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Relationship between Iron Storage, Anti Oxidant Status and Number of Transfusion In Beta-Thalassemia Patients: A Cross-Sectional Study

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Abstract:*Blood transfusionis the most common therapeutic aspect as well as life-saving measure for β-thalassaemic major patients but repeated transfusion is related to iron overload and storage in the body and if untreated it may cause considerable morbidity and enhance early mortality. All serum anti-oxidant including glutathione is also altered due to iron-induced peroxidative injury. However, few studies can be found in India describing the relationship between iron storage, antioxidant status and number of transfusion in β-thalassemia major patients. The present study was carried out to evaluate a relationship between iron storage, anti-oxidant status and number of transfusion in proved cases of β-thalassemia major of Indian origin. Present study confirms thatincreased no of transfusion led to more iron strorage in body and Red blood cells- reduced glutathione (RBC- GSH) is directly correlated with iron storage.*

Keywords*:Anti-oxidant status, β-thalassemia, Ferritin, Iron storage, RBC-GSH.*

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I. Introduction

Thalassemia is one of the commonestmonogenic disorders in the world. Mutation in gene encoding globin chain leads to reduction or absence of globin chainin hemoglobin.[1]Approximately 20 common alleles constitute 80% of known thalassemia cases worldwide. Almost 3% of the world's population carries genes for β-thalassemia and 5-10% carries genes for α-thalassemia in South-East Asia.[2]On an average, worldwide >4 lacs newborns are affected bythalassemia per year.[1]Migration of population is one of the factors causing such high prevalence rate.[3]Blood transfusion is the mainstay of therapy and also life-saving measure in thalassemia major. Each unit of whole blood contains approximately 200 mg of iron. In patients who undergo multiple transfusions, are prone to develop iron overload andif untreated excess stored iron causes irreversible damage to different organs in the body and hence rises morbidity and mortality.[4] The hyperactive bone marrow favors increased iron absorption that contributes to total iron store in the body[5],which result inappropriate suppression of the iron regulating peptide hepcidin. Overexpression of Growth Differentiation Factor-15 fromerythroid compartment enhances iron accumulation bythe inhibition of hepcidin expression.[6]In patients having fully saturated transferrin,a significant fraction of iron circulates in plasma in the form of low molecular weight complexes, not bound to transferrin.[7]Although the exact mechanism of tissue damage remains unclear,ironinduced peroxidative injury (by producing free radicals) to the phospholipids present inlysozomes and mitochondria is probably the most important pathogenic cause.[8]Reduced glutathione (GSH), along with other enzymes and anti-oxidants are the major defenses againstthe free radicals.[9-12]Sulphydryl group of reduced Glutathione (gamma-Glutamyl-Cystinyl-Glycin) is the most importantphysiological anti-oxidant that prevents cellular damage caused by Reactive Oxygen Species (ROS). In healthy tissue,>90% of the total glutathione pool is in the reduced form(GSH) and <10% exists in oxidizedform with disulphide bond (GS-SG).Formation of increased GS-SGfrom GSH is indicative of oxidative stress. Thus, iron induced oxidative stress altered serum anti-oxidant level.[13] In physiologic conditions, ferric ion is bound to protein and thus prevented to participate in reactions leading to cellular injury. Inpathological conditionsrelated to iron overload (like thalassemia),there are evidences of increase in non-protein bound low molecular weight iron in serum and in the intracellular transit pool of iron.[14-18]Only few studies are available relating the changes in the redox balance with progression of disease. Keeping that in mind, we framed out the study to evaluate the relationship between iron storage,

antioxidant status and number of transfusion in diagnosed cases of β-thalassemia major who had undergone multiple blood transfusions.

II. Materials And Methods

A cross-sectional,observational, tertiary care hospital based study was conducted in the department of Biochemistry inassociation with department of Pathology of C.N. MedicalCollege, Kolkata during the period of 1 year 6 months (February, 2012 to July, 2013).Forty β-thalassemia major children were selected randomly from the Thalassemia Day Care Unit as per the inclusion and exclusion criteria and other 40age and sex matched normal children were selected as the control subjects, who were not suffering from any obvious or documented chronic illness; after taking the written consent from the legal guardians of the participants.

Inclusion criteria

1. Age: 3 to 10years.
2. Commencing age of blood transfusion was about 6 months.
3. Cases were receiving regular transfusion for thalassemia treatment.
4. Last date of blood transfusion was within 15 days fromsample collection day.

Exclusion criteria

1. Any other hematological disorder other than thalassemia.
2. Patients who had received Iron-chelation therapy.
3. Patients who were taking extra iron therapy.
4. Any acute illness.
5. Controls were having any documented chronic illness or on any therapy (chronically) for any illness or malnutrition.

