Anatolian Journal of Emergency Medicine 2024;7(2):63-66

ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA MAKALESİ

Impact of Erythrocyte Transfusion on Thiol/Disulfide Homeostasis in Anemic Patients

Eritrosit Transfüzyonunun Anemik Hastalarda Tiyol/Disülfit Homeostazı Üzerine Etkisi

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ABSTRACT

Aim: Anemia limits oxygen delivery to tissues, potentially leading to increased oxidative stress. This study examined the impact of erythrocyte transfusion on thiol/disulfide homeostasis, a marker of oxidative stress, in anemic patients.

Material and Methods: Sixty anemic patients receiving transfusions in emergency department were included in this study. We measured total thiol, native thiol, and disulfide levels in blood samples collected before and after red blood cell transfusion. To isolate the effects of anemia, a subgroup analysis that excluded patients with acute/subacute hemorrhage or aplastic anemia was also done.

Results: The concentrations of total thiol (374.24±94.84 to 344.6±88.5) and native thiol (338.91±90.51 to 304.91±90.95) significantly decreased after erythrocyte suspension treatment compared to baseline (P<0.0001). However, the disulfide level increased (17.75±6.63 to 20.97±7.25; p = 0.009). Our findings suggest a potential increase in overall oxidative stress.

Conclusion: By giving transfusions to anemic patients one may think that oxidative stress will be reduced due to increased oxygen carrying capacity but our results show the contrary. Our results support that oxidative stress increases in vivo in patients who receive transfusion right after the procedure and this is probably due to increased oxidative stress in stored erythrocyte suspensions.

Keywords: Anemia, disulfides, erythrocyte transfusion, sulfhydryl compounds, oxidative stress

ÖZ

Amaç: Anemi, dokulara oksijen taşınmasını kısıtlar, bu da potansiyel olarak artmış oksidatif stres ile sonuçlanabilir. Bu çalışmada, anemik hastalarda eritrosit transfüzyonunun oksidatif stresin bir göstergesi olan tiyol/disülfit homeostazı üzerindeki etkisinin incelenmesi amaçlandı.

Gereç ve Yöntemler: Çalışmaya acil serviste transfüzyon alan altmış anemik hasta dahil edildi. Eritrosit transfüzyonu öncesi ve sonrası toplanan kan örneklerinde toplam tiyol, nativ tiyol ve disülfid seviyeleri ölçüldü. Aneminin etkilerini izole etmek için, akut/subakut kanama veya aplastik anemi olan hastalar hariç tutularak alt grup analizi de yapıldı.

Bulgular: Total tiyol (374.24±94.84'ten 344.6±88.5'e) ve nativ tiyol (338.91±90.51'den 304.91±90.95'e) konsantrasyonları, eritrosit süspansiyonu transfüzyonu sonrası başlangıca göre anlamlı olarak azaldı (P<0.0001). Ancak, disülfid seviyesi arttı (17.75±6.63'ten 20.97±7.25'e; p = 0.009). Bulgularımız hastalarda oksidatif stresin transfüzyon sonrası arttığını göstermektedir.

Sonuç: Anemik hastalara eritrosit transfüzyonu yapıldığında oksidatif stresin azalacağı düşünülebilir çünkü transfüzyonla oksijen taşıma kapasitesi artar, ancak sonuçlarımız bunun aksini göstermektedir. Sonuçlarımız, transfüzyon alan hastalarda işlemden hemen sonra oksidatif stresin arttığını göstermektedir. Bu durum muhtemelen depolanan eritrosit süspansiyonlarında artan oksidatif stresle ilgili olabilir.

Anahtar Kelimeler: Anemi, disulfide, eritrosit transfüzyonu, sulfhidrid bileşikleri, oksidatif stres

Received: 17 April 2024 Accepted: 09 June 2024

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Atıf için/Cited as: Coskun S, Icme F, Yuzbasioglu Y, et al. Impact of Erythrocyte Transfusion on Thiol/Disulfide Homeostasis in Anemic Patients . Anatolian J Emerg Med 2024;7(2):63-66. https://doi.org/10.54996/anatolianjem.1468418.

Introduction

Anemia, a condition characterized by a deficiency in red blood cells, disrupts oxygen delivery throughout the body (1). This limitation in oxygen supply can lead to a cellular imbalance known as oxidative stress. Oxidative stress occurs when the production of free radicals and other reactive oxygen species (ROS) outpaces the body's natural antioxidant defenses (2).

