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# Effect of Erythrocyte Suspension Transfusion on Thiol-Disulfide Homeostasis in Critically ill Children

## Eritrosit Süspansiyonu Transfüzyonunun Kritik Hasta Çocuklarda Tiyol-Disülfid Homeostazı Üzerindeki Etkisi

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## Abstract

**Aim**: Our study aimed to investigate the potential relation between dynamic thiol homeostasis and blood transfusion in the pediatric intensive care unit.

**Material and Method**: Blood samples were collected from pediatric intensive care patients before and after erythrocyte suspension transfusion and from donor blood additionally to measure thiol levels. The study involved 30 patients, including nine females, and a total of 90 blood samples from patients and donors were analyzed.

**Results**: Prior to transfusion, Total Thiol (TT) and Native Thiol (NT) were 414.77±156.14 (µmol/L) and 272.63±115.75 (µmol/L), respectively, and post-transfusion, they were found to decrease to 398.07±187.38 (µmol/L) and 258.97±136.2 (µmol/L), respectively. However, no statistically significant difference was observed between pre- and post-transfusion values. In post-transfusion blood samples, there was a significant increase in Disulfide/TT and Disulfide/NT ratios, indicating an increase in oxidation (34.79±92.34 and 51.89±68.51, respectively), yet no statistical difference was noted.

**Conclusion**: Transfusions administered in the Pediatric Intensive Care Unit were associated with a decrease in total and native thiol levels, indicative of increased oxidative stress, despite the lack of statistically significant differences. To mitigate the potential negative impact on patients with high oxidative properties after transfusions, strengthening the antioxidant defense system is recommended. Research should be planned to develop suitable strategies for enhancing the antioxidant defense system and ensuring patients' resilience to this condition.

**Keywords**: Children, erythrocyte suspension transfusion, pediatric intensive care, dynamic thiol homeostasis

## Öz

**Amaç**: Çocuk yoğun bakım ünitesinde kan transfüzyonu ile dinamik thiol homeostasisi arasında bir bağlantı olup olmadığını araştırmak.

**Gereç ve Yöntem**: Çocuk yoğun bakım ünitesinde yatan hastalarda kan transfüzyonundan önce ve sonra ek olarak donör kanından thiol düzeylerini ölçmek için kan örnekleri alındı. Dokuzu kız toplam 30 hastanın ve ek olarak donörlerin 90 kan örneğinde araştırma yapıldı.

**Bulgular**: Transfüzyondan önceki Total tiyol (TT) ve Nativ tiyol (NT) sırasıyla 414,77±156,14 (µmol/L) ve 272,63±115,75 (µmol/L) idi, transfüzyon sonrasında ise yine sırasıyla 398,07±187,38(µmol/L) ve 258,97±136,2(µmol/L) transfüzyon öncesine göre azalmış olarak bulundu. Ancak, transfüzyon öncesi ve sonrası arasında istatistiksel olarak anlamlı bir fark gösterilemedi. Transfüzyondan sonraki kan örneklerinde ise oksidasyonun artışına delalet eden Disülfid/TT ve Disülfid/NT oranlarında önemli bir artış oldu, (sırasıyla 34,79±92,34 ve 51,89±68,51) yine, istatistiksel olarak bir fark gösterilemedi.

**Sonuç**: Çocuk Yoğun Bakım ünitesinde uygulanan kan transfüzyonları, istatistiksel olarak anlamlı fark olmasa da artmış oksidatif stresi gösteren total ve nativ tiyolde azalma ve disülfid oranlarında artış ile ilişkilendirildi. Oksidatif özelliği fazla olan ES ile transfüzyon sonrası hastaların bu durumdan olumsuz etkilenmemesi için antioksidan savunma sisteminin güçlendirilmesi düşünülmeli ve uygun stratejilerin geliştirilmesi için araştırmalar planlanmalıdır.