The whole study and the study procedures were strictly adhered tothe "Helsinki declaration"(1975) for human studies andcarried out after getting the informed written consent fromthe appropriately formed Institutional Ethical Committee.Five mLvenous blood was collected from each cases and controls.Serum ferritin estimated by sandwich ELISA method[19], haemoglobin estimation of prepared RBC suspension was doneby cyanomethemoglobin method[20], RBC- GSH level wasmeasured in accordance with the method developed by Buetler et. al.[21]

Data found from history and laboratory investigations, were codified and compiled in MS excel sheet and analyzed by applicable statistical methods, as per need. Data display was done by chartsand tables. Data were described by mean, SD,range, median etc. Statistical tests like independent-'t' test, Spearman'scorrelation coefficient (for non-parametric data), Chi-squareetc. were done by suitable statistical software program to explore therelationship between variables. p value of <0.05 wasconsidered to be significant to discard the null hypothesis at 5%precision and 95% confidence interval.

III. Results

Table-1: Descriptive statistics of different attributes and significance of difference between cases and controls

Attributes	Age (years)		No of Transfusions in cases	Ferritin (ng/mL)		GSH (µmol/g of Hb)	
	Cases	Controls		Cases	Controls	Cases	Controls
Mean	32.8	30.5	15.25	923.53	66.300	28.662	8.412
SE of Mean	0.487	0.664	0.288	3.707	2.875	0.465	0.164
Median	33.0	32.0	15.00	925.50	68.50	28.308	8.268
SD	3.082	4.200	1.822	23.446	18.182	2.942	1.036
Variance	9.497	17.641	3.321	549.692	330.574	8.656	1.073
Minimum	28	21	13	881	26.00	21.46	6.33
Maximum	38	38	18	962	112.00	35.71	10.58
Significance of difference	--		--	**<0.001**		**<0.001**	

Table-2: Crosstabulation between Number of transfusion and Ferritin level

No of Transfusions		Ferritin in cases after transfusion (ng/mL)			
		<900	900-925	925-950	≥950
13	Count	8	0	0	0
	% within No of Transfusion	**100.0%**	0.0%	0.0%	0.0%
14	Count	2	6	1	0
	% within No of Transfusion	22.2%	**66.7%**	11.1%	0.0%
15	Count	0	4	5	0
	% within No of Transfusion	0.0%	**44.4%**	**55.6%**	0.0%
17	Count	0	0	7	0
	% within No of Transfusion	0.0%	0.0%	**100.0%**	0.0%

18	Count	0	0	1	6
	% within No of Transfusion	0.0%	0.0%	14.3%	**85.7%**

Pearson Chi-Square significance **<0.001**.Hence, it was obvious that the more the no of transfusion, the more the storage of iron (ferritin) in the body.

Table-3: Correlation between cases in different parameters

Spearman's Correlations in cases after transfusion (N=40)		Age (years)	No of Transfusions	Ferritin (ng/mL)	GSH (µmol/g of Hb)
Age	Correlation Coefficient	1.000	0.446	0.355	0.419
	Significance (2-tailed)		**0.004**	**0.025**	**0.007**
No of Transfusions	Correlation Coefficient	0.446	1.000	0.966	0.363
	Significance (2-tailed)	**0.004**		**<0.001**	**0.021**
Ferritin	Correlation Coefficient	0.355	0.966	1.000	0.366
	Significance (2-tailed)	**0.025**	**<0.001**		**0.020**

Number of transfusions, ferritin level and reduce glutathione level in RBC- all were found to be significantly and positively correlated to age of the participants. Ferritin and GSH were correlated positively and significantly to no of transfusions. GSH was also in significant positive correlation with ferritin level.

IV. Discussion

Various studies showed that, oxidative stress (OS) is present in β-thalassemia major patients receiving chronic blood transfusion. ROS plays an important role in the etiology and/or progression of such human diseases. Haber –Weiss and Fentonreactions produce potent hydroxy radicals[22]. Present study firmly validated the result in Indian scenario that there was a significant relationship in between iron storage and number of transfusions.

It has been found that there is significantly compensatory rise in the glutathione peroxidase and GSH for counter acting oxidative stress inside the cells in the beta thalassemia patients who had showed a significantelevation in oxidative stress.[23]The variation of RBC- GSH is found to occur in a direct manner (positive correlation) with serum ferritin level. So, an increase in oxidative stress is counter acted by the antioxidant activity of reduced glutathione and it simultaneously led to an increase in GSH level as reflected in our study. However, this compensatory increase value was not fully capable of counteracting the oxidative stress that induced damage generated by huge iron overload in our body. It had been supported by a recent animal studywhich showed that an increased intracellular oxidative stress induces an elevation of the reduced glutathione activity.[24]Multiple blood transfusions also could be an explanation of increased RBC- GSH level obtained from fresh blood. In response to oxidative stress, there was compensatory elevation in antioxidant defensive mechanism butthe overall redox balance was skewed in favor of the oxidative stress induced damage.

V. Conclusion

It can be concluded that an anti-oxidant therapy can not prevent the oxidative stress induced damage in the β-thalassemia patients receiving regular blood transfusions and as a consequence having huge iron storage. Iron- chelation therapy is very much needed as an effective therapy to reduce theiron overload. Periodical assessment of the anti-oxidant enzymes and markers of oxidative stressare needed for estimating the cellular damage and monitoring of the effectivity of iron- chelation therapy. A larger multicentre placebo controlled studies and a battery of other antioxidant enzyme measurement is required to reach more conclusive evidence.

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