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Biochemistry Section

Effect of Blood Transfusions on Oxidant/Antioxidants Balance in Beta Thalassaemia Major Patients

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ABSTRACT

Introduction: Thalassaemia is the major health problem all over India which is prevalent amongst all population groups irrespective of caste, religion and creed. Number of studies from different parts of the country provides the data on distribution of various haemoglobinopathies but there are limited data from Northern Maharashtra, India. Regular blood transfusion is one of the conventional treatments for survival in patients with Beta Thalassaemia Major (BTM). This may cause oxidative stress and tissue injury due to iron overload and altered antioxidant enzymes.

Aim: To assess the effect of number of blood transfusions on oxidant/antioxidant balance in patients with beta thalassaemia major of North Maharashtra region.

Materials and Methods: Patients were divided into two groups on the basis of number of blood transfusions. Group A having number of transfusion ≤140 and Group B having number of transfusion >140. These groups were compared on the basis of haematological and biochemical parameters.

Results: Iron overload in Group B than Group A was indicated by significantly ($p<0.0001$) high levels of iron, ferritin and Transferrin Saturation (TS) with significantly low level of Total Iron Binding Capacity (TIBC). Oxidative stress in Group B is higher indicated by significantly ($p<0.0001$) high level of Malondialdehyde (MDA) and Copper (Cu) with significantly low levels of Glutathione Peroxidase (GPx), Zinc (Zn) and vitamin C than Group A. We also observed non significant difference in Superoxide Dismutase (SOD) and serum Ceruloplasmin (CP) in both groups.

Conclusion: The above data implies that after each blood transfusions, accumulation of free iron in the body of thalassaemic patients increases. This excess iron deposited in body tissues leads to many pathophysiological conditions like expanded plasma volume, cardiac output, reduced glucose tolerance, hepatitis, various endocrine abnormalities, cardiac and renal dysfunctions. Estimation of these biochemical parameters along with blood transfusion would help in early detection of the associated complications in these patients. This would be quite helpful to reduce the burden of this disease through preventive measures.

Keywords: Free iron accumulation, Iron overload, Malondialdehyde, Oxidative stress, Superoxide dismutase

INTRODUCTION

Beta thalassaemia is a major single gene disorder resulting from a reduced synthesis or absence of β-globin chain. The carrier rate of beta thalassaemia varies from 1% to 3% in Southern India and 3% to 15% in Northern India [1]. This mutant gene is common in certain communities of India like Sindhis and Punjabis from Northern India they have considerable high frequency i.e., 9.27% [2]. A number of studies from different parts of the country provide the data of distribution of various haemoglobinopathies but there are limited data from Northern Maharashtra [3,4]. The North Maharashtra region especially Dhule, Nandurbar and Jalgaon districts are tribal belts where sickle cell disease and beta thalassaemia are prevalent [5]. The severity of the disease depends on the amount of HbA (Adult Haemoglobin) and HbF (foetal Haemoglobin) present in the blood [6].

Beta thalassaemia is a genetic disease characterised by reduced or absent β globin chain. The first consequence of reduced β chain production is reduced production of the adult haemoglobin (HbA_1: $\alpha_2\beta_2$). A second consequence is imbalanced globin chain synthesis, in which non β chain synthesis proceeds at a normal rate and hence there is an excess of non β chain in the erythrocytes. These excess non β chains are unstable and precipitate in the bone marrow red cell precursors, giving rise to large intracellular inclusions that interfere with the red cell maturation, function and survival. However, those red cells that become mature and enter the circulation contain non β chain inclusions, which interfere with their passage through the micro circulation, particularly in the spleen and hence extramedullarly destruction of red cells takes place. Thus, the anaemia of beta thalassaemia results from ineffective erythropoiesis and a shortened red cell survival [7,8]. Hence, to keep the haemoglobin concentration within normal range i.e., 13-16 gm/dL, the transfusion therapy should be started when diagnosis is made and the haemoglobin level falls below 7 gm/dL [9,10]. Each blood unit introduces 200-250 mg iron into the body of thalassaemic patients and ferritin increases as an index of iron excess. This leads to many pathophysiological conditions like expanded plasma volume, cardiac output, reduced glucose tolerance, hepatitis, various endocrine abnormalities, cardiac and renal dysfunctions [11-13]. Highly increased iron in these patients has a catalytic role to produce powerful Reactive Oxidant Species (ROS) and free radicals, which lead to oxidative damage [14]. This oxidative stress and a possible consequential accelerated apoptosis may contribute to shortened life span of erythrocytes. MDA, a product of lipid peroxidation is generated in excess amounts in supporting the fact that large amounts of membrane bound iron is present in thalassaemic erythrocytes [15,16]. In such condition, depletion of endogenous antioxidants may be expected. Antioxidants are those complex and diverse group of molecules that protect biological sites from oxidative damage. They scavenge free radicals and other ROS [17]. Here we tried to assess the effect of number of blood transfusions on oxidant and antioxidants balance in patients with BTM.