**Anahtar Kelimeler**: Çocuklar, eritrosit süspansiyonu transfüzyonu, pediatrik yoğun bakım, dinamik tiyol homeostazisi

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## INTRODUCTION

Pediatric patients with critical illness commonly experience a high incidence of anemia both upon admission and throughout their stay in the pediatric intensive care unit (PICU). Anemia develops in approximately 95% of intensive care patients before the third day of admission.[1] Around 17% of all PICU patients receive transfusions, and it has been reported that almost 50% of patients with stays exceeding 48 hours in the PICU receive blood products.[2,3] However, the administration of erythrocyte suspension transfusion should be carefully considered when the benefits outweigh the risks.

While transfusion-related infections (TRIs) initially garnered attention in studies on transfusion safety, other factors such as immunomodulation and blood age have also become noteworthy. The risk assessment of transfusion has necessitated exploration into the benefits of this procedure.[4].

Oxidative stress is a key factor in various physiological and pathological events. Despite erythrocytes containing an extensive antioxidant defense system, oxidative stress contributes to oxidative membrane protein and lipid damage, normal cell aging, and shortened cell lifespan.[5,6] Due to its association with worse prognosis in critically ill patients, oxidative stress measurements have become clinically significant in intensive care patients.[7, 8]

To protect cell organelles and membranes from the harmful effects of free radicals, cells possess various enzymatic and non-enzymatic antioxidant defense systems. The antioxidant defense system includes substances such as transferrin, ceruloplasmin, lactoferrin, albumin, haptoglobins, hemopexin, bilirubin, carotenoids, glutathione, vitamin C, vitamin E, uric acid, and acute-phase proteins (C3, antiproteases, CRP, and serum amyloid A).[9] Oxidative stress, on the other hand, manifests itself through an increase in reactive oxygen species like hydroxyl radicals, superoxide radicals, and hydrogen peroxide during cellular metabolism.[10]

Studies have shown that during the storage process of erythrocyte suspension, iron is released from transferrin, leading to an increase in non-transferrin-bound iron (NTBI) levels (toxic form). It is reported that NTBI levels reach their maximum on the 35th day of storage. This increase in oxidative stress during storage is speculated to contribute to the elevated frequency of diseases like premature retinopathy and necrotizing enterocolitis in newborns receiving erythrocyte suspension.[11-13] The increased burden of free iron is also suggested to harm the recipient's lungs, increasing the risk of chronic lung disease.[13]

Hemoglobin (Hb) in erythrocyte suspension is generally well preserved with a range of antioxidants and other protective molecules. However, when erythrocyte suspensions are stored under cold conditions, the mechanisms that protect the cells lose their effectiveness over time. Hb becomes sensitive to oxidation and is released outside the cell. The increase of extracellular Hb during the storage of erythrocyte suspensions is used as an indicator of the degree of hemolysis.[13,14]

### Thiol-Disulfide Balance

Thiols are organic compounds consisting of a sulfur atom and a hydrogen atom attached to a carbon atom, containing a sulfhydryl group.[15] The plasma thiol pool primarily consists of albumin thiols, protein thiols, and low molecular weight thiols. Plasma thiols, which are of great importance in physiological and biological events, can exhibit pro-oxidant or predominantly antioxidant effects.[16,17] Antioxidants containing thiol (-SH) groups in their structures include pyridoxine, methionine, S-adenosylmethionine, N-acetylcysteine (NAC), alpha-lipoic acid, captopril, taurine, and homocysteine. Cysteine, homocysteine, and glutathione (GSH) are found in abundance among plasma thiols.[18] Thiol and disulfide (-S-S-) groups play a crucial role in stabilizing protein configurations, regulating the functions of proteins and enzymes, as well as in carriers and receptors, Na-K transmission, and transcription.[19]

The antioxidant or pro-oxidant effects of -SH groups depend on the level of oxidative stress in the environment, the physiological and biological state, and the levels of sulfur-containing amino acids. Compounds containing -SH groups exhibit their antioxidant effects by inhibiting the impact of free radicals.[18] Dynamic thiol-disulfide homeostasis plays a significant role in various mechanisms such as detoxification, apoptosis, antioxidant protection, signal transmission, and enzyme activity regulation. Furthermore, anomalous thiol-disulfide balance has been linked to a range of illnesses such as cardiovascular disease, cancer, rheumatoid arthritis, diabetes, chronic kidney disease, and multiple sclerosis.[20-22] The development of sepsis is intricately influenced by oxidative stress and the body's antioxidant defense mechanisms.[23-25]

