BLOOD COMPONENTS AND REGENERATIVE MEDICINE

Original article

GSTP1 rs1695 and rs1871042, and *SOD2* rs4880 as molecular markers of lipid peroxidation in blood storage

Marcelo Reis do Nascimento[1], Rodrigo O. Silva de Souza[2], Alexander L. Silva Junior[1], Emerson Silva Lima[2], Marilda Souza Gonçalves[3], José Pereira de Moura Neto[1]

[1]*Haematology and Haemotherapy Hospital Foundation of Amazonas, Manaus, Amazonas;*
[2]*Faculty of Pharmaceutical Sciences, Federal University of Amazonas, Manaus, Amazonas;*
[3]*Oswaldo Cruz Foundation - Gonçalo Moniz Research Centre, Salvador, Bahia, Brazil*

Background - Red blood cells (RBC) are subject to oxidative stress by reactive oxygen species (ROS) during storage. Molecular characterisation of oxidative stress in stored RBC, which may also occur in other blood components during long periods of storage, is rare.

Materials and methods - Our study included 45 healthy RBC donors recruited in Brazil. Blood was collected into standard Grifols® Triple Bags containing CPD SAG-M. Haematological values, biochemical data, and oxidative stress markers were assessed weekly during storage until 42 days after collection. *GSTM1* and *GSTT1* were determined by multiplex-polymerase chain reaction (PCR), while *GSTP1* rs1695 and rs1871042, CAT rs1001179, and *SOD2* rs4880 were evaluated by real-time PCR.

Results - A direct proportional relationship was found between storage time and levels of ROS and thiobarbituric acid reactive substances (TBARS, indicators of lipid peroxidation) ($p<0.001$). These parameters were indirectly proportional to ABTS values ($p<0.001$). The plasma concentration of TBARS was associated with GSTP1 $303^{AG/GG}$, *GSTP1* $-16^{CT/TT}$, and *SOD2* $47^{CT/TT}$ genotypes. Single-nucleotide polymorphisms at the CAT C-262T gene were not associated with TBARS, nor were oxidative markers of ROS.

Discussion - Prolonged storage may result in the onset of erythrocyte deterioration. Our results clearly indicate that erythrocytes are capable of attenuating ROS for 2 weeks of storage. We observed an association between elevated TBARS levels and the presence of *GSTP1* and *SOD2* variants in stored RBC. Although notable for heterozygous variants, this association was even stronger for the homozygous variants *GSTP1* rs1695 (303^{GG}), *GSTP1* rs1871042 (-16^{TT}), and *SOD2* rs4880 (47^{TT}). These findings accentuate the importance of genetic factors in storage lesions and will expand our understanding and consideration of endogenous and exogenous causes in improving clinical treatment with blood transfusions.

Keywords: *GSTP1, SOD2, lipid peroxidation, blood storage, storage lesions.*

INTRODUCTION

Packed red blood cells (RBC) must be stored in optimum conditions in order to preserve their efficacy[1,2]. Several recent surveys have renewed interest in studies on RBC during refrigerated storage aimed at improving the stability, transfusion efficacy, and safety up to

Arrived: 13 March 2020
Revision accepted: 12 June 2020
Correspondence: José Pereira de Moura Neto
e-mail: jpmn@ufam.edu.br

© SIMTIPRO Srl

All rights reserved - For personal use only
No other use without premission

© SIMTIPRO Srl

42 days of this blood product[3]. In fact, many retrospective studies have demonstrated multiple complications associated with transfusion of old RBC stored for longer than 28 days, compared with the transfusion of fresh RBC stored for less than 14 days. These effects are linked to *in vitro* specification during the processing and the blood storage. Moreover, they are determined by the donors' characteristics[4,5].

Some of these studies, however, failed to propose plausible pathophysiological mechanisms and their conclusions have been rather controversial. In addition, some findings show weak associations: in the most widely discussed study, the incidence of in-hospital mortality in patients receiving RBC transfusions showed a statistically significant increase from only 1.7 to 2.8% in those who received old RBC[6]. In another study, transfusion of blood stored for more than 2 weeks was associated with significantly increased risks of post-operative complications and infection after major injury[7].

Any research on the mechanisms associated with complications arising from the transfusion of old RBC should explain this relatively subtle variance in the incidence. Several randomised trials are now underway to address this issue, including the Red Cell Storage Duration Study (RECESS), the Age of Blood Evaluation (ABLE) study, and the Age of Red Blood Cells in Premature Infants (ARIPI) study[8-10]. Although these studies may provide very useful information, they are not specifically designed to determine particular pathophysiological mechanisms.

Several study reports agree that negative consequences of the transfusion of old RBC are a result of a series of biochemical changes in the stored cells, defined as storage lesions[11,12]. Tinmouth *et al*. observed various changes in erythrocytes during storage, including changes in shape that led to decreased membrane deformability, increased aggregability, and increased intracellular viscosity; membrane loss; lysis; reduced levels of glutathione (GSH) and nitric oxide; increased oxidation of cellular lipids and proteins; higher levels of extracellular haemoglobin, potassium, and lactate dehydrogenase; and lower extracellular pH[13].