Hypothesis

Null hypothesis: There is no association between multiple transfusions and biochemical parameters like Iron indices, oxidant and antioxidants in patients with BTM.

Alternative hypothesis: There is an association between multiple transfusions and biochemical parameters like Iron indices, oxidant and antioxidants in patients with BTM.

MATERIALS AND METHODS

This comparative study was carried out on 123 multi transfused patients with BTM aged between 6 months up to 20 years who received regular blood transfusions at two major blood banks and thalassaemia center of North Maharashtra region during the period of October 2011 to December 2013. Written consent was taken from parents of study participants. The clinical status like the basic information of age, occupation, duration of disease, family history and number of blood transfusions was confirmed with the help of physician and taken into account for the study. Ethical committee approval (Ref. No.2439/ACPM/Dhule) was taken before conducting the study.

Patients who are being transfused and managed for the clinical symptoms and manifestations of the disease were included in this present study. Patients were excluded if they had diseases like Hepatitis B or C infection, HIV infection, chronic renal/heart failure and splenectomy. Also, on special diet including vegetarian diet, or consumed multivitamin plus factitious mineral water. After an overnight fast of 10-12 hours 6 mL blood samples in plain bulb and 2 mL in heparinised bulb were taken from eligible those who were fulfilling inclusion criteria. Blood samples from plain bulbs were allowed to clot at room temperature for about 30 minutes and then centrifuged at 3000 rpm for 10 minutes. The separated serum was used for biochemical analysis by using Coralab plus Semi-automatic Analyser (Tulip Diagnostics (P) Ltd.,).

For this study we divided our study population into two groups on the basis of number of blood transfusions Group A having number of transfusion ≤140 and Group B having number of transfusion >140 and tried to assess the effect of number of blood transfusions on oxidant/antioxidant balance in these patients.

Haemoglobin concentration of blood was measured by cell counter (Councell 21). The concentrations of iron and TIBC in serum were measured by Ferrozine method, using Crest Biosystems kit [18]. TS were calculated by formula: Iron/TIBC×100 [19]. Serum ferritin was estimated by ELISA method, using Fortress Diagnostics Kit [20]. Lipid peroxide level in serum was measured by thiobarbituric acid assay and results were expressed as nmol of MDA formed [21,22]. SOD was measured by using RANSOD kit [23]. GPx was measured by using RANSEL kit [24]. Serum CP and Cu was measured by standard diagnostic kits [25,26]. Serum Zn level was measured by using Centronic GmbH-Germany kit [27] and vitamin C level was measured by the method of Ayekyaw by colorimetry [28].

STATISTICAL ANALYSIS

All data obtained were entered into an SPSS version 20.0 and quantitative data were presented as Mean±SD. The unpaired Student's t-test was used for statistical analysis of data. All tests were two tailed. For all comparisons, p-value less than 0.05 were considered to be statistically significant.

RESULTS

Group A patients (60) has number of transfusions ≤140 and Group B patients (63) have number of transfusions >140. The observations and inference obtained from this study were summarised in tables.

[Table/Fig-1] shows comparison of demographic characteristics and iron indices in Group A and Group B. There was highly significant difference found in age and weight between Group A and Group B (p<0.0001). Mean haemoglobin, serum iron, ferritin and TS levels of Group B was significantly higher than Group A (p<0.0001) while TIBC level of Group B was significantly lower than Group A (p<0.0001). These parameters shows that iron overload increases with each blood transfusion.

[Table/Fig-2] shows comparison of oxidant marker MDA and antioxidants in Group A and Group B. The mean level of serum MDA and Cu in Group B was significantly higher than Group A (p<0.0001). Antioxidants like GPx, Zn and vitamin C were significantly lower in Group B than Group A. (p<0.0001). There was non significant difference observed in SOD and CP in both groups (p=0.339 and p=0.0601).