In conducted studies, it is known that the -SH groups in proteinaceous compounds such as cysteine and GSH are oxidized by oxidizing molecules in the environment, leading to reversible -S-S- structures. Subsequently, these formed -S-S- structures are reduced back to -SH groups, thereby maintaining thiol-disulfide homeostasis.[25] N-acetyl cysteine, found as a substrate in GSH synthesis and serving as a source of -SH groups in cells, affects oxygen-derived radicals such as OH-, inhibiting reactive oxygen molecules, preserving protein structure to extend metabolic lifespan, and preventing apoptosis.[26]

Conversely, the interaction of GSH with -S-S- groups results in the formation of oxidized glutathione (GSSG). GSSG, an indicator of oxidative stress, is a compound with harmful effects on the structure and metabolism of -SH-containing proteins and is indicative of the presence of oxidative stress. Consequently, the conversion of -SH groups to -S-S- groups and various oxidized compounds such as oxoacids is an initial indication of oxidation caused by reactive oxygen species in proteins.

This study aims to investigate the dynamic thiol levels in donor erythrocyte suspension and their impact on the dynamic thiol balance in post-transfusion period in critically ill children.

## METHODS

The study was conducted in the Pediatric Intensive Care Unit of Selçuk University Faculty of Medicine between January 1, 2018, and January 1, 2021, including 30 consecutive patients who met the inclusion criteria. Patients or their parents or legal guardians were included in the study after reading the information and consent forms provided to them and obtaining their signatures.

To determine the thiol levels of the erythrocyte suspension obtained from the blood bank for each patient, 2 ml of plasma was taken from the donor erythrocyte suspension. Additionally, 2 ml of serum was collected from the patient before and after transfusion (6-12 hours). Elective ES transfusions are administered for 4 hours in our Pediatric Intensive Care unit with a dose of 10 ml/kg. For donors, variables such as repeated donation/age/systemic disease may also affect the thiol balance in ES. However, unfortunately these data could not be obtained. No additional blood products other than ES and ES were transfused to the patients included in the study while they were hospitalized in another clinic in our hospital or at another health center where they applied before being transferred to our unit.

These samples were stored at -80°C until the desired number of patients was reached. Subsequently, biochemical measurements were performed at the Department of Biochemistry, Selçuk University Faculty of Medicine.

### Determination of Thiol-Disulfide Serum Levels

Serum total thiol (TT) and native thiol (NT) levels were determined using an automatic method developed by Erel and Neselioğlu.[25] Thiol/disulfide homeostasis tests were performed using a new automatic and spectrophotometric method that measures the complete thiol/disulfide status. The principle of the thiol/disulfide measurement method involves the reduction of dynamic disulfide bonds (-S-S-) to functional thiol groups (-SH) by NaBH4. Any remaining NaBH4 residues are completely removed with formaldehyde. This process prevents further reduction of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and also eliminates any disulfide bonds resulting from the reaction with DTNB. The modified Ellman reagent is used to measure the total thiol content in the samples. After serum extraction, the test takes approximately 12 minutes to measure all parameters.[25] The disulfide level is calculated as half of the difference between total thiol and native thiol. The disulfide/native thiol ratio (index 1), disulfide/total thiol ratio (index 2), and native thiol/total thiol ratio (index 3) are calculated.

### Statistical Analyses

Statistical analysis was conducted using the SPSS package program (SPSS for Windows, Version 17.0, SPSS Inc., USA). Descriptive statistics, percentages, and appropriate data type graphs were generated. The Chi-square test was applied for cross-tabulations. After evaluating the normal distribution of the data with the Kolmogorov-Smirnov test, analysis of variance (ANOVA) was used for comparisons between groups. For data that did not follow a normal distribution, Kruskal-Wallis-Friedman variance analysis was applied to analyze differences between groups. The relationships between parameters were assessed using Pearson correlation coefficient for normally distributed data and Spearman's rank correlation coefficient for non-normally distributed data. In all analyses, p-values less than 0.05 were considered statistically significant.

