml in the group withVITB12 and 8.9 ng/ml ± 4 ng/ml in the group without VITB12 deficiency respectively. There was no difference between both groups (p: 0.77).

The average Homocysteine levels was 18.2 mol/L ± 9.2 mol/L in the group with VITB12 and 8.52 µmol/L ± 2.87 µmol/L in the group without VITB12 deficiency respectively. There was statistically significant difference between both groups (p: 0.00).

The average MMA levels was 45.2 ng/ml ± 19.9 ng/ml in the group with VITB12 and 9.21 ng/ml ± 5.28 ng/ml in the group without VITB12 deficiency respectively. There was statistically significant difference between both groups (p: 0.00).

The average native thiol levels was 368.81 µmol/L ± 67.3 µmol/L in the group with VITB12 and 328.69 µmol/L ± 70.41 µmol/L in the group without VITB12 deficiency respectively. There was no difference between both groups (p: 0.689).

The average disulfide (total thiol-native thiol)/2 ratios were 17.95 ± 5.94 in the group with VITB12 and 17.03 ± 7.1 in the group without VITB12 deficiency respectively. There was no difference between both groups (p: 0.395).

The average Ischemia Modified Albumin (IMA) levels were 0.56 ± 0.09 ABSU in the group with VITB12 and 0.61 ± 0.07 ABSU in the group without VITB12 deficiency respectively. There was no difference between both groups (p: 0.083).

The average albumin levels were 3.5 g/dl ± 0.36 g/dl in the group with VITB12 and 0.38 g/dl ± 0.33 g/dl in the group without VITB12 deficiency respectively. There was no difference between both groups (p: 0.376).

## DISCUSSION

The aim of this research is to measure the levels of thiol and disulphide in pediatric patients with vitamin B12 deficiency. The results in this study have been reported as follows:

Thiol-disulphide balance is impaired in pediatric patients with vitamin B12 deficiency.

Native thiol level, total thiol level, and dynamic disulphide bond level have been found statistically lower in vitamin B12 deficiency groups.

Native thiol level, total thiol level, and dynamic disulphide bond level have been found higher in vit B12 deficiency group compared to control group but not statistically significant.

Although it has been shown that thiols play a role in the pathogenesis of many diseases, such as ankylosing spondylitis, lung cancer, rheumatoid arthritis, diabetic nephropathy, sickle cell disease, gastric cancer, and juvenile idiopathic arthritis, few studies have been conducted with respect to vit B12 deficiency [7-9].

Ayar G et al. measured thiol-disulphide homeostasis as oxidative stress marker in critically ill children with sepsis [10]. They found that the levels of native thiol, total thiol, and disulphide were significantly decreased in patients with septis shock and sepsis. They found no significant difference in terms of reduced thiol ratio, oxidized thiol ratio, and thiol oxidation reduction ratio between healthy and sepsis groups. In addition, it has been found that levels of native thiol, total thiol, and disulphide were higher in nonsurvivor group compared with survivor group. This indicates that there is no correlation between levels of native thiol, total thiol, disulphide, and disease activity in patients with sepsis [11-13]

Thiols are organic compounds containing sulfhydryl (SH) groups. Thiol structure is most commonly observed in plasma and other proteins [14]. However, fewer amounts of thiol groups can also be seen in molecules containing cysteine, such as glutathione, cysteine, homocysteine, N-acetylcysteine, gamma-glutamylcysteine [15]. ROS is formed as a result of oxidative stress. ROS leads to damages in cells and tissues. Thiols react with free radicals in order to prevent the ROS-mediated cell and tissue damage [16]. Free radicals cause the oxidation of thiol groups of amino acids containing sulfur and disulfide bonds are formed as a result of this oxidation reaction [17]. These disulfide bonds can be converted to thiols via reducing. In this way, the thiol/disulfide balance is ensured in cells and tissues. Thus, the antioxidant defense system, detoxification, apoptosis, and enzyme activities can be regulated, and the regulations of intracellular signal transduction mechanisms are ensured [18]. Decreased thiols and increased disulfide levels lead to decreased clearance of ROS products [19]. In this way apoptosis and cellular damage frequency increase [20, 21].

Erel et al. have reported that thiol/disulfide balance deteriorated in diseases characterized by intense inflammation and oxidative stress such as hypertension, myocardial infarction, type 1 diabetes, pre-diabetes, metabolic syndrome, autoimmune thyroiditis, and inflammatory bowel disease [22, 23]. This is the status of intense inflammation, increased cytokine, and oxidative stress. In a study which was conducted with Familial Mediterranean Fever (FMF) patients, it was found that NT and TT levels of patients were lower and disulfide levels were higher compared to control individuals. Thiol/disulfide balance deteriorated in FMF disease because of the increased oxidative stress, macrophage Migration İnhibitory Factor (MIF) increase and the paraoxonase enzyme activity (an antioxidant mechanism) [24].

