Original Research Paper

Volume-8 | Issue-8 | August-2018 | PRINT ISSN No 2249-555X

Biochemistry

STATUS OF EXOGENOUS ANTIOXIDANT, TOTAL ANTIOXIDANT CAPACITY AND OXIDATIVE STRESS IN SCA PATIENTS.

Vikas K. Gupta — Associate Professor, Department Of Biochemistry, Late Shri Lakhiram Agrawal Memorial Medical College, Raigarh (C.G.)

Rajesh Kumar Shrivastava* — Professor, Department Of Biochemistry, Sri Arbindo Medical College & PGI, Indore (M.P.) *Corresponding Author

Neelima Singh — Professor & Head, Department Of Biochemistry, Gajraraja Medical College, Gwalior (M.P.)

ABSTRACT In India, haemoglobinopathies, especially sickle haemoglobin are the commonest genetic disorders in the tribal belt of Central and southern India. Madhya Pradesh and Chhattisgarh harbours the largest tribal population in India, which is about one fourth of the total tribal population of the county. The prevalence of sickle haemoglobin from various parts of Madhya Pradesh and Chhattisgarh varied from 15 to 30 percent. The study was carried out in Chhattisgarh Institute of Medical Sciences, Bilaspur (C.G.) Since the Eastern region of central India is prone to Sickle Cell Anemia. Hence samples preferably collected from this area. The study was conducted on 90 human subjects. Out of which 30 were suffering from Sickle cell disease, 30 were Sickle Cell Trait and 30 were taken as control group. Haemoglobin, Malondialdehyde & antioxidants parameters were estimated. In subjects with homozygous sickle cell anemia, level of Plasma Malondialdehyde (MDA) were significantl ($P<0.001$) increased as compare to controls. The level of antioxidants vitamins C, E and TAC ($P<0.001$) decrease in group III as compare to controls. In group II the level of Vitamin C & Vitamin E decreased significantly ($P<0.05$) as compare to controls. Thus our study shows decreased Total antioxidant capacity , elevated plasma MDA and depleted antioxidant vitamins indicate increased oxidative stress in Sickle cell anemia patients. Therefore supplementation with antioxidants vitamin may improve some of the sickle cell symptoms.

KEYWORDS : Oxidative stress, TAC, MDA, SCA,.

Introduction:

Sickle cell Anemia refer to a genetic disorder characterized by a abnormal haemoglobin called HbS[1] and clinically characterized by intermittent vaso-occlusive event, chronic hemolysis, & increased susceptibility to infection[2]. In India, Sickle haemoglobin are the commonest genetic blood disorders in the tribal belt of Central and Southern India. In central India , Madhya Pradesh and Chhattisgarh harbour the largest tribal population in country, which is about one fourth of the total tribal population of the country. The prevalence of sickle cell anemia from various parts of Madhya Pradesh and Chhattisgarh varied from 15% to 30%t. It was also found that the non-tribal people of these belt especially Scheduled Castes and other backward class communities have Hb S in similar proportion as that of tribal Population of the area[3].

In sickle cell disease, free radicals formation increased resulting increased oxidative stress and might play a significant role in the pathophysiology of SCD related microvescular dysfunction, vaso-occlusion and development of organ damage[2]. Reactive oxygen species are produced as the result of intracellular catabolism that requires oxygen as a terminal acceptor (oxidants), normally there is a balance between the production of Reactive Oxygen Species and the defence mechanism of antioxidants (SOD, CAT, GPX, ascorbate, carotonoid, flavaonoid) thereby preventing or limiting oxidative damage[4]. Antioxidants, a substance which slow the oxidation rate by several mechanisms, including regeneration of primary antioxidants, chelation of metals, scavenging of oxygen, and decomposition of hydroperoxides among others. These substances may occur naturally in foods, such as Tocopherol (vitamin E) and ascorbic acid (vitamin C). These antioxidants are mainly vitamins which play an significant role in antioxidant defence system[5,6].