### Ethics

Ethical approval was obtained from the Local Ethics Committee of the Selçuk University Faculty of Medicine (Date: 13-01-2021, approval number 2021/01).

## RESULTS

Of the patients included in the study, 21 (70%) were male and 9 (30%) were female.

Mean age±SD was 34.36±7.66 months (median: 22.80; min-max: 3.6-192 months). When the mean age of the patients was examined according to gender, the mean age of the boys was 40.97±10.60 months (median: 21.66; min-max: 3.6-192 months), while the mean age of the girls was 18.93±3.18 months. (median: 24,000; min-max: 3.6-28.8) months. When the mean age was compared statistically by gender, no significant difference was detected (p: 0.192).

When the diagnoses of the patients included in the study were classified at the time of admission, 10 (33.3%) of the patients had respiratory system diseases, 12 (40%) had infectious diseases, 5 (16.7%) had central nervous system diseases. It was determined that 2 (6.6%) of the patients had gastroenterology system diseases and 1 (3.3%) had urogenital system diseases.

The average values of some hematological parameters (HB, HTC, MCV, RDW and PLT) determined before and after ES transfusion in the study are shown in **Table 1**.

Thiol values in the transfused erythrocyte suspension are given in **Table 2**.

**Table 1. Comparison of the average hematological examinations of the patients before and after erythrocyte suspension**

| | Before Transfusion | | After Transfuion | | p |
|---|---|---|---|---|---|
| | Mean±SD | Median (min-max) | Mean±SD | Median (min-max) | |
| Hb | 7.38±0.82 | 7.2 (5.7-8.9) | 10.91±1.78 | 10.85 (6.6-15.3) | <0.001 |
| MCV | 84.36±5.54 | 85.6 (71.5-95.7) | 84.59±3.79 | 84.35 (76.4-91.8) | 0.464 |
| RDW | 16.66±1.71 | 16.45 (13.1-20.3) | 16.05±1.43 | 16.05 (13.8-19.1) | 0.002 |
| Plt | 222.7±141.12 | 204.5 (5-598) | 243.83±142.71 | 237.5 (36-591) | 0.365 |

(Hb: Hemoglobin, MCV: Mean erythrocyte volume, RDW: red cell distribution width, PLT: Platelet

**Table 2. Thiol values in erythrocyte suspension**

| Thiols in donor erythrocyte suspension | Mean±SD | Median (min-max) |
|---|---|---|
| Total thiol (µmol/L) | 201.68±190.43 | 136.5 (64-989) |
| Native thiol (µmol/L) | 123.11±104.57 | 93 (31-518) |
| Disülfide (µmol/L) | 39.29±44.5 | 28.5 (6-235.5) |
| Reduced thiol (µmol/L) | 62.98±9.08 | 64.2 (43.1-88.2) |
| Oxidized thiol (µmol/L) | 18.5±4.54 | 17.9 (5.9-28.5) |
| Oxidation reduction (µmol/L) | 354.66±122.13 | 358.95 (151.2-777.8) |

Comparison of thiol measurement values of patients who underwent ES transfusion before and after transfusion is shown in **Table 3**.

No correlation was found between the amount of transfused erythrocyte suspension and hemoglobin difference. (r=0.312 p: 0.93) The difference in hemoglobin and dynamic thiol values was calculated as the difference in hemoglobin and thiol values after and before transfusion. While the total