Several studies have focused on damage caused by oxidative stress as a factor in storage lesions, since RBC are very susceptible to oxidative stress. Some of the storage lesions, including membrane loss, are attributed to lipid peroxidation of membrane and cytoskeletal proteins[14,15]. Storage of RBC progressively deregulates redox poise, decreasing GSH activity and increasing glutathione disulphide levels, which result in a rise in levels of reactive oxygen species (ROS) in the form of hydroxyl radicals and superoxide[16,17]. Previous studies on reducing oxidative damage have examined storage under carbon monoxide to stabilise haemoglobin and prevention of oxygen diffusion into the bags during storage; however, no such studies have reported acceptable RBC recovery in transfusion[18,19].

Plasma antioxidant and RBC antioxidant enzyme levels decrease gradually in relation to the duration of storage, inducing progressive pathological alterations in RBC membrane proteins[20]. Glutathione-S-transferases (GST) are a family of phase II enzymes that play special roles in metabolism and detoxification[21]. Glutathione S-transferase P1 (GSTP1) is involved in the detoxification of xenobiotics, metabolism of many drugs, apoptosis, and cell cycle regulation[22,23]. Common *GSTP1* genetic polymorphisms have been widely studied; the single-nucleotide polymorphisms (SNP) A313G and IVS6-C16T express a protein with altered thermal instability and catalytic activity[24,25].

Mitochondrial superoxide dismutase 2 (SOD2) along with catalase (CAT) are first-line antioxidants in the defence against ROS[26]. While mitochondrial *SOD2* acts in the conversion of O^{2-} to H_2O_2, catalase converts H_2O_2 into water and oxygen[27,28]. The *SOD2* C47T SNP alters the structure of the enzyme, decreasing the efficiency of its entry into the mitochondrial matrix and consequently reduces H_2O_2 production. Catalase is expressed in all tissue types, but in particular in the liver and kidney; it exists in a soluble state in erythrocytes[29]. The *CAT* C-262T SNP is found in the promoter region of the gene and is associated with low enzyme activity[30].

The relationships between the storage period of erythrocytes and their haemoglobin level and oxidative status remain unclear. Studies have demonstrated the involvement of polymorphisms of several genes in improvements or decreases in oxidative status. Including genetic variant information in blood transfusion management is essential for risk prediction and avoiding transfusion complications, and may allow individualised approaches to transfusions for at-risk recipients[31,32].

All rights reserved - For personal use only
No other use without premission

Therefore, the purpose of this study was to identify oxidative changes that may compromise the physiological functions of erythrocytes in stored blood and their antioxidative defences. To this end, we explored the contributions of the *SNP* of *GSTP1 A303G*, *GSTP1 IVS -16CT*, *SOD2 C47T*, and *CAT C-262T* to modification of the levels of oxidative stress markers correlated with antioxidant status in erythrocytes of stored blood.

MATERIALS AND METHODS

Experimental design

The analyses were conducted using 45 blood bags from healthy volunteer donors recruited from the Haematology and Haemotherapy Hospital Foundation of Amazonas (Manaus, Amazonas, Brazil) (age: 35.4±15.11 years; 36 males and 9 females). Healthy volunteer donors were considered fit for study inclusion following a clinical interview. We excluded donors with positive serological tests and carriers of structural haemoglobinopathies, as well as haemolysed, irradiated, or washed specimens with positive microbiology results.

Blood samples

Our study was conducted on whole blood (450±50 mL) collected in a Triple Bag CPD SAG-M system containing citrate-phosphate-dextrose as an anticoagulant in the collection container (Grifols, São Paulo, Brazil) and meeting the requisites set out in the "Consolidation Ordinance n. 5, September 2017 - Consolidation of the rules on health actions and services of the Unified Health System - Brazil"[33] for blood donation. Immediately after collection, the whole blood units were centrifuged at 2,750 rpm for 4 min at 22 °C, and the resulting packed RBC were stored. During 42 days of storage, plasma aliquots were collected weekly from a total of 45 RBC units for haematological, biochemical, and oxidative stress analyses.

ABTS antioxidant assay

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) was used according to the method of Erel[34]. Using this method, assays were performed in 96-well test plates. Each well was loaded with a plasma sample and ABTS solution and incubated at room temperature in ambient light for 15 min. Samples were read at 714 nm on a test plate reader (TP-Reader, Thermoplate, Italy).

Lipid peroxidation assay

Lipid peroxidation by-products in the form of thiobarbituric acid reactive substances (TBARS) were examined using the method of Ohkawa *et al.*[35] adapted by Draper & Hadley[36]. Sodium dodecylsulphate 8.1% was added to the sample, which was vortexed and incubated at room temperature. After addition of 20% acetic acid and 0.6% thiobarbituric acid, the sample was placed in a boiling water bath. Samples were allowed to cool, then butanol-pyridine was added and the mixture was centrifuged. Absorbance of the coloured layer was measured at 532 nm.