Since the various membrane abnormalities of sickle RBCs might result from excessive accumulation of oxidant damage and decrease activity of antioxidant defence in sickle cell anaemia patients. Therefore, we planned our study to know the level of exogenous antioxidants and antioxidant capacity in Sickle cell disorder.

Material and methods:

The study was carried out in CIMS, Bilaspur (C.G.). Since the Eastern region of central India is prone to Sickle Cell Anemia. Hence samples preferably collected from this area. The study was conducted on 90 human subjects. Out of which 30 were suffering from Sickle cell disease , 30 were Sickle Cell trait and 30 were taken as control group (Healthy age matched, having no blood disorder). The written consent were also obtained before starting the study. The family history, clinical manifestations pertaining to disease was collected in the study Performa. The study was approved by the Institutional Ethical committee of Chhattisgarh institute of Medical sciences , Bilaspur (C.G.) A blood sample for the estimation of all the parameters was collected from the subjects. Six ml (06ml) Fasting blood samples were freshly withdrawn from the anticubital vein and collected. One part is distributed into EDTA vial and other part was kept for clotting from both subjects and controls. Each sample was centrifuged at 3000 rpm and the serum was stored for further estimation but hemoglobin indices were taken to measure immediately. Solubility test was carried out by commercially available kit (HIMEDIA). Hemoglobin Electrophoresis (Alkaline) by GENIOS (fully automated electrophoresis), The assay was performed exactly as recommended by the manufacturer. Hematological parameter, hemoblobin indices were estimated by Hematological using hematology analyzer make Orphee, France. Plasma Malondialdehyde by Jean C.D.et al (1983)[7]. Total Antioxidant Capacity by D Koracevic (2001)[8] ,Vitamin C By A kyaw (1978)[9] and Vitamin E By Baker and Frank (1968)[10], Using PC based double beam UV, VIS spectrophotometer Systronic (Type 2202). Data analyses were performed with the SPSS, Chicago, Illinois, USA for window version 16.0.

Results:

The haematological and biochemical parameters of these patients and controls are listed in Table 1 & 2. The level vitamin E, C and , Total antioxidant capacity decreased significantly ($P<0.001$) in Sickle cell disease patients (table 1) and Sickle cell traits ($P<0.05$) as compare to controls (Table 2). In subjects with homozygous sickle cell anemia. Level of Plasma MDA were significantly

($P<0.001$) increased as compare to controls (Table no.2).

Table 1: Antioxidant vitamin, TAC and MDA measurements for subjects of Group I and Group II.

Parameters	Group I (Mean ± SD)	Group II (Mean ± SD)
Age (yrs)	24.2 ± 5.2	24.8 ± 6.7
Haemoglobin (g/dl)	14.3 ± .80	13.1 ± 1.2
Vitamin E (mg/dl)	1.07 ± .40	0.78±.28*

Vitamin C (mg/dl)	0.98 ± .25	0.84±.18*
TAC (mmol/l)	1.96± 00.21	0.98± 00.24*
MDA (nmol/ml)	2.35±.48	3.20±1.16NS

NS= not significant, * P<0.05, **P<.001,

Table 2: Antioxidant vitamin E & C, Total antioxidant capacity and MDA measurements for subjects of Group III and Group I.

Parameters	Group I (Mean ± SD)	Group III (Mean ± SD)
Age (yrs)	24.2 ± 5.2	17.2 ± 5.5
Hb (g/dl)	14.3 ± .80	8.7 ± 2.1
Vitamin E (mg/dl)	1.07 ± .40	0.48 ±.19**
Vitamin C (mg/dl)	0.98 ± .25	0.51±.11**
TAC (mmol/l)	1.96± 00.21	00.82± 0.24**
MDA (nmol/ml)	2.35 ±.48	7.44±2.57**

NS= not significant, * P<0.05, **P<.001,

Discussion:

