Hematological Parameters and RBC TBARS Level of Q 10 Supplemented Tribal Sickle Cell Patients: A Hospital Based Study

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Abstract The study has been undertaken as number of sickle cell patients in Chhattisgarh tribal population is 23.7 %. The Co enzyme Q10 is a strong antioxidant and energy producing compound. The patients were divided into three groups group A homozygous (SS), group B heterozygous (AS) and group C controls for TBARS study. The age group is 10–55 years and 200 mg of CoQ10 was given to A and B groups. The hematological parameters, C reactive protein as well as RBC TBARS level were performed by usual and standard techniques. The results were obtained as 25.37 % increased RBC level in group A and 23.24 % in group B. The increased hemoglobin level was observed as 16.73 % in group A and 10.7 % in group B. In case of WBC it was observed increased 24.38 % in group

A and 12.0 % in group B. C-reactive protein was observed 7.8 times decreased in group A and 1.54 times in group B. The RBC TBARS level was also found decreased 48 % in group A and 51 % in group B as compared to group C. During the supplementation of coenzyme Q10 the pain caused by vaso-occlusive events has reduced. This significant increase in hematological parameters as well as decreased C-reactive protein and TBARS level suggest that the Q10 should be included in the diet of sickle cell patients.

Keywords TBARS·Thiobarbituric acid reactive species · CoQ10 · Coenzyme Q10

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Introduction

Sickle cell disease (SCD), an inherited disorder of hemoglobin synthesis has been traced to a single point mutation that substitutes valine for glutamic acid in the β -globin subunit. This induces the polymerization of sickle hemoglobin (HBS) and a resultant elongation and stiffening of sickle erythrocytes (RBC). However phenotypic expression of the disorder is complicated and is characterized by episodic vaso-occlusive events that elicit ischemia reperfusion related inflammatory responses in multiple organ systems, producing pain crises and end organ damage [1, 2]. The reactive oxygen species (ROS) are also recognized major players in ischemia–reperfusion injury. It is unsurprising that oxidative stress has also been implicated in the pathogenesis of SCD [3].

The antioxidant nature of CoQ10 derives from its energy carrier function [4]. Coenzyme Q10 affinity for the enzymes is not high enough to saturate them, at the physiological concentration. The velocity of respiratory

chain will strongly depend on the Coenzyme Q10 concentrations [5]. CoQ10 inhibits lipid peroxidation by preventing the production of lipid peroxyl radicals, reduces the initial perferyl radical and singlet oxygen and also regenerates other antioxidants such as vitamin E [4]. The normal plasma coenzyme Q10 level is $0.79 \pm 0.2 \mu\text{g/ml}$ [6]. Definite levels of CoQ10 are also found in white and red blood cell components, as well as in platelets. Plasma and erythrocyte CoQ10 has a well assessed antioxidant role, which was demonstrated through a series of experiments. Several enzymatic activities of erythrocyte ghosts were also protected by different side chain CoQ homologues, both when reduced and, although at a lesser extent, in the oxidized state [7]. By observing a wonderful property of coenzyme Q10 i.e., a strong antioxidant we have utilized it for study in the disease like sickle cell anemia. The study has been undertaken as there is more prevalence of Sickle cell disorders in the Chhattisgarh state, India and also their dietary pattern contains less amount of Co Q10 and other antioxidants.

Materials and Methods

Before the study, approval was taken from medical college ethical committee. The study subject selected from Maharani Hospital, GMC, Jagdalpur, who were attending medicine OPD. Inclusion criteria for the subjects were only known cases of sickle cell disease and sickle cell trait. The subjects having crisis, required repeated transfusion, kidney and liver disease or infection were excluded from the study. 130 subjects were selected after their written consent but only 34 patients were follow up the study up to 6 months. The age group was taken 10–55 years. The age groups of the 32 patients were 10–30 years and two sickle cell trait patients were 51 and 55 years. and they were not included in statistical analysis. The patients were divided into three groups. Group A. Homozygous (HbSS, 15 no's), Group B Heterozygous (HbAS, 19 no's) and Group C Control (HbA, 20 no's). The 200 mg of Co Q10 was given to the group A and group B patients for 6 months. SCD patients were confirmed by electrophoresis. The following parameters were performed every 2 months for consecutive 6 months. Statistical analysis was done by using *t* test.

