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Correlation between Plasma Protein Carbonyl Adducts and RBC Reduced Glutathione Level in Beta Thalassemia Major Patients in Southern Asian Region

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Abstract: ***Blood transfusion therapy is mainstay of therapy and life-saving for patients with beta thalassaemia major. It is associated with an iron overload and the resultant increase in oxidative stress. The evaluation of carbonyl group contain blood protein is useful bio-marker for oxidative stress. All serum antioxidant including glutathione is also altered due to oxidative injury. However, no studies could be found in India describe the correlation between plasma protein carbonyl adducts (PC adducts) and RBC reduced glutathione(RBC GSH). Accordingly we carried out the present study to evaluate a correlation between plasma PC adducts and RBC GSH in proved cases of beta thalassemia major of Indian origin . In this present study, it was found that in patients of beta thalassemia major RBC GSH is inversely correlated with OS marker plasma PC adducts.***

Keywords: Beta thalassemia , oxidative stress , Protein carbonyl adducts, RBC GSH.

1. Introduction

The thalassemias refer to a heterogeneous group of inherited diseases characterized by defect in synthesis of alpha or beta globin chains of hemoglobin. Beta thalassemia is one of the most common inherited single gene disorder with >400,000 newborns affected per year worldwide [1]. This disorder is found in the malarial, tropical and sub-tropical regions of Mediterranean countries, the Middle East, Trans Caucasus, Central Asia, the Indian Subcontinent (South Asia) and Southeast Asia [2]. Indian Subcontinent (or South Asia), which includes the countries of Pakistan, SriLanka and India, there are ~45 million carriers (carrier rate of ~1:20) of beta thalassemia [3]They are seen in countries to which these high-frequency populations immigrate is very common [4]. Patients who received transfusion therapy for several years, the accumulation of iron, if untreated, promotes peroxidative damage to cell and organelle membranes that accumulate excess iron, including liver, pituitary gland, pancreas, and heart. Iron-induced peroxidative injury to the phospholipids of lysosomes and mitochondria, produced by free hydroxyl radicals, is probably the most important pathogenetic factor [5]. So, to combat free radical injury, reduced glutathione (GSH), along with other enzymes & antioxidant become the major defendants [6-9]. Carbonyl group in blood proteins is considered a useful biomarker of oxidation induced by reactive oxygen species (ROS) [10]. Different cross sectional and longitudinal studies revealed that oxidative stress (OS) in these patient produce a significant amount of damage to cell membrane lipids as reflection of an increase in malondialdehyde (MDA) in the patients [11-17].Regarding the relationship between plasma PC adducts and RBC GSH with thalassemia , few studies are available till now relating the changes in the redox balance with progression of disease. Accordingly we carried out the present study to evaluate a correlation between plasma PC adducts and RBC GSH in proved cases of beta thalassemia major of Indian origin .

2. Materials and Methods

The present study was undertaken as a cross-sectional observation study in the department of Biochemistry in association with department of pathology of C.N. Medical College, Kolkata. During study period (February 2012- July 2013) 53 subjects were selected as control subjects following screening for exclusion and inclusion criteria. On the other hand, 83 patients diagnosed as beta thalassemia major were selected as case group after meeting the requisite inclusion and exclusion criteria.

Inclusion criteria

1) Aged 3 to 10yrs.
2) Receiving regular transfusion management.
3) At least two blood transfusion per month.
4) Last blood transfusion at least 15days ago.

Exclusion criteria

1) Any hematological disorder other than thalassemia , e.g., anaemia due to other causes, G6PD deficiency, sickle cell anemia.

2) Active infection.

The whole study and the study procedure strictly adhered to the Helsinki declaration 1975 for human studies and was carried out after getting the informed written consent from the appropriately formed institutional ethical committee. Venous blood samples were collected from case and control and estimated for serum ferritin by sandwich ELISA method [18], haemoglobin estimation of prepared RBC suspension by cyanomethemoglobin method [19], RBC GSH level was measured by the method of Buetler et. al.[20] , plasma PC adducts was performed by the method of Levine et .al [21] and total protein estimation by Biuret method [22,23].