**Table 3. Comparison of Thiol Levels before and after ES**

| | Before Transfusion | | After Transfusiion | | p |
|---|---|---|---|---|---|
| | Mean±SD | Median (min-max) | Mean±SD | Median (min-max) | |
| Total thiol (µmol/L) | 414.77±156.14 | 372.5 (187-815) | 398.07±187.38 | 378.5 (25-801) | 0.696 |
| Native thiol (µmol/L) | 272.63±115.75 | 281.5 (52-457) | 258.97±136.2 | 242 (29-479) | 0.789 |
| Disülfide (µmol/L) | 71.07±51.67 | 67.25 (1-184) | 74.87±55.58 | 52.25 (5-204) | 0.439 |
| Reduced thiol (µmol/L) | 66.23±20.68 | 69.85 (27.8-99.6) | 73.08±55.29 | 68.05 (15-340) | 0.447 |
| Oxidized thiol (µmol/L) | 16.89±10.35 | 15.1 (0.2-36.1) | 21.45±21.8 | 17.65 (2-120) | 0.166 |
| Oxidation reduction (µmol/L) | 772.45±778.86 | 466 (77-2500) | 735.11±927.72 | 369.95 (35.2-4266.7) | 0.673 |
| Disulfide/total thiol (%) | 16.88± 10.34 | 15.07 ( 0-36) | 34.79±92.34 | 17.63 (2-520) | 0.277 |
| Disulfide/native thiol (%) | 34.87± 32.97 | 21.61 (0-130() | 51.89±68.51 | 27.36 (2-284) | 0.056 |
| Native thiol/total thiol (%) | 66.23±20.68 | 69.87 (28-100) | 73.07±55.29 | 68.51 (15-340) | 0.527 |

