ORIGINAL ARTICLE

ASSESSMENT OF OXIDATIVE STRESS IN RECIPIENTS OF CHRONIC TRANSFUSION THERAPY

Rupak Chattopadhyay[1], Jayanta De[2], Kaushik Bhowmick[3], Utpal Chowdhury[4], Gopaldev Chattopadhyay[5], Indranil Chakraborty[3]

[1]Department of Biochemistry, Medical College Kolkata, [2]Department of Biochemistry, Midnapore Medical College, Midnapore, [3]Department of Biochemistry, Malda Medical College, Malda, [4]Institute of Hematology and Transfusion Medicine, Medical College Kolkata, West Bengal, India, [5]Bureau of Applied Economics and Statistics, Government of West Bengal, India

ABSTRACT

Background: Excess iron deposition in chronically transfused patients may cause organ damage through generation of free radicals and resultant oxidative stress. The objective of study was to assess the oxidative stress in recipients of chronic transfusion therapy.

Material & Methods: This cross-sectional study was undertaken in Department of Biochemistry, Medical College & Hospital, Kolkata, India in collaboration with Institute of Hematology & Transfusion Medicine of the same institute from May, 2007 to April, 2008. Serum Malondialdehyde, Vitamin E, erythrocyte reduced glutathione, osmotic fragility of red blood cells and serum ferritin were measured.

Results: Serum malondialdehyde concentration in cases 7.87±1.44nmol/ml was significantly higher than that of controls 5.00±1.27nmol/ml ($p<0.05$). A significantly higher osmotic fragility in cases 4.57±0.44 g/l NaCl as compared to that of controls 4.37+ 0.46g/l NaCl was also noted ($p<0.05$). However, vitamin E concentration was not significantly different between these two groups in cases 10.05±2.20 mg/l as compared to controls 13.04±1.94 mg/l ($p>0.05$). Similarly, no significant difference was observed in erythrocyte reduced glutathione concentration in cases 3.94±1.20ìmol/gHb compared to 5.80±1.08ìmol/gHb in controls. The cases demonstrated significantly higher ($p<0.05$) serum ferritin concentration (197.98±61.65ng/ml) as compared to controls (24.51±38.47ng/ml).

Conclusion: There was increased oxidative stress in cases receiving chronic transfusion therapy as signified by markers of oxidative damage, the increase in serum ferritin concentration might not have been sufficient enough to produce a concomitant decrease in serum vitamin E and reduced glutathione concentration, probably reflecting a transitory phase of ongoing cellular oxidative damage.

KEY WORDS: Oxidative stress, Transfusion therapy, Reduced glutathione, Vitamin E, Malondialdehyde, Ferritin.

This article may be cited as: Chattopadhyay R, De J, Bhowmick K, Chowdhury U, Chattopadhyay G, Chakraborty I. Assessment of oxidative stress in recipients of chronic transfusion therapy. Gomal J Med Sci 2013; 11:105-9.

INTRODUCTION

Despite significant improvement in transfusion technology, clear understanding of its indications and prudent use of safety measures in modern medicine, blood transfusion continues to have its limitations and potential hazards.[1,2] At present, chronic blood transfusion is indicated in various conditions like thalassemia, hypoplastic/aplastic anemia, myelodysplastic syndrome, Chronic myeloid leukemia.

Chronic blood transfusion may lead to a variety of adverse effects including blood borne infections, alloimmunization, febrile reactions and lethal iron overload. Iron accumulates in chronically transfused patients and iron overload has been documented to be a major problem in patients requiring long term red cell transfusion support for chronic anemia. It can affect the functions of heart, liver and other organs.[3,4] It has been suggested that excess iron deposition causes organ damage through generation of free radicals and resultant oxidative

Corresponding author:
Kaushik Bhowmick
Associate Professor
Department of Biochemistry
Malda Medical College & Hospital
West Bengal, India.
e-mail: drkbhowmick@gmail.com

stress. Free iron has been documented to catalyse conversion of hydrogen peroxide to more reactive free radical ions via the Fenton reactions that attack cellular membranes, proteins and DNA.[5]

Aerobic life is characterized by continuous production of oxidants which is balanced by equivalent synthesis or intake of antioxidants.[6] Antioxidant enzymes Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Catalase (CAT) form first line defense against free radical chain reactions, whereas vitamin E, beta carotene, vitamin C etc. act as a second line defense. A shift in the balance in favour of pro oxidants may trigger a cascade of reactions leading to the formation of highly reactive cytotoxic compounds such as reactive oxygen metabolites (ROM).[7] The effect of this oxidative stress has become a matter of growing interest. Oxidations and reduction modulate all cellular events including modification of signaling molecules in the inter and intra cellular communications.[8] Thus oxidative stress has been described as a double edged sword which is necessary at low grade for bodily functions, but becomes excessively activated in many pathological conditions.

