Original Article

Respiratory Burst Enzymes, Pro-Oxidants and Antioxidants Status in Bangladeshi Population with β-Thalassemia Major

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Abstract

Background: Oxidative stress is intimately associated with many diseases, including β-thalassemia. **Aim:** The study was to estimate the status of respiratory burst enzymes, pro-oxidants, and antioxidants in β-thalassemia major patients in Bangladesh and to compare with apparently healthy individuals. **Materials and Methods:** A total of 49 subjects were recruited which included 25 patients (age range 5 to 40 years) with β-thalassemia major and 24 controls (age and sex matched). Superoxide dismutase (SOD) and catalase (CAT) represented respiratory burst enzymes; malondialdehyde (MDA), lipid hydroperoxide (LHP), and xanthine oxidase (XO) were measured as pro-oxidants; and glutathione S transferase (GST), vitamin C (Vit.C), and glutathione (GSH) were the measured antioxidants. **Results:** The activity of SOD was significantly ($P < 0.001$) increased by about 79% and the activity of CAT was significantly ($P < 0.001$) decreased by more than 34% in the blood of β-thalassemia major patients compared to the control group. The content of pro-oxidants such as MDA, LHP, and XO was significantly ($P < 0.001$) higher in patients by about 228%, 241.3% and 148.1% respectively compared to control group. The level of GSH and Vit.C were significantly ($P = 0.000$) decreased in patients by about 59% and 81% versus the healthy group, respectively; and GST activity was significantly ($P < 0.001$) declined by 44.25% in patients group. **Conclusion:** β-thalassemia major patients demonstrate raised oxidative stress compared to healthy subjects.

Keywords: Antioxidants, Children, Free radicals, Respiratory burst enzymes, Sickle cell disease

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Introduction

Beta-thalassemias (both homo- and heterozygote), one common form of hemoglobinopathy, are a group of hereditary blood disorders caused by moderately low levels of hemoglobin synthesis or the absence of the beta chains of hemoglobin.[1,2] Beta-thalassemias are more common in Mediterranean countries, the Middle East, the Indian subcontinent, and many parts of Southeast Asia.[3] Since there is no effective treatment of β-thalassemia except frequent blood transfusions and bone marrow transplants, the purpose of this study was to provide some valuable information about the patients to treat them properly, though this will not reduce the number of patients except declining those with oxidative stress. Beta-thalassemia is accompanied with heart diseases, liver fibrosis and cirrhosis, diabetes mellitus, hypogonadism, and thyroid gland-related disorders.[4,5] More common complications include metabolic irregulation, iron overload, chronic hypoxia, and cell damage.[6] The terminology "oxidative stress" points to a shift in the equilibrium between oxidants and antioxidants, in favor of oxidants.[7] Oxidative stress is a common mechanism in the progression of many disorders like β-thalassemia major, cardiovascular failure, cancer, renal and neurological diseases, infections, etc.[8-10] Excess production of reactive oxygen species (ROS) as well as the induced lipid peroxidation (LPO) are the markers of this process that exceed the capacity of the antioxidant

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defenses, consequently, actuates various oxidations in both intracellular and extracellular components of the red blood cells in β-thalassemia major patients.[11-13] ROS are generated during the intracellular catabolism that requires oxygen as a terminal electron acceptor[14] and produce intermediates such as O^{-2}, H_2O_2 and OH^- radicals, even in healthy individuals.[15,16] Enzymes such as xanthine oxidase (XO), NADPH oxidase, nitric oxide synthase (NOS), cytochrome P450, cyclo-oxygenase, and lipoxygenase are also responsible for generating ROS during the repeated cycles of hypoxia/re-oxygenation or ischemia/reperfusion.[17,18] Furthermore, malondialdehyde (MDA) is produced due to the degradation of polyunsaturated lipids by ROS[19] that cause toxic stress in cells.[20] Major ROS defense mechanisms include enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The non-enzymatic system includes antioxidants like reduced glutathione (GSH), ascorbic acid (Vit. C), riboflavin, zinc, carotenoids, and uric acid, as well as metal-binding proteins.[21]

Blood transfusion is the typical treatment of beta-thalassemia major that can cause complications of iron overload[22] but the introduction of chelating agents can control and combat it, resulting in the inhibition of ROS-generation and regulation of LPO-processes that leads to improve life expectancy.[23] The aim of this work is to study the level of oxidative stress as a central pathological process in the blood of β-Thalassemia major patients in the form of the status of respiratory burst enzymes, pro-oxidants and antioxidants for the better treatment of Bangladeshi patients.