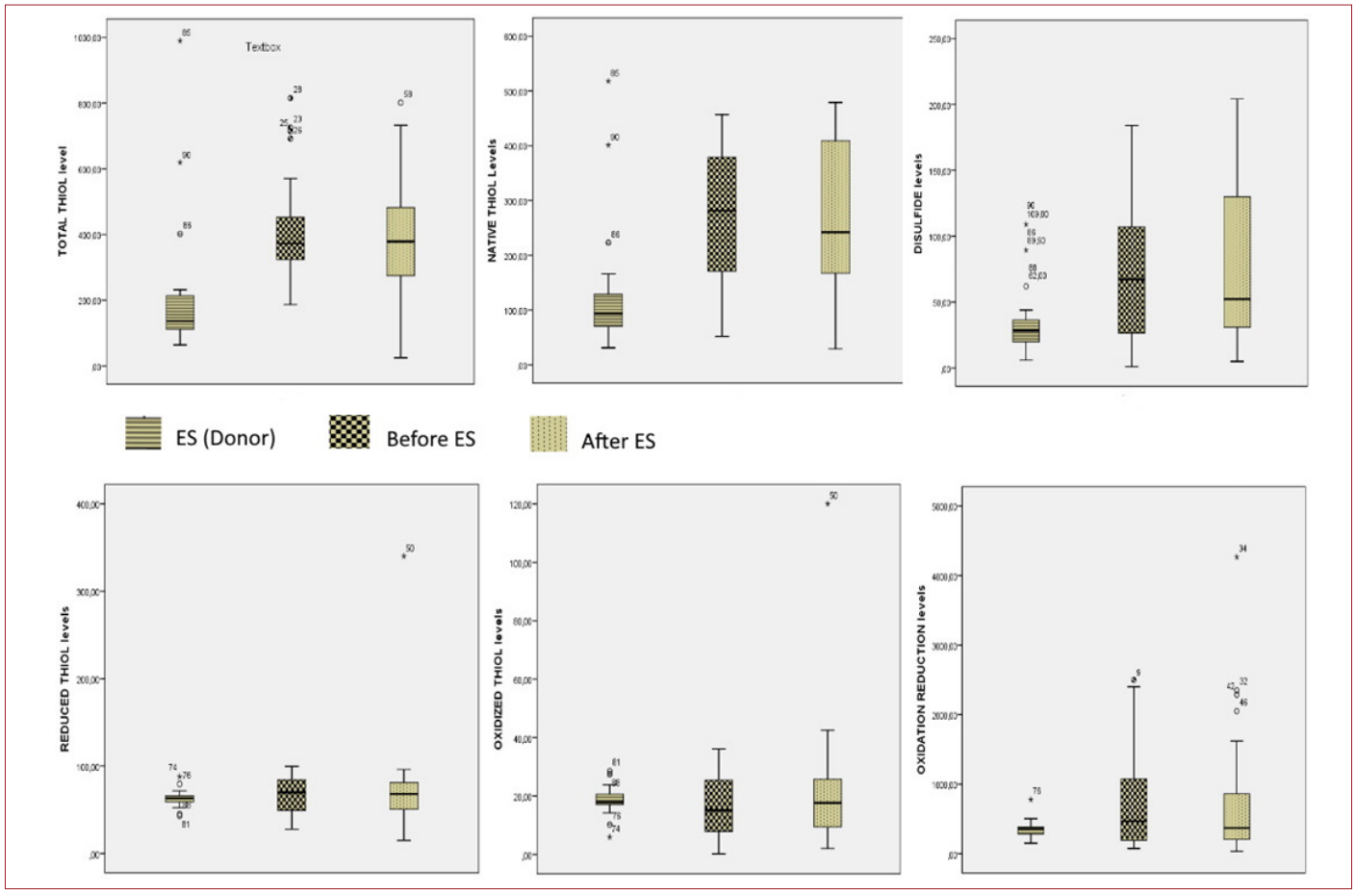


**Figure 1.** Serum total Thiol, native Thiol, disulfide levels, and reduced Thiol, oxidized thiol, and oxidation reduction levels on three samples: in donor eritrocyte suspension (ES), patients serum before ES and patients serum after ES. Lower and upper margin of each box represents 25th and 75th percentiles, horizontal lines in the middle of the boxes represents median value, and whiskers represent lowest and highest values, circles represent outliers and stars represent extreme values. Transfused donor ES had lower Total Thiol, Native Thiol, and disulfide levels in patients' serum before and after ES transfusion. Oxidized thiol level was found to be higher. However, no statistically significant difference was found. This situation may be associated with the high level of oxidation in the stored blood obtained from the blood center.

thiol difference and native thiol difference show a normal distribution, other dynamic thiol value differences do not show a normal distribution. When the relationship between hemoglobin difference and dynamic thiol value differences was examined, no statistically significant difference was detected (**Table 4**).

**Table 4. Correlation between post-transfusion hemoglobin increase and thiol change**

| | | Δtotal_ thiol | UTL | Δdisülfid | Δreduced_ thiol | Δoxidized_ thiol |
|---|---|---|---|---|---|---|
| ΔHb | Correlation | .263* | .254* | .173** | .066** | .062** |
| | p | .159 | .175 | .362 | .727 | .743 |
| | n | 30 | 30 | 30 | 30 | 30 |

*: Pearson r correlation coefficient. **: Spearman's Rho correlation coefficient. Δ: calculated by subtracting serum thiol values measured before transfusion from serum thiol values after transfusion. In terms of Hb, it is the difference between Hb after and before transfusion
Hb: Hemoglobin, UTL: Undepleted thiol level (UTL=serum native thiol value measured after transfusion – serum native thiol value measured before transfusion).

## DISCUSSION

Anemia in children can result from various chronic diseases such as trauma, surgical procedures, anemia due to frequent blood draws, coagulopathy, hemolysis, nutritional deficiencies, and bone marrow depression. Erythrocyte replacement is performed in patients due to a decrease in hemoglobin levels caused by these factors. Given the increased susceptibility to anemia in pediatric intensive care patients, studies on the changes in clinical and laboratory data of patients undergoing erythrocyte replacement become significant.[2]

Transfusion rates and indications for erythrocyte transfusions vary in pediatric intensive care units. In a single-center study by Demaret et al. involving 842 patients, erythrocyte transfusion was administered to 144 (17.1%) patients, with the most common indication being low hemoglobin levels and impaired hemodynamics.[27] Additionally, in several studies, ES transfusion rates during hospitalization in pediatric intensive care units have been reported to range from 49% to 61.7%.[3,28,29] In our study, the ES transfusion rate was determined to be 57.45%. Differences in transfusion rates may be explained by the fact that (1) the studies were conducted in different time periods,(2) the characteristics of included patients, and (3) differences in applied guidelines.