This study investigated the impact of erythrocyte transfusion, a common treatment for deep anemia, on thiol/disulfide homeostasis in anemic patients. Thiol/disulfide homeostasis is a marker of oxidative stress and reflects the balance between reduced thiol groups (antioxidant potential) and oxidized disulfide bonds within proteins. By measuring thiol/disulfide levels before and after transfusions, we aimed to determine if improving oxygen delivery through erythrocyte transfusion influences oxidative stress in anemic patients.

Material and Methods

The study was conducted in the emergency department of a Training and Research Hospital which has 140000 patient visits annually. Study period was between June 2015 to December 2015. This is a prospective, analytic, observational study.

To investigate the influence of erythrocyte transfusion on oxidative stress in anemia, this study enrolled sixty anemic patients admitted to a hospital emergency department who required transfusions. Studies regarding human use were conducted in accordance with all relevant national regulations, institutional policies and the principles of the Declaration of Helsinki and were approved by the authors' institutional ethics committee (Yildirim Beyazit University Etic committee 26379996/214) Written informed consent was obtained from the patients.

Inclusion Criteria: During the study period we included all patients who were admitted to emergency department due to any acute condition and had anemia that needed erythrocyte transfusion in the emergency department. Patients were included if their hemoglobin (Hb) level was below 7 g/dL and their hematocrit (Hct) was less than 21% upon admission.

Exclusion Criteria: Patients with Hb level above 7 were excluded even if they were symptomatic and required transfusion in emergency department. To isolate the effects of anemia on oxidative stress, we excluded patients with pre-existing conditions that could independently contribute to oxidative stress, we excluded patients who met any of the following criteria: Electrocardiogram (ECG) showing ST-segment elevation or Q waves; Known or suspected thrombotic disorder; pre-existing treatment with a medication affecting blood coagulation before sample collection; Uncontrolled medical conditions within the past month, including diabetes mellitus, thyroid dysfunction, surgery, trauma, malignancy, autoimmune disease, inflammatory disease, electrolyte imbalance, chronic kidney disease, chronic liver disease, or active infection. By excluding patients with these conditions, we minimized the influence of confounding factors that could contribute to oxidative stress. Patients with incomplete study forms were excluded from the analysis.

Initial blood samples for complete blood count (CBC) were taken within 2 hours of presentation to emergency room. Decision to give erythrocyte transfusion and how many units will be given was made by the attending emergency physician responsible for the patients care and the number of units given was not standard.

Transfusion Blood samples were collected from each patient at two time points: immediately before the erythrocyte transfusion and again shortly after the transfusion was completed. These samples were then analyzed using a standardized method to measure their levels of total thiol, native thiol, and disulfide bonds in unrefrigerated samples. The spectrophotometric method defined by Erel O et al was utilized to assess the serum disulfide/thiol homeostasis (3). Standardized venous blood was obtained without stasis through an antecubital vein for studies of complete blood count (CBC) and biochemical parameters. Blood samples for measurements of thiol/disulfide homeostasis were obtained with the patient supine via a 21-gauge needle inserted into an antecubital vein.

Biochemical Assay Summary

This assay measures the concentration of dynamic disulfide bonds (-S-S-) in a sample. Sodium borohydride (NaBH4) is used to reduce these bonds, converting them into free functional thiol groups (-SH). Formaldehyde is then added to completely consume and remove any remaining NaBH4. This prevents further reduction of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and the disulfide bonds formed after its reaction with the sample. Finally, DTNB identifies all thiol groups (both reduced and native) at the end of the reaction.

Statistical analysis

All analyses were performed with SPSS software Version 24.0 (SPSS Inc., Chicago, IL) and p value <0.05 was considered statistically significant. Categorical variables are expressed as numbers (%), and continuous variables are presented as mean ± SD or median (minimum-maximum values). Continuous data were tested for normal distribution with the Kolmogorov-Smirnov test. Differences between ratios were compared by Paired Sample T test. Sample size was not calculated but a convenience sample was used according to the sample size of previous studies about thiol disulfide homeostasis. Post hoc power analysis revealed a power of 0.693 for total thiol measurement, 0.815 for native thiol and 0.942 for dynamic disulfide for the 60 patients.