Vitamin B12 is a vitamin that is synthesized by major microorganisms and has various derivatives. Man cannot synthesize the vitamin B12 he needs. Insufficient intake in the diet is an important cause of vitamin B12 deficiency [25]. It is important to consider, diagnose and treat vitamin B12 deficiency in childhood. Although the cost of treatment is quite low, delay in treatment can cause serious complications such as deep anemia, gastrointestinal symptoms, and irreversible neurological damage [26].

Homocysteine is an amino acid containing sulfhydryl which is an intermediate product of methionine metabolism [27]. Vitamin B12; Methyl malonic acid and homocysteine accumulate in vitamin B12 deficiency, as it plays a role in the conversion of methyl malonic acid to succinyl coenzyme A and homocysteine to methionine [28, 29]. Levels of 15 (µmol/L) and less for homocysteine in human plasma are considered normal [30, 31].

There is increasing evidence that homocysteine exerts its effects by creating oxidative damage. When homocysteine is added to the plasma, it rapidly oxidizes to disulfide homocysteine or homocysteine thiolactone. During this reaction, reactive oxygen products such as hydrogen peroxide and superoxide radical are formed. The resulting hydrogen 26 peroxide causes damage to the vascular endothelium, while superoxide radicals initiate lipid peroxidation by affecting both endothelial and LDL particles [32].

Normal endothelial cells secrete nitric oxide, which binds homocysteine to counteract the toxic effects of homocysteine. This protective effect of nitric oxide is impaired by long-term exposure of the endothelium to hyperhomocysteinemia because homocysteine; It reduces endothelial nitric oxide synthase release by causing lipid peroxidation. The result is the disruption in the endothelial production of nitric oxide; exposes the endothelium to homocysteine-mediated oxidative damage and endothelial dysfunction occurs [33]. It is known that plasma homocysteine concentration is closely related with age and gender. Homocysteine levels in men are slightly higher than in women; this effect is attributed to the protective effect of estrogen in women [34]. The fact that the homocysteine level in females is significantly lower than males in our study supports the literature.

In our study, when constituting the patient group, cases with vitamin B12 level below 200 pg/ml and homocysteine level above 15 µmol/L were selected. Looking at the literature; a high level of homocysteine or methyl malonic acid accompanying low vitamin B12 levels strengthens the diagnosis. In the study of Savage et al with 406 cases, it was stated that 10%-26% misdiagnosis could be made with vitamin B12 level alone, and it was stated that the sensitivity could reach 99.8% by studying methyl malonic acid and homocysteine [35, 36]. Homocysteine, which is known to increase in vitamin B12 deficiency, has been reported to cause apoptosis, especially at high concentrations, in studies conducted in various cell cultures *in-vitro*. It has been suggested that homocysteine exerts this effect through its pro-oxidant properties [37].

Oxidative stress is an undesirable condition with adverse consequences for the body. Oxidative stress state is the disruption of the balance between the level of antioxidant and oxidant molecules [38]. Overproduction of reactive oxidative species disrupts the structure of proteins and lipids. Due to this effect, breaks in DNA structures and oxidation in proteins and lipids of cell membrane occur [39]. A breakdown in the mitochondrial electron transmission systems and an increase in the inducible nitric oxide synthesis are the most important changes that occur [40, 41]. These mechanisms in sepsis cause multiple organ failure that lead to myocardial depression, cellular dysfunction, endothelial damage, and vascular catecholamine hyporesponsiveness.

There are lots of commercial tests to evaluate the levels of oxidant and antioxidant molecules in human body. Thiols are antioxidant molecules containing sulfhydryl (-SH) group consisting of hydrogen, sulphide, and carbon atoms. Electrons from reactive oxygen molecules are transferred to thiol in the human body and oxidation of these molecules is made by disulphide bonds. Bonding of disulphide structures is reversible [42, 43]. The amount of this conversion may vary according to the level of oxidative stress. Disruption of thiol-disulphide homeostasis, which develops as a result of the increase of oxidative molecules, should be evaluated as a precursor of possible diseases that may occur in the human body.

Limited numbers of patients, not using another antioxidant parameter for comparison, and being single centered are limitations of this study.

## CONCLUSION

There are studies showing that thiol-disulfide homeostasis changes in conditions such as type-1 diabetes mellitus, sepsis, myocardial infarction, atherosclerosis, idiopathic recurrent miscarriages, attention deficit and hyperactivity. In this study, vitamin B12 deficiency will be evaluated for the first time in the literature. Future studies will enlighten the hemostasis and effects of thiol-disulfide levels on various conditions of metabolism of human.

## DECLARATIONS

Ethics approval and consent to participate have been taken from Health Sciences University.

## ETHICAL COMMITTIE

Consent for publication has been taken from the patients' parents. Patient's parents gave informed written consent for their personal or clinical details along with any identifying images to be published in this study. Ethics approval and consent to participate have been taken from Health Sciences University.

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## AUTHOR'S CONTRIBUTIONS

Author Huseyin Avni Solgun, et al. have read and approved the manuscript.

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