In a study conducted in 2007 in the pediatric intensive care unit on patients with stable general conditions (without active blood loss, cyanotic heart diseases, coronary artery diseases, and severe hypoxemia), the hemoglobin (Hb) thresholds for ES transfusion were compared, with values of 7g/dL and 9.5 g/dL. The study recommended a threshold value of 7 g/dL to prevent unnecessary transfusions.[30] According to the American Association of Blood Banks (AABB) recommendations in 2012 and 2016, the Hb limit for transfusion in hospitalized stable patients is set between 7-8 g/dL, while for patients with a history of cardiovascular disease, it is 8 g/dL.[31,32]

The transfusion guideline for critically ill pediatric patients was last updated in 2018, and, except for specific situations, a hemoglobin level <5 g/dL is recommended.[33] However, both in our study and in the literature, it is frequently observed that transfusions are administered when hemoglobin levels are >7 g/dL. In our study, 40% of the patients (n:12) received transfusion when Hb concentration was between 5-7 g/dL, while 60% (n:18) received transfusion when Hb concentration was between 7-10 g/dL. Most patients who underwent ES transfusion in our study had hemoglobin concentrations between 7-10 g/dL, aligning with similar findings in the literature.[30,34,35]

In the literature, the mean hemoglobin level before ES transfusion ranged from 7.6 to 9.7 g/dL, and the mean hemoglobin level after transfusion ranged from 10.86 to 14.6 g/dL.[3,13,27,28,36] Our study found that the average hemoglobin level before ES transfusion was 7.38±0.82 g/dL, and the average hemoglobin level after transfusion was 10.91±1.78 g/dL, consistent with literature values.

Erythrocytes, despite having high antioxidant properties, require additional antioxidants during storage due to exposure to various factors. Among these factors are agitation, high glucose concentrations, light, and free radicals released by leukocytes.[37,38] When analyzing research on alterations in oxidant and antioxidant parameters during the storage of ES, various changes in erythrocytes become apparent, including adenosine triphosphate and 2,3-diphosphoglycerate (DPG) depletion, loss of flexibility, vesiculation, phospholipid loss, protein oxidation, and lipid membrane peroxidation. These modifications may contribute to adverse clinical outcomes, such as a reduction in oxygen transport.[35] Furthermore, Wardle and colleagues noted an elevation in urinary malondialdehyde levels in preterm newborns following transfusion, suggesting a correlation between transfusion and lipid peroxidation.[39] Another study analyzing levels of TBARS (thiobarbituric acid reactive substances) and protein carbonyls before and after transfusion demonstrated an increase in these levels following ES transfusions, with a positive correlation to mortality. These findings suggest that blood transfusions in critically ill patients may negatively impact patient outcomes.[40]

In the literature, it has been demonstrated that the levels of non-transferrin-bound iron (NTBI), a significant marker of oxidative stress, and malondialdehyde (MDA), a crucial indicator of lipid peroxidation, increase after ES transfusion in premature infants.[13,41] Following ES replacement in premature infants, an increase in free iron load due to ceruloplasmin and transferrin deficiencies leads to catalyzation of the Fenton reaction. This results in an elevated quantity of free radicals in the environment, causing damage to the lungs and retina, and a decrease in total antioxidant status (TAS).[13] However, the high oxygen affinity of fetal hemoglobin in premature infants is thought to play a role in the reduced oxygen distribution to tissues, contributing to retinopathy of prematurity (ROP) and chronic lung disease.[42]

In a study by Ogunro et al., it was shown that on the first day of storage, MDA levels increased by 24.8%, TAS plasma concentration decreased by 27% after 20 days of storage, and the activity of the important antioxidant enzyme glutathione peroxidase decreased by 17.1% after 15 days of storage.[43] During the storage of erythrocyte suspension, the amount of glutathione (GSH), which protects hemoglobin against excessive oxidation, gradually decreased, MDA levels increased outside the cells, and NTBI levels increased.[11] When evaluating NTBI and transferrin levels after transfusing stored fresh (3-7 days) ES and ES stored for 40-42 days, it was shown that NTBI and transferrin levels significantly increased in stored ES compared to fresh ES.[44] The day-by-day increase in MDA levels, the decrease in average TAS levels, and the increase in lactate dehydrogenase (LDH) levels, a marker of oxidative damage, were demonstrated in stored erythrocyte suspensions.[45] In a study by D'Alessandro et al., it was shown that during the storage process, MDA, lactate, and cellular calcium levels increased as the storage period of ES extended.[46] In our study, similar to the literature, patients who received stored RBCs showed a decrease in average TAS levels, an increase in average LDH and calcium levels after transfusion, indicating oxidative stress.