Single nucleotide polymorphism genotyping

The DNA was extracted from leucocytes using the Brazol® technique according to the manufacturer's instructions. SNP genotyping was conducted using TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) on a StepOnePlus™ Real-Time PCR system (Applied Biosystems). The probes used were: *GSTP1* A303G (rs1695), *GSTP1 IVS* -16CT (rs1871042), *SOD2* C47T (rs4880) and *CAT* C-262T (rs1001179). The genotypic frequencies of polymorphisms were analysed according to frequencies predicted by the Hardy-Weinberg equilibrium.

Statistical analysis

Statistical analysis was carried out using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5.0 (GraphPad Software Inc., La Jolla, CA, USA). The distribution of variables was analysed using the Kolmogorov-Smirnov test. The analysis of variance (ANOVA) parametric test was used to examine the distribution of means of quantitative variables with a normal distribution within categories. The non-parametric Kruskal-Wallis test was used to compare quantitative variables without normal distributions.

RESULTS

Demographic characteristics

The majority of the samples came from male volunteers (36 bags, 80%), with type O blood being the most common (18 bags, 50%), followed by type A (10 bags, 27.78%), type B (7 bags, 19.44%), and type AB (1 bag, 2.78%). The nine female volunteers (20%) also most commonly had type O blood (5 bags, 55.56%), followed by type A (3 bags, 33.33%) and type B (1 bag, 11.11%).

© SIMTI PRO Srl

All rights reserved - For personal use only
No other use without premission

Blood donors' clinic-haematological and biochemical profiles

The haematological and biochemical data as well as oxidative stress parameters of all volunteers on the day of blood donation (day 1) were within reference values. There were no significant correlations between any of these data and sex (data not shown), so all subsequent analyses were performed without gender distinction.

Oxidative stress levels

As expected, TBARS levels increased with days of blood storage, confirming the increased exposure of stored erythrocytes to oxidative stress. Lipid peroxidation increased with increasing days of storage (Pearson's correlation: $r=0.739$; $p<0.001$) (data not shown). In addition, we observed a relationship between decreased levels of ABTS and increased oxidative stress (ROS production) (**Figure 1**), indicating decreased antioxidant capacity. The decrease in ABTS also reflects a greater susceptibility to oxidation and lipid peroxidation.

Correlations between genotypes and oxidative stress

We examined the genotypic frequencies of polymorphisms in *GSTP1*, *SOD2*, and *CAT* among volunteer blood donors in this study and found 41.7% had *GSTP1* 303^{AG}, 18.3% had *GSTP1* 303^{GG}; 26.7% had *GSTP1 IVS* -16^{CT} and 5.0% had *GSTP1 IVS* -16^{TT}; 48.3% had *SOD2* 47CT and 30.0% had *SOD2* 47^{TT}; and 21.7% had *CAT* -262CT and 5.0% had *CAT* -262^{TT}.

The plasma concentration of TBARS was associated with the *GSTP1* rs1695/rs1871042 and *SOD2* rs4880 genotypes (**Figures 2-5**). SNP at *CAT* rs1001179 were not associated with TBARS levels. The oxidative markers of ROS and ABTS were not associated with any of the SNP examined. For the first time, we describe an association of *GSTP1* $303^{AG/GG}$ and $-16^{CT/TT}$ variants with increased TBARS levels, which reflect lipid peroxidation during blood storage. For both *GSTP1* variants, we found higher TBARS levels in heterozygotes ($p<0.001$) and highest levels in the homozygotes for the mutant genotypes ($p<0.001$) (**Figures 2 and 3**). Notably, the synergy between the *GSTP1* A303G and IVS-C16T polymorphisms was reflected by elevated TBARS levels with increasing storage time (**Figure 4**). Our results also indicate an association between the genetic variation *SOD2* $47^{CT/TT}$ and increased lipid peroxidation, a potential molecular marker of oxidative stress in stored blood (**Figure 5**).