The data were compiled in MS excel and analyzed by different statistical methods. Data display was done by charts and tables. Data were described by proportion, mean, SD, range etc. Statistical tests like independent't' test, Pearson's correlation coefficient (r) etc. were used to explore the relationship between variables. P value of <0.05 was considered significant to discard the null hypothesis at 5% precision and 95% confidence interval.

3. Results

Table 1 shows distribution of gender in both case and control groups. It is evident from the data that there is no significant difference between case and control groups as per as distribution of male and females are concerned. The Pearson Chi -Square value is 1.234 and p value is 0.347 which is statistically non significant.

Table1: Distribution of male and female in both case and control groups

	Value	Df	Asymp. Sig(2-sided)	Exact Sig (2-sided)	Exact Sig (1-sided)
Pearson Chi-Square	1.234[a]	1	.267		
Continuity Correction [b]	.851	1	.356		
Likelihood Ratio	1.252	1	.263		
Fisher's Exact Test				.347	.178
N of Valid Cases	137				

a. 0 cells (.0%) have expected count less than 5.The minimum expected count is 16.95

b. Computed only for a 2x2 table

In **Table 2**, significance of differences between test parameters in case and control group is obtained by parametric analysis (independent t test). It shows a high dispersion value in the distribution of serum ferritin in the case group (mean+/_SD=1249.14+/_707.79) that has signified, the distribution of serum ferritin may not followed the pattern of normal distribution in case group. So, non parametric analysis is done.

Table 2: Significance of differences between test parameter in case and control group as obtained by parametric analysis(independent t test)

	Case(n=83) mean+/-SD	Control(n=54) mean+/-SD	t value	p value
Age (in months)	49.2+/- 22.6	50.4+/- 12.12	-0.354	0.72
Plasma protein carbonyl in nmol/mg of protein	0.43+/- 0.07	0.18+/-0.01	25.35	<0.001
RBC GSH in um/g of Hb	27.55+/-4.00	8.60+/-1.20	33.77	<0.001
Serum ferritin in ng/ml	1249.14+/-707.79	63.62+/-15.12	12.29	<0.001

p value is considered to be significant at the level of p≤0.05 at 95% confidence interval.

In the **Table 3**, distribution of different test parameters are shown. Group 1 and group 0 indicated the case and control subjects respectively. Differences between the mean rank values of the parameter suggest a significant difference in the distribution of study parameters between the case and control population. The result of the Table 3 were validated by the Man-Whitney test in Table 4.

Table 3: Rank distribution of the test parameter in case and control group in independent non Parametric analysis. Case=1, Control=0

Group		*N*	*Mean Rank*	*Sum of Rank*
Plasma protein Carbonyl in nmol/mg of protein	.00	54	27.50	1485.00
	1.00	83	96.00	7968.00
	Total	137		
RBC GSH in um/g of Hb	.00	54	27.50	1485.00
	1.00	83	96.00	7968.00
	Total	137		
Serum ferritin in ng/ml	.00	54	27.50	1485.00
	1.00	83	96.00	7968.00
	Total	137		

In **Table 4**, significance of differences between study parameters in case and control group is analyzed. Significant difference is found between case and control group in these study parameter: plasma PC adducts, RBC GSH and serum ferritin.

Table 4: Significance of differences between test parameter in case and control group as obtained by non parametric analysis (Man –Whitney test)

	Case(n=83) median	Control (n=54) median	Z value	Significance (2-tailed)
Plasma protein carbonyl in nmol/mg of protein	0.43	0.18	-9.871	<0.001
RBC GSH in um/g of Hb	28.1	8.65	-9.871	<0.001
Serum ferritin in ng/ml	980	67	-9.872	<0.001

In **Table 5**, the strength of association between different parameter in the case group, Pearson bivariate correlation analysis is performed. It is observed that there is a proportional increased PC adducts in plasma with and increased in serum ferritin value and vice-versa. Correlation co efficient r =0.459, p value=<0.001(2-tailed) is highly significant. However, the variation of RBC GSH is found to occurred in a inverse manner with rising serum ferritin level as well as plasma PC adducts (Correlation coefficient r = -0.181 and p value =<0.001 and 0.01 respectively) . This data has signified that there is significant decreased in RBC GSH level along with an increased in serum ferritin value. Althrough RBC GSH is showed an inverse correlation in the plasma PC adducts and the p value is non significant i.e 0.102.