SCD is inherited disorder of hemoglobin. Mostly prevalent in central and southern region of India [11]. It may present itself either in heterozygous (HbAS) or in homozygous form (HbSS) due to autosomal inheritance. In severe homozygous (Hb SS) form all Hb present is sickle hemoglobin, in milder heterozygous (HbAS) form up to 40 percent of Hb is sickle [12]. In Sickle cell disorder acidic amino acid glutamine (Gln) substitutes neutral amino acid valine at 6th position of beta hemoglobin chain. The substitution of a positively charge amino acid glutamine, for valine, a neutral amino acid results in the formation of Hb S and cases a multeity of pathological conditions that affects the haemoglobin, with the sickle hemoglobin, tamper the oxygenation of Hemoglobin and subsequently sickling of RBC (sickle shape) and hemolysis. This causes physiological changes that affect the hemoglobin molecule in its deoxygenated state through the sickling of red blood cells, this triggers the formation of Hb S polymer, oxidative degeneration of the Hb S molecule and the generation of oxidized free radicals[13]. SCD is known to be associated with membrane lipid abnormality long chain unsaturated FAs are known to increase membrane fluidity, decrease adhesion and aggregation of Red Blood Cells. Both Vitamin E and C have protective role against oxidative membrane attack, while caroteniods act at low oxygen tension, mainly derived from food and other dietary source [14].

In the present study we have considered the role of vitamin E & C, in SCA and found that all these vitamins levels decreased significantly in Group II and group III as compared to control group I (table no. 1 & 2). This is same as quoted by Deves Ray et al[15] 2006, Rain Het al 2008[16], Wali U et al 2013[17].

Vitamin E (Tocopherol) has a protective role in almost all cells and tissues of the body. It removes free radical by its ability to transfer phenolic hydrogen to a peroxyl free radical of peroxidized polyunsaturated fatty acid. Since SCD is associated with multiple membrane defects in this condition[18], it is possible that membrane damage might be a critical factor in this disorder. Oxidatively modified membrane associated protein are currently implicated in the formation of irreversible sickle cell, which is leading to a paradigm shift from the older cross liking theory[19]. Due to intravascular hemolysis, low Hb are found in the SCA patients which act as potent catalyst for lipid peroxidation. So, plasma haemoglobin catalyze the oxidative destruction of Tocopherol and account for the lower plasma Tocopherol levels found in the subjects (table no. 2).

In homozygous state vit E level is significantly reduced as when compared to control while the heterozygous group showed less change as compared to control group (table no. 1. & 2). So, we interpret that in the presence of oxidative stress vit E get depleted in proportion.

We also observed in our study that vitamin C level change was almost similar to that of vitamin E. This suggest that vit C level change was almost similar to vit E but vitamin C is not involve in first line of antioxidant system but it might play a significant role as a replenishing agent for vit E[20]. Ascorbic acid (Vitamin C), and aqueous phase antioxidant has excellent protective role in regeneration of the reduced form of other powerful antioxidants of the body named GPx and Vit E. thus halting the free radical chain reaction[21].. Lower level of vit C in SCA patients indicates its exhausted status to remove generated free radicals.

As SCD (SS) patients are more prone to haemolysis and have a greater tendency for the formation of superoxide anion and hydrogen peroxide, a decreased the vit C level show a negative correlation with serum MDA level. Wali U et al reported the same. The decreased level of antioxidants vitamins are associated with increased oxidative stress in SCA patients, leading to higher utilization of these vitamins and consequently leading to their deficiency in sickle cell anemia. This result is also consistent with[22] and he reported that deficiency of these vitamins could for some of the observed manifestation of SCD such as increased susceptibility to infection and hemolysis[23].

In our study we measured Total antioxidant capacity (TAC) in both cases i.e. homozygous (SS) and heterozygous (AS) and found significantly decreased level of antioxidant in Sickle cell anemia patients as compared to control subjects.

TAC reflects the collective contribution to the reducing property of non protein antioxidant or electron donating component. Total antioxidant capacity values are more informative than the knowledge of individual antioxidants. Reduced antioxidant defence both enzymatic and low molecular weight antioxidant have decrease activity and their concentration in plasma of SCA patients[24,25].

So it is clearly evident that Sickle cell anemia patients are exposed to oxidative stress and this oxidative stress could be, to some extent, be relieved by supplementation these antioxidant vitamins.