Materials and Methods

Subjects

A total of 49 subjects were recruited: 25 β-thalassemia major patients, aged 5 to 40 years, from Bangladesh Thalassemia Hospital, Dhaka, Bangladesh and 24 age- and sex-matched, apparently healthy subjects who served as controls. Institutional ethical clearance was obtained and conset was obtained from patients and parents of patients.

Sample collection and analysis

A blood sample (6 ml) was collected, then spun for serum, and was used to measure the serum concentration of lipid hydroperoxide (LHP), Vit. C, GSH, MDA, as well as serum activities of CAT, glutathione S transferase (GST), SOD, and XO. All the parameters were measured using colorimetric methods and reagents used were of analytical grade. MDA was measured with a method based on the thiobarbituric acid reactive substance (TBARS) assay, a colorimetric method described by Satoh.[24] XO activity was determined using the method described by Shintani.[25] LHP was estimated by the method of Yagi.[26] The serum activities of SOD and CAT were assessed by the method of Beyer and Fridovich[27] and L. Goth,[28] respectively. The activity of serum GST and the content of GSH were determined by using 1-Chloro-2,4-dinitrobenzene[29] and by Beutler *et al*.[30] using 5,5′-dithiobis-(2- nitrobenzoic acid) respectively. Vit. C was estimated by the method of Lowry *et al*.[31]

Statistical analysis

Statistical analysis was carried out using the SPSS, version 16 (SPSS Inc., Chicago, IL, USA) and *P*-values were set at 0.05. The independent T — test was performed to explore the statistically significant difference between β-thalassemia major patients and controls. Results were expressed as Mean ± SD (standard deviation). Bivariate analysis was carried out to find out the relationship among the target variables.

Subjects gave their consent. All authors declare that written assent was obtained from each subject before being enrolled into the study. The authors declare that this study was performed under the ethical standards of the ethical review committee of the β-Thalassemia Hospital and the study protocol was approved by the Human Ethics Committee of Dhaka University, Dhaka, Bangladesh.

Results

Baseline features of beta-thalassemia major patients and controls are presented in Table 1. The average age (in years) of the study subjects was almost similar and the sex was also matched. The mean heights and weights of the controls were higher by 2.95% and 18.4%, respectively, compared to patients. So the body mass index (BMI) of patients (19.85 kg/m^2) was 13.36% lower than the controls (22.91 kg/m^2).

Table 2 represents the results of respiratory burst enzymes, pro-oxidants, and antioxidants in beta-thalassemia major patients compared with healthy individuals. The activity of SOD was significantly lower in patients, whereas

Table 1: Baseline characteristic of study subjects

Parameters	Controls	Patients
Number of subjects (n)	24	25
No. of Females	11	12
No. of Males	13	13
Age* (years)	18.43±5.35	19.46±8.67
Height* (cm)	136.11±2.31	132.09±5.42
Weight* (kg)	42.38±8.79	34.58±11.68
BMI* (kg/m^2)	22.91±0.86	19.85±1.80

*Data are presented as Mean ± SD

the CAT activity was significantly higher in patients compared to the controls. Serum XO activity was found significantly higher in beta-thalassemia subjects compared to controls. The level of pro-oxidants, MDA and LHP, were significantly higher in patients in comparison to healthy subjects. Serum levels of GSH and Vit. C, and GST activity were significantly lower in patients with β-thalassemia major as compared to controls.

The comparison of all the parameters in males and females is presented in Table 3. The activity of respiratory burst enzymes (i.e., SOD and CAT) was significantly different in males and females of both groups except for CAT activity of the control group. The content of LHP in males and females was significantly different between both groups. XO activity and level of MDA in males and female were significantly different in the control group but not in patients group. Although there is no any significant difference of GSH and Vit. C level in males and females of both the studied groups, the activity of GST was significantly different between both groups.

In the control group, moderately positive correlations were found between SOD and MDA (r = 0.136), MDA and CAT (r = 0.209), MDA and LHP (r = 0.194), and between GSH and GST (r = 0.340). Highly significant negative correlations were found between GST and Vit. C (r = −0.561), but moderate negative correlations were found between SOD and LHP (r = −0.292), LHP and GSH (r = −0.292), MDA and GSH (r = −0.272), CAT and Vit. C (r = −0.244), GSH and XO (r = −0.277), and SOD and GSH (r = −0.211). In the patient group, significant positive correlation was found between MDA and LHP (r = 0.410); moderate positive correlations were found between CAT and LHP (r = 0.238), XO and SOD (r = 0.363) and CAT and Vit. C (r = 0.274); moderate negative correlations were found between GSH and CAT (r = −0.372), XO and CAT (r = −0.388), and XO and Vit. C (r = −0.274).