Parameters	Groups	Number	Mean	Std. Deviation	t-test value	p-value
Age (years)	Group A Group B	60 63	6.60 12.00	2.68 2.57	11.389	<0.0001***
Weight (kg)	Group A Group B	60 63	20.86 33.17	7.29 5.16	10.842	<0.0001***
Hb (gm/dL)	Group A Group B	60 63	7.68 10.11	0.65 1.44	11.941	<0.0001***
Sr. Iron (µg/dL)	Group A Group B	60 63	147.95 199.30	19.47 43.97	8.302	<0.0001***
Ferritin (µg/L)	Group A Group B	60 63	2494.00 3583.96	300.19 854.99	9.340	<0.0001***
TIBC (µg/dL)	Group A Group B	60 63	239.93 203.93	6.81 12.13	20.146	<0.0001***
TS (%)	Group A Group B	60 63	61.63 97.92	8.59 22.59	11.659	<0.0001***

[Table/Fig-1]: Comparison of iron indices in patients on the basis of blood transfusions.
***Highly significant, p<0.0001;
Unpaired Student's t-test, p<0.05 significant
Group A having number of transfusion <140 and Group B having number of transfusion >140
Hb: Haemoglobin; TIBC: Total iron binding capacity; TS: Transferrin saturation; Sr.: Serum

Parameters	Groups	Number	Mean	Std. Deviation	t-test value	p-value
MDA (nmol/mL)	Group A Group B	60 63	3.54 6.39	1.02 1.34	13.194	<0.0001***
SOD (U/mL)	Group A Group B	60 63	161.83 155.87	43.90 21.89	0.960	0.339
GPx (U/L)	Group A Group B	60 63	3967.41 3330.23	466.12 588.03	6.639	<0.0001***
Sr. Ceruloplasmin (mg/dL)	Group A Group B	60 63	26.73 29.14	7.30 6.75	1.898	0.0601
Zinc (µg/dL)	Group A Group B	60 63	91.05 76.77	11.55 14.43	6.035	<0.0001***
Sr. Copper (µg/dL)	Group A Group B	60 63	142.63 163.06	35.67 32.71	3.306	0.0013*
Vitamin C (mg/dL)	Group A Group B	60 63	0.50 0.42	0.06 0.08	6.003	<0.0001***

[Table/Fig-2]: Comparison of oxidant and antioxidants on the basis of blood transfusions in patients.
*Significant, p<0.005 *** Highly significant, p<0.0001
Group A having number of transfusion <140 and Group B having number of transfusion >140
MDA: Malondialdehyde; SOD: Superoxide dismutase; GPx: Glutathione peroxidase, Sr.: Serum

DISCUSSION

Regular blood transfusion is one of the conventional treatments of thalassaemia to keep the haemoglobin levels close to normal. It was observed that, there is significant difference in age and weight between Groups A and B (p<0.0001). Mean haemoglobin level, serum Iron, ferritin, TS were significantly higher while TIBC was significantly lower in Group B when compared with Group A. These finding indicates that patients of Group B develops higher iron overload than Group A [Table/Fig-1]. This iron overload is the joint outcome of multiple blood transfusions and inappropriately increased iron absorption associated with ineffective erythropoiesis. Treatment with transfusion programs and chelating therapy has considerably prolonged survival in thalassaemic patients. However, as a result of hyper transfusion therapy and increased longevity, iron tissue toxicity has become more common and contributes significantly to morbidity in these patients [29].

As per the study of Fangion S et al., Tos SC et al., and Brissot P et al., multiple blood transfusions developed severe iron load with increased number of blood transfusions in thalassaemic patients. This was indicated particular by the raised serum ferritin levels [30-32]. Karamifar H et al., also supported a significant increase (p<0.001) in the mean serum ferritin level in patients with multiple blood transfusions as compared to values in patients without transfusions [33]. Moreover, they showed an incidence of hypothyroidism in patients with (22%) and without (21%) blood transfusion. The number of blood units and the amount of the accumulated iron justify that older BTM patients accumulate an increasing amounts of iron during their life more than the younger patients [33].