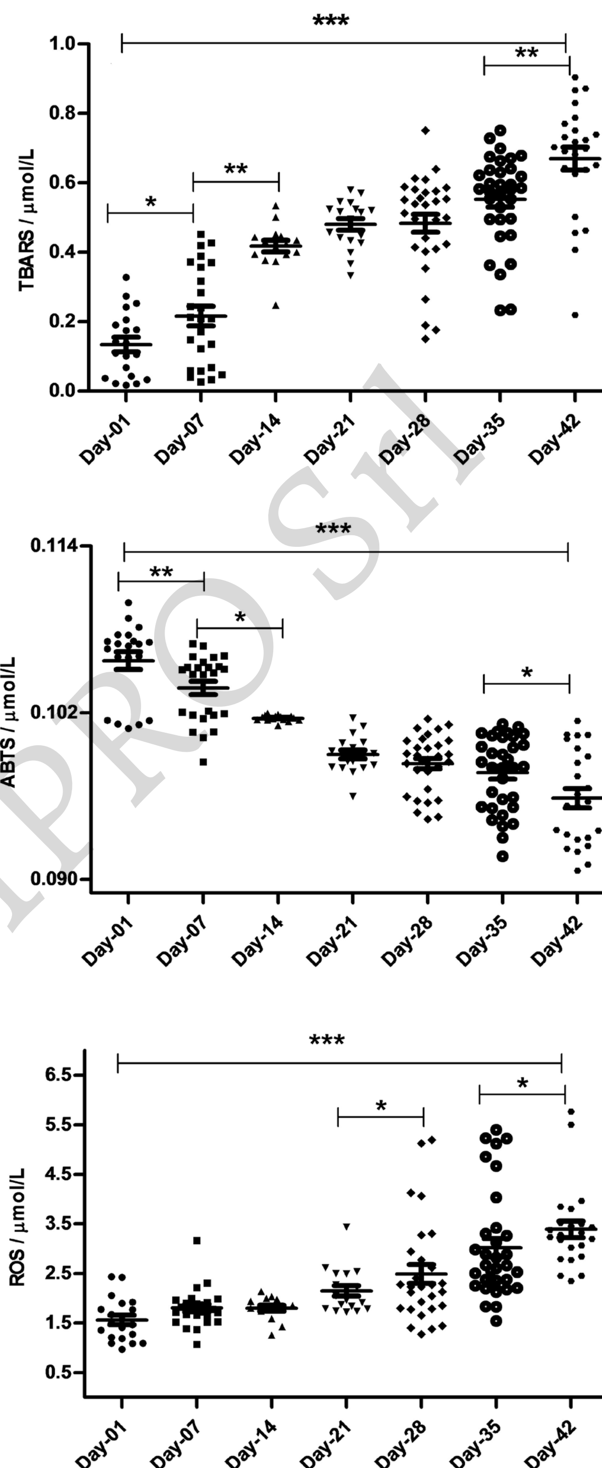
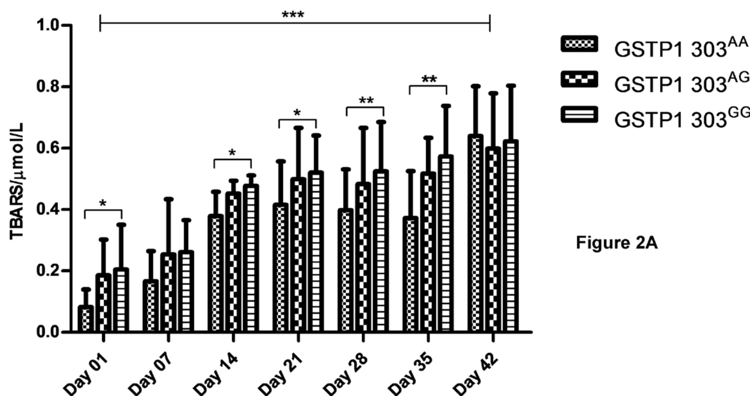


Figure 1 - Evaluation of thiobarbituric acid reactive substances (TBARS), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and reactive oxygen species (ROS) levels in the plasma of stored red blood cells over 42 days of storage

© SIMTI PRO Srl

All rights reserved - For personal use only
No other use without premission



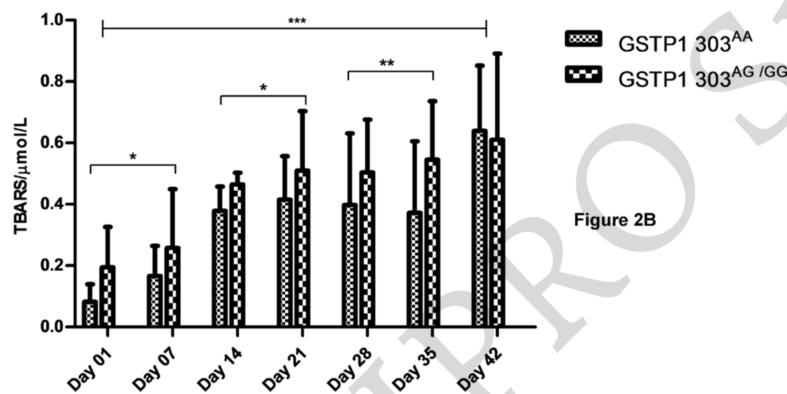


Figure 2 - Evaluation of the association between the levels of thiobarbituric acid reactive substances (TBARS) and the *GSTP1* A303G polymorphism. Genotypes modify TBARS concentration according to time in storage

(A) Associations of TBARS level with wild-type (AA), heterozygous (AG), and homozygous (GG) genotypes. (B) Associations of TBARS level with wild-type (AA) vs mutant genotypes [heterozygous (AG) plus homozygous (GG)]. Positive correlations were demonstrated between parameters and days of storage. Statistically significant differences: *p<0.05; **p<0.01; ***p<0.001.