Lipid peroxidation was assayed by measuring TBARS products by the method of Walls et al. (1976): 0.2 ml of 50 % (w/v) trichloroacetic acid was added to 2 ml of the 5 % erythrocyte suspension after incubation, mixed and centrifuged at 3,000 rpm/min for 10 min. Then 1 ml of the supernatant was removed and 0.5 ml of 0.75 % thiobarbituric acid in 0.1 M-HCl was added. The samples were heated at 90–95 °C for 20 min and centrifuged for 10 min. The supernatant was removed and the pink chromophore

was assayed spectrophotometrically at 532 nm. The standard curve was prepared with malonaldehyde bis(dimethylacetal) (Aldrich) hydrolyzed with 6 M-HCl [8]. The test for CBC, Hb, C-reactive proteins and fragility of RBC were performed by standard methods with the help of Minaro Vactar cell counter and Erbachem-50 clinical analyzer.

Results and Discussion

See Figs. 1, 2, 3, 4, 5, and Table 1.

Fig. 1 Hemoglobin (gm/dl)

Fig. 2 RBC (mill/cumm)

Fig. 3 WBC (cells/cumm)

Fig. 4 CRP (mg/dl)

Fig. 5 TBARS Level (nmol/10¹⁰cells)

Patients with SCD are subject to increase oxidative stress, particularly during vaso-occlusive crisis and acute chest pain [9]. Several aspects of the abnormalities in SCD are thought to be result from the oxidative stress of RBC, WBC and endothelial cells and activation of platelets [9]. Incubation of RBC's and platelets derived from SCD patients with antioxidants reduces their status. The oxidative stress of SCD-RBC could be attributable to the

inherent instability of Hbs. It was shown that SCD-RBC's produces greater quantities of O_2^- , H_2O_2 and OH^- than normal RBC's. Another cause of oxidative stress in SCD is the high concentration of iron and iron containing compounds forms ROS which makes RBC more susceptible to endogenous ROS mediated damage. The vaso-occlusive process is a characteristic of SCD might also be affected by oxidative stress and cells become activated and adhere to the endothelium forms thrombus [10].

In the present study we have supplemented Sick cell disease and trait individuals with 200 mg coenzyme Q10. The TBARS level decreases 48 and 51 % in group A (SS) and group B (AS) as compared to group C (AA), after supplementation of Co Q10. This may be due to reduced oxidative stress by coenzyme Q10. The RBC level increases 25.37 and 23.24 % in group A (SS) and group B (AS) respectively. The WBC level increases 24.38 and 12 % in group A and group B respectively. The increased and maintenance of RBC and WBC level and their decreased breakdown might be due to the reduced oxidative stress by coenzyme Q10 [7].

The hemoglobin level was maintained and found increased 16.73 and 10.7 % in group A (SS) and group B (AS) respectively. The Hb of SCD-RBC is oxidized 1.7 times the rate of HbA [10]. This oxidation of Hbs might be reduced due to antioxidant nature of coenzyme Q10 which reduces the oxidative stress in RBCs.

The C-reactive protein concentration was 7.8 times decreased in group A (SS) and 1.54 times in group B (AS) after coenzyme Q10 supplementation. The maintenance of blood cells level and decreased oxidative stress might have reduced break down of cells, decreasing vaso-occlusive events and pain crisis leads to decrease CRP level significantly [1, 2]. The patients have reported decrease in pain as well as tired less. The dietary pattern of Chhattisgarh tribal population contains less antioxidant as well as coenzyme Q10 level. Hence the above study suggest that the rationale of using Co Q10 in the treatment of SCD patients of Chhattisgarh tribal population.

Table 1 Hematological parameters and TBARS (RBC) of sickle cell patients before and after Q 10 supplementation

S. no	Parameters	Group A (SS)		Group B (AS)		Group C
		Before Q10 (n = 15)	After Q10 (average value of 3 analysis) (n = 15)	Before Q10 (n = 17)	AfterQ10 (average value of 3 analysis) (n = 17)	Control without SCD (n = 20)
1.	TBARS in erythrocytes	11.17 ± 1.28	5.83 ± 0.79	10.31 ± 1.18	5.05 ± 0.63	6.55 ± 1.30
2.	RBC count	2.64 ± 0.42	3.31 ± 0.67	3.14 ± 0.70	3.87 ± 0.89	4.62 ± 0.95
3.	WBC count	7493 ± 2539	9320 ± 3329	7131 ± 1811	7989 ± 2557	7682 ± 2442
4.	HB count	7.89 ± 1.13	9.21 ± 1.43	9.06 ± 1.54	10.03 ± 1.75	15.14 ± 1.52
5.	CRP	23.22 ± 10.47	2.62 ± 1.90	5.95 ± 2.59	2.34 ± 0.75	1.2 ± 0.45

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