Regular blood transfusion is one of the conventional treatments of thalassaemia to keep the haemoglobin levels close to normal [34]. This transfusion therapy have increased the life expectancy with beta thalassaemia major but caused a progressive iron overload [35]. It is well documented that disturbances of oxidant and antioxidant balance occur in haemoglobinopathies, especially in thalassaemia. MDA is a good indicator of oxidative damage [36]. In present study population we observed higher MDA and lower level of antioxidants like SOD, GPx, Zn, and vitamin C in Group B than Group A, which confirms that oxidative stress increases with number of blood transfusions. When we focused on mean values of serum CP and Cu level, we observed that Cu was significantly higher while ceruloplasmin was non significantly higher in Group B as compared to Group A.

In spite of the iron overload due to repeated blood transfusion, oxidants also originate from other sources for example, the excess unpaired α haemoglobin chains denature and autoxidise, contributing to increased oxidants, ineffective erythropoiesis, haemolysis and shortened erythrocyte survival [37]. Therefore, MDA, a product of lipid peroxidation and protein carbonyls, representing oxidation of the circulating proteins, is elevated in beta thalassaemia [38].

Our findings were supported by Livrea MA et al., and Attia MA et al., who found increased MDA in beta thalassaemia major patients and concluded that, as a result of continuous blood transfusions the patients might be subjected to peroxidative tissue injury by the secondary iron overload [17,39]. These findings might support the idea of iron overload in beta thalassaemia leads to an enhanced generation of reactive oxygen species and oxidative stress. Under normal circumstances, there is virtually no free iron. However, the presence of iron complex (like vitamin C, vitamin B12, folic acid) stimulate peroxidation by peroxide decomposition of unsaturated fatty acids generating alkoxyl (·OL) and peroxyl (LOO·) radicals [40]. Normally, the superoxide anion is converted by the enzyme SOD to produce H_2O_2 [41] which in turn is converted to innocuous compounds by the action of catalase and peroxidase. However, if free ferrous iron is available it reacts with H_2O_2 to produce hydroxyl radical an extremely reactive species which is leading to depolymerisation of polysaccharide [42]. The production of free radicals due to iron overload was associated with a significant decrease in enzymatic antioxidants like SOD and GPx as shown in the present study. Moreover, marked changes in the other antioxidant pattern were also observed in all patients. Evidence is presented of a net drop in the concentration of Zn and vitamin C in all patients those are transfused with more than 140 blood units when compared with those are transfused with less than 140 blood units. Vitamin C deficiency commonly occurs in beta thalassaemia major especially in the older and more transfused patients. The levels of cellular antioxidant vitamins like vitamin A, C and E, as well as the activities of enzymatic antioxidants such as catalase, GPx and glutathione reductase, were found to be considerably lower in thalassaemic patients compared to normal subjects. These results suggest a major consumption of antioxidants under iron overload

from continuous blood transfusions or oxidative stress in BTM patients [43-45].

Livrea MA et al., also observed a significant decline in the concentration of vitamin A, C and E in all patients affected with thalassaemia due to continuous blood transfusions. Hypercupremia in blood transfused thalassaemic patients occurs due to acute, chronic infections and haemochromatosis which is a principal complication in thalassaemia [17]. Claster S et al., found serum Cu and ceruloplasmin were higher and lower side respectively in chronically transfused patients of thalassaemia major due to haemolysis, iron toxicity, ineffective erythropoiesis, inflammation, anaemia, diet, and absorption [46]. However, Sabah N and Sherien M were reported non significant decrease in serum levels of Cu and Zn [47]. Patel HV et al., observed that endocrinopathies such as poor growth, delayed puberty, impaired glucose tolerance and osteoporosis are some complications that can occur in thalassaemia patients due to oxidative stress [48].

LIMITATION

The dose and frequency of iron chelators therapy were not investigated in this study. Genetic changes may interfere in biochemical parameters which were not studied in this study due to limited resources. Gender difference was also not considered during study. Exact prevalence of this disease in our study area was limited. Hence, it does not represent all thalassaemic population of this area. To know exact prevalence, community based studies are recommended.

CONCLUSION

Main complication of blood transfusion therapy is iron overload which increases with increasing number of blood transfusions. The complications of iron overload like cardiovascular risk, liver cirrhosis, and oxidative stress will be monitored by evaluation of iron indices, oxidant and antioxidants. Blood transfusions increase life expectancy of the BTM patients however it affects the biochemical parameters. These alterations lead to further complications and it may be preventable and helpful if these parameters evaluate regularly with blood transfusions.

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