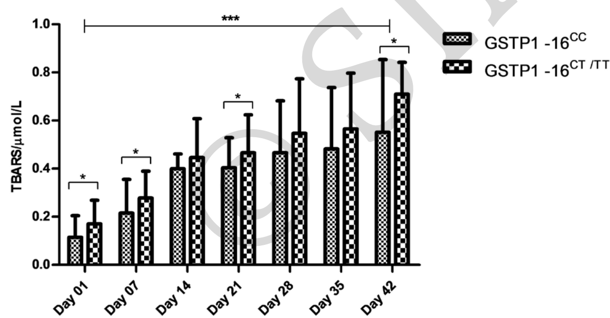


Figure 3 - Evaluation of the association between the levels of thiobarbituric acid reactive substances (TBARS) and *GSTP1* IVS-C16T polymorphisms

Lower levels of TBARS were present in the plasma of samples with the wild-type (CC) genotype. Positive correlations were demonstrated between the parameter and days of storage. Statistically significant differences: *p<0.05; **p<0.01; ***p<0.001.

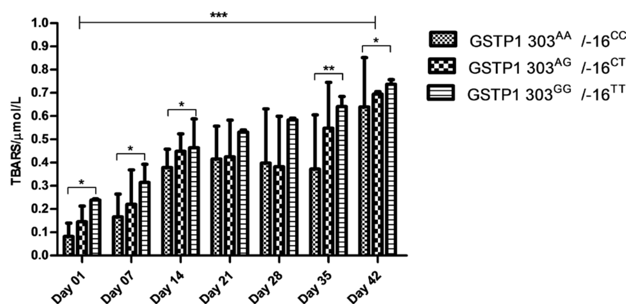


Figure 4 - Synergy between GSTP1 A303G and IVS-C16T polymorphisms in the association with levels of thiobarbituric acid reactive substances (TBARS) and storage time. Lower levels of TBARS were associated with wild-type genotypes (303^{AA}/-16^{CC})

Positive correlations were demonstrated between the parameter and days of storage. Statistically significant differences: *p<0.05; **p<0.01; ***p<0.001).

© SIMTI PRO Srl

All rights reserved - For personal use only
No other use without premission

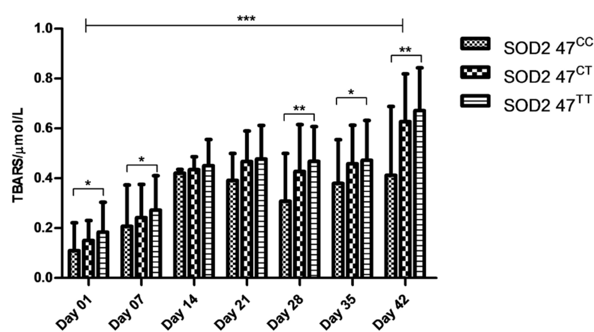


Figure 5 - Evaluation of the association between levels of thiobarbituric acid reactive substances (TBARS) and the SOD2 C47T polymorphism

TBARS levels were lower in samples from individuals with the wild-type (CC) genotype than in those from individuals with the heterozygous (CT) and homozygous (TT) genotypes. Positive correlations were demonstrated between the parameter and days of storage. Statistically significant differences: *p<0.05; **p<0.01; ***p<0.001.

DISCUSSION

This study was conducted to explore oxidative changes that may compromise the physiological functions of erythrocytes in stored blood and the contributions of various SNP to these modifications.

With regards to the participants, it is important to note that none of the nine female volunteer donors in this study was post-menopausal, used hormone replacement therapy, was a smoker or current drinker, or was taking aspirin. An overview of world blood donors showed that the distribution of female and male donors is similar around the world, with the average frequency of female donors being less than 35%[37,38].

Blood storage induces haemolysis, releasing free alpha chains and intra-erythrocytic haem, which trigger a haemoglobin oxidation pathway, thus causing damage to the RBC. This auto-oxidation reflects the formation of superoxide radicals (O^{2-}) and hydrogen peroxide (H_2O_2), resulting in oxidative stress[39]. There are limited studies assessing plasma total antioxidant capacity in stored blood. Some authors have shown depletion of antioxidants in haemolytic diseases, such as alpha and beta thalassaemia, with a significant decrease in total antioxidant capacity reported in patients with thalassaemia compared to that in healthy controls[40-42].

Polymorphisms in GST are distributed heterogeneously in different populations around the world[43,44]. The distribution of A303G variants of *GSTP1* in our study is similar to that in a study conducted among indigenous populations of Amazonas: wild-type, heterozygous, and homozygous frequencies were 40%, 41%, and 18%, respectively[45,46]. The genotypic frequencies of *SOD2* C47T and *CAT* C-262T are also in accordance with those in different Brazilian populations[47]. Taufer *et al.*[48] found that the frequencies of the 47^{CT} and 47^{TT} genotypes of *SOD2* were approximately 60.6% and 24.4%, respectively, in a population of mixed ethnicity, consistently with our study. GSTP regulates the activity of glutathione peroxidase VI (PRDX6) by initial heterodimerisation and subsequent S-glutathionylation. PRDX6 is a dual-functioning antioxidant enzyme that protects biological membranes against damage caused by lipid peroxidation[49,50]. We believe that *GSTP1* mediates activation of PRDX6, providing power contingent upon the GSTP genotype. Individual *GSTP1* mutants are expected to have significantly different antioxidant responses, particularly affecting protection of cell membranes against lipid peroxidation. Moreover, previous studies have suggested that GSTP1 polymorphisms are associated with cancer risk. Such differences will be important in the analysis of epidemiological data on differences in population sensitivities to oxidative stress when considering long-term blood storage[51,52].