The objective of study was to assess the oxidative stress in recipients of chronic transfusion therapy.

MATERIAL AND METHODS

This hospital-based, comparative, cross-sectional study was undertaken in the Department of Biochemistry, Medical College and Hospital, Kolkata, West Bengal in collaboration with Institute of Hematology & Transfusion Medicine of the same institute. The study was conducted from May, 2007 to April, 2008. The study was approved by the Ethical Committee, Medical College and Hospital, Kolkata. A sample of 65 cases with a sample of 59 age and sex matched healthy controls (from the attendants of these patients) was selected by convenience sampling from the Out Patient Department of Institute of Hematology and Transfusion Medicine. Inclusion criterion was cases requiring repeated whole blood or erythrocyte transfusion with different hematological diseases like myelodysplastic syndrome, aplastic anemia, chronic myeloid leukemia. All cases of thalassemia were excluded from the study as globin chains have been documented to aggregate and deposit in the erythrocyte membrane of thalassemic patients contributing to oxidative stress in addition to excess iron deposition.[9] Written informed consent was obtained from all participants of the study.

A 10 ml sample of venous blood was collected from cases and controls with aseptic technique for estimation of five research variables. To assess osmotic fragility and reduced glutathione concentration, 2.5 ml was transferred to a vial containing EDTA. The rest of the sample was allowed to clot. This was used to assess serum malondialdehyde (MDA), serum vitamin E and serum ferritin concentration.

Serum MDA was measured utilising its reaction with thiobarbituric acid (TBA).[10] Estimation of á-tocopherol in serum was done by the method of Baker and Flank.[11] Ferritin was measured employing commercially available assay kit utilizing solid phase enzyme immunoassay based on 'Sandwich' principle.[12] Estimation of reduced glutathione (GSH) concentration was based on the development of a yellow colour when 5,5'-dithiobis (2-nitrobenzoic acid) (Ellman's reagent, DTNB) was added to sulphydryl compounds.[13] Hemoglobin estimation was performed by cyanmethhaemoglobin method. Osmotic fragility of erythrocytes was estimated based on the method of Parpart et al.[14]

All these numeric data were statistically processed. These included measures of central tendency viz. calculation of mean and median, measure of variability viz. standard deviation, calculation of significance of difference viz. standard error, estimation of population parameter viz. confidence interval and measure of probability viz. calculation of p value.

RESULTS

The results of each research variable analyzed in casess and controls are presented below in Table.

It was observed that serum MDA concentration in cases was significantly higher than that of control population (7.87±1.44 nmol/ml and 5.00±1.27 nmol/ml respectively) ($p<0.05$). A significantly higher osmotic fragility compared to that of the control ($p<0.05$) was also noted. However, vitamin E concentration was not significantly different between these two groups (10.05±2.20 mg/L as compared to 13.04±1.94 mg/L in controls ($p>0.05$). Similarly, no significant difference was observed in erythrocyte reduced glutathione concentration (3.94 ±1.20 ìmol/g Hb compared to 5.80±1.08 ìmol/g Hb of controls) ($p>0.05$). Finally, the cases demonstrated significantly higher serum ferritin concentration (197.98±61.65 ng/ml) when compared to age and sex matched controls (24.51±38.47 ng/ml) ($p<0.05$).

Table : Descriptive & Inferential Statistics of 65 Cases and 59 Controls for assessment of Oxidative Stress in Recipients of Chronic Transfusion Therapy.

Research Varables	Groups	Mean	Median	SD	SE	C I (95%)		p-value
						Upper	Lower	
Malondialdehyde (ηmol/ml)	Cases	7.87	7.90	0.72	0.09	8.11	7.64	<0.05
	Controls	5.00	4.90	0.63	0.08	5.16	4.83	
Osmotic Fragility (g/l NaCl)	Cases	4.57	4.50	0.22	0.31	4.65	4.51	<0.05
	Controls	4.37	4.25	0.23	0.03	4.43	4.31	
Vitamin E (mg/l)	Cases	10.05	9.96	1.10	0.14	10.42	9.69	>0.05
	Controls	13.04	13.00	0.97	0.13	13.29	12.78	
Reduced Glutathione (μmol/g Hb)	Cases	3.94	3.89	0.60	0.07	4.15	3.75	<0.05
	Controls	5.80	5.82	0.54	0.07	5.94	5.66	
Ferritin (ηg/ml)	Cases	197.98	204.63	30.83	5.00	208.11	187.85	<0.05
	Controls	28.86	24.51	19.24	2.50	33.87	23.85	

SD=Standard Deviation, SE=Standard Error, CI=Confidence Interval.