Results

Out of 76 patients initially enrolled, 16 were excluded from statistical analysis due to incomplete data, resulting in a final sample size of 60 participants (27 males, 33 females). Patient characteristics, mean Hb levels before and after transfusion and amount of packed red blood cells (pRBC) transfused of 60 patients were shown in Table 1. Minimum amount of pRBC transfusion was 2 units and maximum was 5 units.

The concentrations of total thiol (374.24±94.84 to 344.6±88.5) and native thiol (338.91±90.51 to 304.91±90.95) significantly decreased after erythrocyte suspension treatment compared to baseline (P<0.0001). However, the disulfide level increased (17.75±6.63 to 20.97±7.25; p = 0.009). Test results of total thiol, native thiol and dynamic disulfide concentrations before and after the administration of erythrocyte suspensions are shown in Table 2.

	Means±SD	Minimum-Maximum
Age, year	72.24±13.18	[34-91]
Men/Women, n	27/33	
Hb level on the admission	5.5±0.96	[2.9-6.9 mg/dl]
Iron level on the admission	19.97±9.68	[3-188 gr/dL]
Total iron binding capacity	351.05±95.87	[138-551mcg/dL]
Hb level after the treatment	9.6±1.12	[7.6-11.6 mg/dl]
The amount of Packed RBC transfused	3.11±0.86	[2-5]

Table 1. Demographic and clinical data of the patients

	Baseline	After Replacement	p value
Total Thiol	374.24±94.84	344.6±88.5	<0.001
Native Thiol	338.91±90.51	304.91±90.95	<0.001
Dynamic Disulfide	17.75±6.63	20.97±7.25	0.009

Table 2. Total thiol, native thiol and dynamic disulfide concentrations of group 1 before and after the administration of erythrocyte suspensions and paired sample T test results (n:60)
Values are means±SD; All differences are statistically significant

The statistics were repeated after the removal of a total of 13 patients who had acute or subacute hemorrhage (12 patients), and aplastic anemia (did not cause coagulation disorder) (1 patient), resulting in more specific data. The patient subgroup resulting from excluding these patients is described below. These patients' paired-sample T-test results of total thiol, native thiol and dynamic disulfide concentrations before and after the administration of erythrocyte suspensions are shown in Table 3.

	Baseline	After Replacement	p value
Total Thiol	379.45±97.68	332.54±86.36	<0.001
Native Thiol	344.87±93.07	293.61±90.09	<0.001
Dynamic Disulfide	17.39±6,89	20.91±7.8	0,016

Table 3. Total thiol, native thiol and dynamic disulfide concentrations of group 2 before and after the administration of erythrocyte suspensions and paired sample T test results (n:47)
Values are means±SD; All differences are statistically significant

Discussion

Anemia is a condition characterized by a significant decrease in the number of circulating red blood cells. This decrease can be caused by several factors, including blood loss, increased destruction of red blood cells (hemolysis), or decreased production of red blood cells (1). Oxidative stress is known to increase in various types of anemia, including iron deficiency anemia, hemoglobinopathies like thalassemias, hemolytic anemias like sickle cell disease, conditions requiring hemodialysis, and infection-induced anemias such as malaria (2, 4-9).

The dynamic balance between thiol and disulfide is essential for the antioxidant system. Total thiol (TT), particularly the protein thiol (-SH) groups, are crucial plasma antioxidants (3). The most serious complications of severe anemia arise from tissue hypoxia. Shock, hypotension, or coronary and pulmonary insufficiency can occur. The oxygen carrying capacity of the blood—is, by definition, low in anemic patients. RBC transfusion is indicated to improve oxygen delivery to tissues (10). By giving transfusions one may think that oxidative stress will be reduced due to increased oxygen carrying capacity but our results show the contrary. In our study, the concentrations of total thiol (379,45±97,68 to 332,54±86,36) and native thiol (344,87±93,07 to 293,61±90,09) were decreased significantly after erythrocyte suspension treatment compared with baseline (P<0.0001). This may be because of volume dilution caused by transfusion but then we would expect disulfide levels to also decrease. Since thiol and native thiol levels decreased and disulfide levels increased oxidative stress is increased in our patients.