Table 5: Strength of association between different test parameter in case group as shown by Pearson bivariate correlation study

		Plasma protein carbonyl in nmol/mg of protein	RBC GSH in um/g of Hb	Serum ferritin in ng/ml
Plasma protein carbonyl in nmol/mg of protein	Pearson correlation Sig.((2 tailed) N	1 83	-.181 .102 83	.459** .000 83
RBC GSH in um/g of Hb	Pearson correlation Sig.((2 tailed) N	-.181 .102 83	1 83	-.526** .000 83
Serum ferritin in ng/ml	Pearson correlation Sig.((2 tailed) N	.459** .000 83	-.526** .000 83	1 83

**Correlation is significant at the 0.01 level (2- tailed).

4. Discussion

It is well known from various studies that oxidative stress (OS) in beta thalassemia patients who have received regular blood transfusion. OS and reactive oxygen species (ROSs) play an important role in the etiology and/or progression of a number of human diseases. By Fenton's and Haber –Weiss reaction much potent hydroxyl radical is formed [24]. It is notorious to damage the lipid membrane, intracellular protein and nucleic acid too leading to covalent attack formation between the damaged carbonyl group and increased label of PC adducts is formed in the tissue fluid. It has been documented earlier that OS in beta thalassemia patients who have received regular blood transfusion produce a significant amount of damage to the cell membrane lipids as reflection of an increase in malondialdehyde (MDA) in the patients [11-17].

The present study was proposed to validate the result in Indian scenario with an object to explore a correlation between plasma PC adducts and RBC GSH in proved cases of beta thalassemia major patients of Indian origin . The value from the (Table no.4) shows protein carbonyl levels are found to be significantly increased in case group (p value<0.001). These findings are strengthened by the observation of a direct and significant correlation between the serum ferritin and plasma PC adducts which suggest that generation of OS induced protein damage occur in a linear fashion with the total body iron store (Table no. 5 ,Correlation co-efficient r = 0.459 and p value < 0.001). It has been found that there is significantly compensatory rise in the glutathione peroxidase and GSH for counter acting OS inside the cells [25]. Keeping in track, a significant increase in the RBC GSH has been observed in the patients who have showed a significant elevation in OS (Table no. 3). However, the variation of RBC GSH is found to occure in a inverse manner with rising serum ferritin level as well as plasma PC adducts (Table no. 5 , Correlation co-efficient r = - 0.181 and p value < 0.001 and 0.01 respectively). These data indicate that there is significant decrease in RBC GSH level along with an increased in serum ferritin value. Althrough, RBC GSH values show an inverse correlation to the plasma PC adducts, the p value is not significant i.e 0.102 (Table no.5). It indicates clearly that an increase in OS is counteracted by the antioxidant activity of GSH and it simultaneously leads to a compensatory increase in the GSH levels as reflected in our study. However, this compensatory increased value is not fully capable of counteracting the OS that induce damage generated by huge iron overload in our body. It has been corroborated in recent studies involving animal models that an increased intracellular OS induces an elevation of the GSH activity[26]. All these findings importantly stress on the fact that in spite of a compensatory increase in the antioxidant defensive mechanism in our body in response to the OS, the overall redox balance is skewed in favour of the OS induced tissue damage.

5. Conclusion

Results of the present study can be concluded to the fact that a mere antioxidant therapy can't combat the OS induced damage in the beta thalassemia patients receiving regular blood transfusions and consequently having huge iron storage. Rather more importantly, an effective chelation therapy is needed to reduce the tissue ferritin level and thereby reduce iron over load in the body. Furthermore, determination of the antioxidant enzymes and markers of OS is needed periodically for assessing the cellular damage and monitoring the chelation therapy success. However, this study has few limitations that must be mentioned. We could not measure the tissue ferritin level for having a more realistic value of intra-cellular iron storage. Furthermore, a battery of other antioxidant enzyme measurement in larger multicentre placebo controlled studies is needed to reach conclusive evidence.

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