DISCUSSION

The objective of the present study was based on the assumption that the patients suffering from different hematological conditions who get regular blood or erythrocyte transfusion, may suffer from iron overload. Only those patients were chosen who were not suffering from any diseases that are documented to produce any membrane related pathology such that any increase in osmotic fragility could be predicted as a reflection of increased damage of erythrocyte membrane.

Under physiological condition, ferric iron bound to proteins, prevents it from participating in reactions that could lead to cellular injury. Elevation in low molecular weight iron-binding complex in serum and intracellular transit pool have been associated in pathological condition of iron overload.[15-17] This promotes peroxidative damage to cell and organelle membrane. *Files* et al showed serum ferritin concentrations to increase linearly with cumulative transfusion volume.[18] The cases demonstrated statistically significantly higher ($p<0.05$) serum ferritin concentration when compared to age and sex matched controls. Thus it may be reasonably inferred that serum ferritin may be used as an indirect estimate of body iron store.

However, Harmatz et al observed a poor correlation between serum ferritin and the quantitative iron on liver biopsy.[19] Their data suggested that, in patients with sickle cell disease, ferritin was a poor marker for accurate assessment of iron overload. The measurement of plasma non transferrin bound iron (NTBI) has been proposed to be a more useful indicator of body iron status.

MDA has been established as a good indicator of oxidative stress. In the present study serum MDA concentration in cases was observed to be significantly higher ($p<0.05$) than that of control population. Earlier studies suggested that repeated transfusion lead to iron overload, which increased the MDA concentration suggesting oxidative stress. Livea et al, documented that because of repeated blood transfusion, thalassemia patients were subjected to peroxidative tissue injury by the secondary iron overload.[20] They showed that MDA/TBA adduct increased about twofold as compared to control. They showed that plasma ferritin level was positively correlated with the amount of MDA present in the plasma.

In the present study, there was no significant difference in vitamin E concentration between the two groups ($p>0.05$). However, Livea et al[20] and Das[21] reported vitamin E level to decrease significantly among thalassemic subjects getting regular transfusions. They suggested that liver damage due to iron deposition may play a major role in the depletion of lipid soluble vitamin E. It may be inferred that in the present study, the insignificant fall in vitamin E may have been due to sub-critical inflammatory changes in liver arising from iron burden.

Osmotic fragility is a test to detect red blood cells that are more fragile than that of normal erythrocytes. As, the red blood cells are subjected to oxidative stress in case of transfusion, it might be assumed that oxidative stress will make erythrocytes osmotically labile, as was indeed the observation in the present study. Ghosh et al[22] showed that erythrocytes from acute lymphoblastic leukemia patients had increased osmotic fragility of erythrocytes aris-

ing from oxidative stress. They showed that increased TBARS activity was correlated with increased osmotic fragility. In the present study the noted significantly higher osmotic fragility in cases compared to that in the controls ($p<0.05$) may be ascribed to increased oxidative stress which was evident by rise in MDA.

Reduced glutathione acts as one of the most important intracellular antioxidant. Kiessling et al, documented that red cells from patients of Sickle cell disease exhibited increased oxidative damage to both membrane and cytoskeletal proteins, as well as lipids.[23] This significantly decreased the level of reduced glutathione in erythrocytes. However, Livea et al, showed that although there was increased oxidative stress, reduced glutathione concentration did not decrease significantly.[20] No significant difference ($p>0.05$) was observed in erythrocyte reduced glutathione concentration between the cases and controls in spite of there being evidence of increased oxidative stress indicated by increased TBARS level, as well as increased osmotic fragility of erythrocytes. It may be assumed that although ferritin level increased significantly, total increase in ferritin was not sufficiently high to produce significant decrease of reduced glutathione.

Thus, in the present study, TBARS, Vitamin E, ferritin, reduced glutathione and erythrocyte osmotic fragility were assayed in patients suffering from myelodysplastic syndrome, aplastic anemia, chronic myeloid leukemia, etc, requiring repeated blood or erythrocyte transfusion. It was observed that oxidative stress was increased due to iron overload, as indicated by significant elevation of TBARS and ferritin concentration, resulting in increased osmotic fragility of erythrocytes. Vitamin E and reduced glutathione concentration were observed to be decreased, though this decrease was statistically insignificant.

CONCLUSION

It may be concluded from the finding of the study that though there was increased oxidative stress in patients receiving chronic transfusion therapy as signified by markers of oxidative damage, the increase in serum ferritin concentration might not have been sufficient enough to produce a concomitant decrease in serum vitamin E and reduced glutathione concentration, probably reflecting a transitory phase of ongoing cellular oxidative damage.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.