There are studies that show oxidative stress increase with storage period in erythrocyte suspensions in literature. Blood storage inflicts a series of changes on red blood cells (RBC) that compromise the cell survival and functionality. Largely these alterations (storage lesions) are due to oxidative modifications. Donor RBCs are fundamentally altered during processing and storage, in a fashion that impairs oxygen transport efficacy (10,11). It is harder to show the effect of these changes in clinical practice. Our results support that oxidative stress increases in vivo in patients who receive transfusion right after the procedure and this is probably due to increased oxidative stress in stored erythrocyte suspensions. Likewise, in their study Yılmaz et. al found that oxidative stress levels increase after erythrocyte transfusions in pediatric intensive care patients (12).

Acute blood loss can confound the diagnosis of anemia due to changes in plasma volume as in blood loss. We thought the physiological response to acute anemia may have changed the physiological response to erythrocyte replacement. Therefore; after the removal of patients with acute or subacute hemorrhage, the statistical analysis was repeated. test results of total thiol, native thiol and dynamic disulfide concentrations of group 2 before and after the administration of erythrocyte suspensions were more significant. (total thiol 374,24±94,84 to 344,6±88,5) and native thiol (338,91±90,51to 304,91±90,95) Increase in oxidative stress was also found statistically significant in this group of patients.

Excluding patients with certain pre-existing conditions allowed us to isolate the effects of anemia on oxidative stress. However, including a more diverse patient population in future research could provide a more comprehensive picture of how erythrocyte transfusion affects thiol/disulfide homeostasis across various medical backgrounds.

In literature, the possibility of improving the quality of packed RBC stored for transfusion including N-acetyl cysteine (NAC) in the preservation solutions are under investigation. Amen et al reported that relatively high concentrations of NAC (20-25 mM) were necessary to prevent the progressive leakage of hemoglobin, while lower concentrations (2.5 mM) were enough to prevent the loss of reduced glutathione during the first 21 days of storage. They also reported that peroxiredoxin-2 was also affected during storage, with a progressive accumulation of disulfide-linked dimers and hetero-protein complexes in the cytosol and also in the membrane of stored RBC. There is an increasing point of view on potential use of NAC as an additive in the

preservation solution to improve RBC performance after transfusion (13,14,15).

Thiol/disulfide homeostasis offers several advantages for studying oxidative stress. First, it can be measured using a relatively simple and accessible method compared to other markers, making it a practical tool for clinical research. Second, it reflects the dynamic interplay between disulfide bond formation and reduction in proteins and provides valuable insight into ongoing oxidative stress processes. Finally, although not entirely specific to anemia, changes in thiol/disulfide homeostasis may provide clues to oxidative stress, particularly like our study associated with red blood cell dysfunction or deficiency.

Limitations

Due to small sample size we could not make sub group comparisons between patients with initial different Hb levels. We also could not compare groups that receive different units of erythrocyte transfusions. We did not calculate the body mass index of the patients that may have an importance in total body blood volume. We did not record the storage time of erythrocytes that may have an effect on oxidative stress in our study. There is no control group included. Although we have excluded patients that has a known factor that increases oxidative stress treatment other than erythrocyte suspensions given after entering the emergency room might have influenced the results. Additionally, incorporating a control group of anemic patients who did not receive a transfusion would strengthen the study design by allowing for a direct comparison of changes in oxidative stress levels. Since we choose a convenience sample post hoc power analysis revealed less than desired for total thiol parameter and this should be considered when interpreting our results.

Conclusion

This study provides evidence that erythrocyte transfusion in anemic patients in emergency department may worsen thiol/disulfide homeostasis, potentially reflecting an increase in oxidative stress. Further research is necessary to elucidate the underlying mechanisms responsible for these changes and to explore the potential impact of red blood cell storage duration on oxidative stress after transfusion. Additionally, a control group, including more subjects to improve power for thiol, grouping patients according to their baseline Hb levels and the amount of pRBC transfusion could offer a more comprehensive understanding of the clinical implications of erythrocyte transfusion on oxidative stress in anemia.

Conflict of Interest: The authors declare that there is no conflict of interest.

Financial Support: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' Contribution: Each author contributed significantly to the research process and preparation of the manuscript. All authors reviewed and approved the final version of the manuscript for submission.

Ethical Approval: This retrospective study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Local Ethics Committee approved this study. The study received approval from the Yildirim Beyazit University Ethics Committee (registration number: 26379996/214)

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