Discussion

This study was designed to investigate the respiratory burst enzymes (SOD and CAT), pro-oxidants (MDA, XO, and LHP), antioxidants (GSH, Vit. C, and GST), and the variations in male and female individuals with β-thalassemia major patients compared with normal subjects. Oxidative free radicals are generated due to the disturbance in the redox state of cells and also from environmental pollutants such as X-rays, smokes, chemicals etc.[32] Although the main function of superoxide, nitric oxide and their particularly reactive product, peroxynitrite is to kill processed pathogens by phagocytes,[33] they are involved in damaging DNA, membrane phospholipids, and proteins. β-thalassemia major is the most severe form of β-thalassemias that results from abnormal synthesis or absence of β chain of the hemoglobin molecule. Affected individuals require regular, lifelong blood transfusions, but bone marrow transplants can be curative for some

Table 2: Mean comparison between controls and patients

Parameters	Units	Controls (*n* = 24)	Patients (*n* = 25)	*P*-values
Superoxide dismutase (SOD)	U/mg	82.99±9.85	148.63±13.83	<0.001*
Catalase (CAT)	KU/L	55.6±6.54	36.12±8.88	<0.001*
Xanthine oxidase (XO)	U/mg-protein	0.54±0.10	1.34±0.19	<0.001*
Malondialdehyde (MDA)	nmol/mL	0.68±0.19	2.23±0.42	<0.001*
Lipid hydroperoxide (LHP)	nmol/mL	0.92±0.24	3.14±0.66	<0.001*
GlutathioneStransferase (GST)	U/mg	33.74±5.00	18.81±6.24	<0.001*
Reduced glutathione (GSH)	nmol/mL	912.24±91.54	376.95± 99.17	<0.001*
Vitamin C (Vit. C)	nmol/mL	10.70±1.88	2.04±1.1	<0.001*

*Statistically significant

Table 3: Mean comparison of parameters in males and females of both groups

Parameters	Units	Controls (Mean ± SD)			Patients (Mean ± SD)		
		Males (*n* = 13)	Females (*n* = 11)	*P*-values	Males (*n* = 13)	Females (*n* = 12)	*P*-values
SOD activity	U/mg	78.17±6.98	88.69±9.93	0.009*	141.07±12.60	156.82±10.20	0.002*
CAT activity	KU/L	54.22±4.58	57.22±8.25	0.300	31.85±6.81	41.06±8.22	0.006*
XO activity	U/mg-Protein	0.60±0.09	0.47±0.07	0.001*	1.40±0.15	1.27±0.21	0.093
MDA level	nmol/mL	0.75±0.18	0.57±0.15	0.014*	2.38±0.47	2.07±0.30	0.06
LHP level	nmol/mL	1.06±0.21	0.76±0.16	0.001*	3.42±0.69	2.83±0.49	0.02*
GST activity	U/mg	31.41±2.69	36.50±5.79	0.018*	15.23±3.84	22.69±6.10	0.002*
GSH level	nmol/mL	893.72±105.33	934.14±70.62	0.276	350.46±106.37	405.65±85.93	0.17
Vit.C level	nmol/mL	10.14±1.92	11.36±1.69	0.112	1.78±1.01	2.31±1.13	0.23