Mitochondrial *SOD2* converts superoxide anion radicals generated by the electron transport chain into oxygen and H_2O_2 in the mitochondria[53]. It has been hypothesised that the SNP $47^{CT/TT}$ does not propitiate easier transport of the *SOD2* precursor into the mitochondria, resulting in lower basal activity of the enzyme[54]. The $47^{CT/TT}$ SNP might be associated with partial arrest of the enzyme within the inner mitochondrial membrane impairing its function[55]. However, Bastaki et al. showed that subjects homozygous for the *SOD2* 47^{TT} genotype have lower enzyme activity than $47^{CC/CT}$ individuals and that these latter have the greatest ability to induce oxidative stress in stored RBC, while those with the homozygous 47^{TT} genotype showed the lowest potential[56].

We found two SNP in *GSTP1* and *SOD2* associated with high levels of lipid peroxidation. We believe that there may be other genetic variants in the same genes or other related genes that are causally associated with increases of ROS or TBARS in stored RBC. The processes that maintain

© SIMTI PRO Srl

All rights reserved - For personal use only
No other use without premission

oxidative balance are complex, and our study addressed only a few of their components.

Our project involved a large number of stored blood samples (n=45), which allowed us to examine subgroups based on gender, age, anaemia, leucocytosis, and other factors. The associations between these variables and their importance in storage lesions will be examined in the future.

CONCLUSIONS

Blood transfusion is usually indicated for patients who have an insufficient supply of oxygen as a result of various diseases or acute or chronic blood loss. Thus, blood transfusion plays a role in saving lives. However, progressive lesions acquired during blood storage significantly reduce the clinical efficacy of transfusions and magnify the potential for adverse effects in blood recipients[57].

We found that oxidative stress may be associated with elevated TBARS as a result of lipid peroxidation influenced by the presence of *GSTP1* and *SOD2* variants, and that this association was accentuated in heterozygous carriers of the variants. However, a very strong association was found between TBARS and the homozygous presence of variants rs1695 (303^{GG}) and rs1871042 (-16^{TT}) of *GSTP1* and *SOD2* rs4880 (47^{TT}). Understanding the endogenous and exogenous causes underlying storage lesions of blood cells, including oxidative status and genetic factors, is important in order to improve clinical treatment with blood transfusions.

FUNDING AND RESOURCES

- Fundação de Amparo a Pesquisa do Estado do Amazonas (FAPEAM) [Amazonas State Research Support Foundation] - Process: 1094/2013-FAPEAM;
- Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) [National Council for Scientific and Technological Development] and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) [Coordination for the Improvement of Higher Education Staff];
- The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

AUTHORSHIP CONTRIBUTIONS

MdNR collected samples and performed the practical and laboratory parts of the entire project. He measured the concentrations of oxidative stress markers, including TBARS levels and performed the molecular tests for the genotyping of the *GSTP1* and *SOD2* gene polymorphisms. This project formed the basis of the dissertation for his Master's Degree.; ROSdS assisted in the measurement of lipid peroxidation markers and verified the analytical methods; ALS Jr assisted in the collection of samples and digitisation of results in data analysis programs; ESL assisted in the measurement protocols for oxidative stress markers, mainly in the TBARS assays, and wrote the manuscript with support from JPdMN; MdSG assisted in the development of the project and contributed to the final version of the manuscript; JPdMN conceived the project, designed the study, and advised MNdR. He helped supervise the project, assisted in sample collection and oxidative stress marker analyses and performed the analytical calculations and result simulations. All donors were volunteers and provided (or a legal guardian provided) informed, written consent.

The Authors declare no conflicts of interest.

REFERENCES

1. Hess JR, Greenwalt TG. Storage of red blood cells: new approaches. Transfus Med Rev 2002; **16**: 283-95.
2. Zolla L, D'Alessandro A, Rinalducci S, et al. Classic and alternative red blood cell storage strategies: seven years of "-omics" investigations. Blood Transfus 2015; **13**: 21-31.
3. Wang D, Sun J, Solomon SB, et al. Transfusion of older stored blood and risk of death: a meta-analysis. Transfusion 2012; **52**: 1184-95.
4. Jordan A, Chen D, Yi QL, et al. Assessing the influence of component processing and donor characteristics on quality of red cell concentrates using quality control data. Vox Sang 2016; **111**: 8-15.
5. Tissot JD, Bardyn M, Sonego G, et al. The storage lesions: from past to future. Transfus Clin Biol 2017; **24**: 277-84.
6. Koch CG, Li L, Sessler DI, Figueroa P, et al. Duration of red-cell storage and complications after cardiac surgery. New Engl J Med 2008; **358**: 1229-39.
7. Offner PJ, Moore EE, Biffl WL, et al. Increased rate of infection associated with transfusion of old blood after severe injury. Arch Surg 2002; **137**: 711-6.
8. Fergusson D, Hutton B, Hogan DL, et al. The age of red blood cells in premature infants (ARIPI) randomized controlled trial: study design. Transfus Med Rev 2009; **23**: 55-61.
9. Steiner ME, Assmann SF, Levy JH, et al. Addressing the question of the effect of RBC storage on clinical outcomes: the Red Cell Storage Duration Study (RECESS) (section 7). Transfus Apher Sci 2010; **43**: 107-16.
10. Lacroix J, Hebert P, Fergusson D, et al. The Age of Blood Evaluation (ABLE) randomized controlled trial: study design. Transfus Med Rev 2011; **25**: 197-205.
11. Blasi B, D'Alessandro A, Ramundo N, Zolla L. Red blood cell storage and cell morphology. Transfus Med. 2012; **22**: 90-6.