The dynamic balance of thiol-disulfide homeostasis is pivotal in regulating numerous antioxidants, detoxification processes, apoptosis, enzymatic activity, and cellular signaling mechanisms.[19] Thiols engage with all physiological oxidants in the body, serving as a genuine buffering mechanism. This homeostasis is linked to antioxidant mechanisms, as evidenced in inflammatory diseases.[47] Elevated levels of reactive oxygen species (ROS) have been found in infectious diseases such as septic shock, hepatitis, and HIV.[48] Furthermore, heightened antioxidant activity against reactive oxygen species (ROS) molecules has been demonstrated in numerous infectious diseases.[48,49] It is established that oxidative molecules are generated by oxidative enzymes like nicotinamide adenine dinucleotide phosphate oxidase and myeloperoxidase during granulocyte bacterial infections. Consequently, as serum thiol levels decline, there is an increase in disulfide levels.[47] In a study by Aydoğan et al., the decrease in thiol levels in neonatal sepsis was suggested to be an indicator of oxidative stress.[50]

In our study, it was shown that transfused donor ES had lower TT, NT, and disulfide levels in patients' serum before and after ES transfusion. Oxidized thiol level was found to be higher. However, no statistically significant difference was found. This situation may be associated with the high level of oxidation in the stored blood obtained from the blood center.[11-14]

Although the disulfide level in donor ES was lower than the disulfide levels in the patients' serum before and after ES transfusion, disulfide/TT and disulfide/NT levels in the patients' serum were found to be higher after ES transfusion than before (but no statistically significant difference was found). This suggests that oxidation increases in patients with ES transfusion. Although there are rare studies in the literature investigating the relationship between transfusion and thiol homeostasis, it has been shown that oxidative stress increases and antioxidant capacity decreases.[39,40] It has been reported that native thiol levels are low before transfusion in thalassemia patients and increase after transfusion. This increase was interpreted as oxidative stress already being high in thalassemia patients, and oxidative stress being cleared by transfusion, thus increasing native thiol. Undepleted thiol level (UTL) is a term used for thalassemia patients. UTL is calculated by subtracting serum native thiol values measured before transfusion from serum native thiol values after transfusion (UTL = serum native thiol value measured after transfusion – serum native thiol value measured before transfusion). This mathematical difference may arise depending on the oxidation state of the patient before transfusion, oxidative stress and antioxidant capacity in the donor blood.[51] In the thesis study conducted by one of our researchers, it was shown that total oxidative stress and OSI index increased after ES transfusion, which was statistically significant.[52]

When patients were transfused with ES, which has a high oxidation effect, a decrease in the levels of TT and NT, which are protective against oxidation, was detected compared to before ES transfusion. To prevent patients from being affected after transfusion with ES, which has high oxidative properties, it should be considered that it would be appropriate to strengthen the antioxidant defense system, and research should be planned to develop appropriate strategies.

The most important limitation of our study is that it was studied in a single center and with a very limited number of cases. The waiting time for donor blood in blood banks is not recorded. The effects of oxidative stress may be different in children with critical illnesses, and the fact that there are many different diseases that can increase oxidative stress suggests that there may be differences in the results due to this limited sample size. Multicenter studies with large participation and sufficient number of cases are needed.

## CONCLUSION

Although there was no statistically significant difference, blood transfusions administered in the Pediatric Intensive Care Unit were associated with increased oxidative stress, as indicated by a decrease in total and native thiol and an increase in disulfide ratios. To prevent negative impacts on patients from transfusions with erythrocyte suspensions that have high oxidative properties, consideration should be given to strengthening the antioxidant defense system. Further research is needed, and plans should be made for the development of appropriate strategies.

## ETHICAL DECLARATIONS

**Ethics Committee Approval**: The study was carried out with the permission of Selçuk University Non-interventional Clinical Researches Ethics Committee (Date: 13/01/2021, Decision No: 2021/01).

**Informed Consent:** All patients signed the free and informed consent form.

**Referee Evaluation Process:** Externally peer-reviewed.

**Conflict of Interest Statement:** The authors have no conflicts of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

**Author Contributions:** All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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