*Statistically significant

children.[34] One complication of excess blood transfusions is iron overload[35,36] that ultimately leads to the significant increase in serum ferritin levels, resulting in 37-fold more ferritinemia in β-thalassemia patients compared to controls.[36] Experiment in animal models showed that high liver iron levels induce the elevation of lipid peroxides and oxidants,[37,38] as well as in thalassemia patients[39,40] because thalassemia RBCs were more susceptible to auto-oxidation than normal cells.[41] Serum MDA, LHP and XO were studied as biomarkers of tissue injury and oxidative stress. XO is responsible for the catalysis to generate superoxide radical from hypoxanthine.[32] It has been found that there was an increase production of XO in sickle transgenic mice following hypoxia and has deleterious effects after reperfusion.[32,42] So the increase in XO activity leads to increased production of LHP [Table 2]. Thiobarbituric acid reactive substances (equivalent to MDA) are well-recognized biomarker of lipid peroxidation.[43,44] The significant increase of serum MDA and LHP findings in β-thalassemia major patients and their controls group are presented in Table 2 ($P < 0.001$). Our report is consistent with other different studies, where thalassemia patients showed increased MDA and LHP levels.[36,45,46] There was no significant difference in MDA levels between males and female samples in the patient group that is consistent with the Abdalla *et al*. study.[36] Since SOD helps to out-compete the damaging reactions of superoxide anion by converting to hydrogen peroxide[47] that is further decomposed to simple products, water and oxygen molecule, by CAT;[48] the study of these antioxidant enzymes (respiratory burst enzymes) could be very informative as they are the first line of defense against oxidative stress.[49] In this present study, the findings of serum levels of SOD were significantly ($P < 0.001$) increased in patients as compared to controls [Table 2]; and the activity of CAT was significantly ($P < 0.001$) declined in patients when compared to controls group [Table 2]. Other studies had also found that SOD levels of β-thalassemia patients were significantly higher when compared to controls.[36,49] This increase in SOD indicates the results of oxidative stress in β-thalassemia patients as they may play a compensatory mechanism to scavenge excess superoxide anion.[50,51] On the other hand, the decrease in CAT activity in patients may be due to the decrease in nicotinamide adenine dinucleotide phosphatase (NADPH) that is crucial for the maintenance of CAT activity because CAT monomer contains a high affinity binding site for NADPH. A second contributing cause may be at play. It is possible that iron may deplete H_2O_2 through Fenton chemistry, which would result in loss of induction of CAT expression by H_2O_2. Iron converts hydrogen peroxide to hydroxyl radical, peroxyl radical, and hydroxyl anion. It is notable that production of hydroxyl and peroxyl radicals may accentuate lipid peroxidation chain reactions; this would explain the association of this disorder with lipid peroxidation.[52-55] Findings by Walter *et al*.[56] and by Cheng *et al*.[57] suggest that β-thalassemia major patients have significantly decreased levels of NADPH. So our finding was obvious. When the samples were divided into male and female samples, female sample exhibited significantly ($P < 0.05$) higher SOD and CAT activities as compared with male sample in both groups [Table 3] except the CAT activity in controls and this finding was similar to the study by Bolzán *et al*.[58] SOD is negatively correlated with the CAT value in the patient group ($r = -0.02$) that is similar to the findings by Bogdanska *et al*. [Table 4].[59] Many other studies showed that the patients with β-Thalassemia major have high lipid peroxidation products and low level of antioxidants compared to the normal individuals.[36,60,61] In our study, a significant ($P < 0.001$) decreased GST activity was found in the patients as compared to the healthy subjects [Table 2]. When the samples were studied as male and female samples, the female samples displayed higher GST activities than the male samples in both studied samples [Table 3], and it was consistent with the report by Hunaiti and al-Shareef.[62] The contents of GSH and Vit. C in β-thalassemia major patients were significantly ($P < 0.001$) lower when compared to control group [Table 2]. There was no significant ($P > 0.05$) difference in GSH and Vit. C levels in males and females of both the studied groups [Table 3]. The results suggest that there was a significant increase in free radicals or ROS levels in β-thalassemia major patients, and as the level of antioxidants were very low, the patient group suffers more from oxidative stress. Demographic data analysis has also played a very important role in the

Table 4: Bivariate analysis between patients and controls

Controls	Patients							
	CAT	GSH	GST	LHP	MDA	SOD	Vit. C	XO
CAT	1	−0.372	−0.119	0.234	0.26	−0.064	0.274	−0.388
GSH	.147	1	0.208	−0.141	0.19	−0.141	−0.279	−0.042
GST	.097	.340	1	0.004	−0.221	0.022	−0.233	0.238
LHP	−.061	−.292	.014	1	.410*	0.142	−0.013	0.13
MDA	.209	−.272	−.067	.194	1	−0.02	−0.128	−0.237
SOD	−.046	−.211	−.154	−.292	.136	1	−0.076	0.363
Vit. C	−.244	−.201	−.561**	−.025	.173	.007	1	−0.274
XO	−.021	−.277	−.170	−.002	.003	.068	−.042	1

*$P < 0.05$, **$P < 0.01$. Significant correlations are highlighted in bold

diagnosis and confirmation of β-thalassemia. The patients' family histories, BMI, duration of transfusions, transfusion intervals, and age were very much important for the quick diagnosis of β-thalassemia. Finally, it can be inferred that overall experiment of this research was performed to evaluate the overall status of respiratory burst enzymes, pro-oxidants, and antioxidant level for the proper and better treatment, and management of beta-thalassemia patients as the findings by Attia *et al*.[60] suggest that proper treatment with antioxidant vitamins like vitamins A, C, and E could decrease the pro-oxidant level and increase the antioxidant levels, and even could solve the activities of CAT and SOD. As Bangladesh is an economically poor country, costly treatments (frequent blood transfusions and bone marrow transplantss) of β-thalassemia is very much impossible for the general people.

Conclusion

The findings from this study show that patients with β-thalassemia major demonstrate increased oxidative stress compared to the control groups.

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