© SIMTI PRO Srl

All rights reserved - For personal use only
No other use without premission

12. Kato GJ. Understanding the erythrocyte storage lesion. Anesthesiology 2012; **117**: 1159-61.
13. Tinmouth A, Fergusson D, Yee IC, Hebert PC. Clinical consequences of red cell storage in the critically ill. Transfusion 2006; **46**: 2014-27.
14. Wolfe LC. Oxidative injuries to the red cell membrane during conventional blood preservation. Semin Hematol 1989; **26**: 307-12.
15. Bardyn M, Tissot JD, Prudent M. Oxidative stress and antioxidant defenses during blood processing and storage of erythrocyte concentrates. Transfus Clin Biol 2018; **25**: 96-100.
16. D'Amici GM, Rinalducci S, Zolla L. Proteomic analysis of RBC membrane protein degradation during blood storage. J Proteome Res 2007; **6**: 3242-55.
17. D'Alessandro A, Kriebardis AG, Rinalducci S, et al. An update on red blood cell storage lesions, as gleaned through biochemistry and omics technologies. Transfusion. 2015; **55**: 205-19.
18. Hogman CF, de Verdier CH, Ericson A, et al. Effects of oxygen on red cells during liquid storage at +4 degrees C. Vox Sang 1986; **51**: 27-34.
19. Wolfe L, Byrne A, Chiu D. The storage lesion of liquid preserved erythrocytes is blocked in vivo by treatment with carbon monoxide. Blood 1987; **70** (Suppl): 335a.
20. Ogunro PS, Ogungbamigbe TO, Muhibi MA. The influence of storage period on the antioxidants level of red blood cells and the plasma before transfusion. Afr J Med Med Sci 2010; 39: 99-104.
21. Ye Z, Song H. Glutathione S-transferase polymorphisms (GSTM1, GSTP1 and GSTT1) and the risk of acute leukaemia: a systematic review and meta-analysis. Eur J Cancer 2005; **41**: 980-9.
22. Fundia AF, Weich N, Crivelli A, et al. Glutathione S-transferase gene polymorphisms in celiac disease and their correlation with genomic instability phenotype. Clin Res Hepatol Gastroenterol. 2014; **38**: 379-84.
23. Pégeot H, Mathiot S, Perrot T, et al. Structural plasticity among glutathione transferase Phi members: natural combination of catalytic residues confers dual biochemical activities. FEBS J. 2017; **284**: 2442-2463.
24. Arevalo JA, Vázquez-Medina JP. The role of peroxiredoxin 6 in cell signaling. Antioxidants (Basel). 2018; **7**: 172.
25. Spurdle AB, Webb PM, Purdie DM, et al. Polymorphisms at the glutathione S-transferase GSTM1, GSTT1 and GSTP1 loci: risk of ovarian cancer by histological subtype. Carcinogenesis 2001; **22**: 67-72.
26. Crawford A, Fassett RG, Coombes JS, et al. Glutathione peroxidase, superoxide dismutase and catalase genotypes and activities and the progression of chronic kidney disease. Nephrol Dial Transpl 2011; **26**: 2806-13.
27. Xu Z, Zhu H, Luk JM, et al. Clinical significance of SOD2 and GSTP1 gene polymorphisms in Chinese patients with gastric cancer. Cancer 2012; **118**: 5489-96.
28. Zhan CD1, Sindhu RK, Pang J, et al. Superoxide dismutase, catalase and glutathione peroxidase in the spontaneously hypertensive rat kidney: effect of antioxidant-rich diet. J Hypertens 2004; **22**: 2025-33.
29. Crawford A, Fassett RG, Geraghty DP, et al. Relationships between single nucleotide polymorphisms of antioxidant enzymes and disease. Gene 2012; **501**: 89-103.
30. dos Santos KG, Canani LH, Gross JL, et al. The catalase -262C/T promoter polymorphism and diabetic complications in Caucasians with type 2 diabetes. Dis Markers 2006; **22**: 355-9.
31. Kanias T, Gladwin MT. Nitric oxide, hemolysis, and the red blood cell storage lesion: interactions between transfusion, donor, and recipient. Transfusion. 2012; **52**: 1388-92.
32. Edgren G, Rostgaard K, Vasan SK, et al. The new Scandinavian Donations and Transfusions database (SCANDAT2): a blood safety resource with added versatility. Transfusion 2015; **55**: 1600-6.
33. Ministério da Saúde, Gabinete do Ministro. [Portaria de Consolidação Nº 5, de 28 de Setembro de 2017.] Available at: https://portalarquivos2.saude.gov.br/images/pdf/2018/marco/29/PRC-5-Portaria-de-Consolida----o-n---5--de-28-de-setembro-de-2017.pdf. Accessed on 16/03/2020. [In Portuguese.]
34. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004; **37**: 277-85.
35. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; **95**: 351-8.
36. Drapper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990; **186**: 421-31.
37. Goldman M, Steele WR, Di Angelantonio E, et al. Comparison of donor and general population demographics over time: a BEST Collaborative group study. Transfusion 2017; **57**: 2469-76.
38. Veldhuizen IJ, Doggen CJ, Atsma F, De Kort WL. Donor profiles: demographic factors and their influence on the donor career. Vox Sang 2009; **97**: 129-38.
39. Scott MD. H2O2 injury in beta thalassemic erythrocytes: protective role of catalase and the prooxidant effects of GSH. Free Radic Biol Med 2006; **40**: 1264-72.
40. Ghone RA, Kumbar KM, Suryakar AN, et al. Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. Ind J Clini Biochem 2008; **23**: 337-40.
41. Hamed EA, ElMelegy NT. Renal functions in pediatric patients with beta-thalassemia major: relation to chelation therapy: original prospective study. Italian J Pediatr 2010; **36**: 39.
42. Cakmak A, Soker M, Koc A, Aksoy N. Prolidase activity and oxidative status in patients with thalassemia major. J Clin Lab Analysis 2010; **24**: 6-11.
43. Zhong S, Huang M, Yang X, et al. Relationship of glutathione S-transferase genotypes with side-effects of pulsed cyclophosphamide therapy in patients with systemic lupus erythematosus. Br J Clin Pharmacol 2006; **62**: 457-72.
44. Karaca S, Karaca M, Cesuroglu T, et al. GSTM1, GSTP1, and GSTT1 genetic variability in Turkish and worldwide populations. Am J Hum Biol 2015; **27**: 310-6.
45. Hiragi CdeO, de Oliveira SF, Hatagima A, et al. Glutathione S-transferase M1 and T1 polymorphisms in Brazilian African descendants. Hum Biol 2007; **79**: 131-40.
46. Klautau-Guimarães MDN, Hiragi CDO, Ascenção RFD, et al. Distribution of glutathione S-transferase GSTM1 and GSTT1 null phenotypes in Brazilian Amerindians. Genet Mol Biol 2005; **28**: 32-5.
47. Hiragi CdeO, Miranda-Vilela AL, Rocha DMS, et al. Superoxide dismutase, catalase, glutathione peroxidase and gluthatione S-transferases M1 and T1 gene polymorphisms in three Brazilian population groups. Genet Mol Biol 2011; **34**: 11-8.
48. Taufer M, Peres A, Andrade VM De, et al. Is the Val16Ala manganese superoxide dismutase polymorphism associated with the aging process? J Gerontol 2005; **60A**: 1-7.
49. Manevich Y, Sweitzer T, Pak JH, et al. 1-Cys peroxiredoxin overexpression protects cells against phospholipid peroxidation-mediated membrane damage. Proc Natl Acad Sci U S A 2002; **99**: 11599-604.
50. Manevich Y, Fisher AB. Peroxiredoxin 6, a 1-Cys peroxiredoxin, functions in antioxidant defense and lung phospholipid metabolism. Free Radic Biol Med 2005; **38**: 1422-32.
51. Harris MJ, Coggan M, Langton L, et al. Polymorphism of the Pi class glutathione S-transferase in normal populations and cancer patients. Pharmacogenetics. 1998; **8**: 27-31.
52. Townsend DM1, Findlay VJ, Fazilev F, et al. A glutathione S-transferase pi-activated prodrug causes kinase activation concurrent with S-glutathionylation of proteins. Mol Pharmacol 2006; **69**: 501-8.
53. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 4th ed. Oxford: Oxford University Press; 2007. xxxvi, 851 pp.
54. Sutton A, Khoury H, Prip-Buus C, et al. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacogenetics 2003; **13**: 145-57.
55. Hiroi S, Harada H, Nishi H, et al. Polymorphisms in the SOD2 and HLA-DRB1 genes are associated with nonfamilial idiopathic dilated cardiomyopathy in Japanese. Biochem Biophys Res Commun 1999; **261**: 332-9.
56. Bastaki M, Huen K, Manzanillo P, et al. Genotype-activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans. Pharmacogenet Genomics 2006; **16**: 279-86.
57. Ning S, Heddle NM, Acker JP. Exploring donor and product factors and their impact on red cell post-transfusion outcomes. Transfus Med Rev 2018; **32**: 28-35.

© SIMTI SERVIZI Srl

All rights reserved - For personal use only
No other use without premission