Reference:

1. Nitin John. A review of clinical Profile in sickle cell trait. OMJ. 2010;25:3-8.
2. Erfan Nur, Bart J. Biemond, Hans-Martin Otten, Dees P. Brandjes, John-Jhon B.Schnog. Oxidative stress in sickle cell disease: pathophysiology andpotential implocations for disease managment. Am. J. Hematol. 2011; 86:484-489.
3. R.B. Gupta, Sickle Cell Disease in Central India- Need for micro Level plannning,. Proceeding of national Symposium on Tribal health. 2004
4. Prakash S. Hundekar, Aadinath N. Suryakar, Aarti C. Karnik, Rajesh Valvi, Rahul A. Ghone, Sonali S. Bhaga The effect of Antioxidant Supplementation on the Oxidant And aAntioxidant Status in Sickle Cell Anaemia Journal of Clinical and Diagnostic Research. 2011 (Suppl-2); 5(7): 1339-1342.
5. Nagababu., Fabry M.E., Nagel R.L., Fifkind J.M. Heme degradation and oxidative stress in murine models for hemoglobinopathies: Thalassemia, sickle cell disease and hemoglobin C Disease, Blood Cells. 2008;41: 61-66.
6. Akohoue S.A., Shankar S., Milne G.L. morrow J., Chen Y.K., Ajay U.W., Buschowski S.M., Energy expenditure, inflammation, and oxidative stress in steady state adolescents with sickle cell anemia. Pediatr Res. 2007;61(2):233-238.
7. Jean CD, Maryse T, Marie JF. Plasma M. alondialdehyde levels durin myocardial infarction. Clin Chim Acta 1983; 129: 319-22.
8. D Koracevic, G Koracevic, V Djordjevic, S Andrejevic, V Cosic . Method for the measurement of antioxidant activity in human fluids. J Clin Pathol 2001; 54: 356–361.
9. Ayekyaw. A simple colorimetric method for ascorbic acid determination in blood plasma. Clin Chim Acta. 1978: 86: 153-157.
10. Baker H Frank O. Warley's Practical Clinical Biochemistry 1968 : sixth edition chapter 35 : 902-905.
11. Agrawal M.B. The burden of haemoglobinopathies in India-time to wake up. J Acad phys Ind. 2005;53:1017-1018.
12. Jyoti Titus, Suresh Chari, Madhur Gupta, and Nitin Parekh. Pro-oxidant and antioxidant status in patients of sickle cell anaemia, Indian Journal of Clinical Biochemistry, 2004;19(2) 168-172.
13. Halliwell B, and Gutteridge J.M.C. Oxygen free radical and iron in relation to biology and medicine: some problems and concepts Arch. Biochem. Biophys. 1986; 246:501-514.
14. Mayes P.A., Murray R.K., Granner D.K., Mayes P.A., Rodwell V.W.(eds). Structure and function of the water-soluble vitamins. In harper's biochemistry. 25th ed. Stamford, Connecticut: Appleton and Lange. 2000:642-52
15. Debes Ray, Pradeep Deshmukh, Kalayan Goswani, Neelam Garg, Antioxidant Vitamin levels in sickle cell disoeder. Natl. Med J India, 2007;20:11-13
16. Ren H., Ghebrameskel K., Okpala I., Lee A., Ibegbulam O., Crawford M. Patients with sickle cell disease have reduced blood antioxidan protection. Nt J Vitam nutr Res. 2008; 78(3): 139-41
17. Wali U. , Yedu, M.H., samial B., And jiya N.M.. Serum antioxidant vitamins level in children with sickle cell anemia in Sokoto western Nigeria. Bajopas. 2013; 6(1).
18. Hebbel R.P. Beyond hemoglobin ploymerization: The red blood cell mebrane and sickle disease pathophysiology. Blood.1991;77: 214-37.
19. Wang W.C., Greer J.P., FoersterJ., Lukens J.N., Rodgers G.M., Paraskevas F., Glader B. (eds). Sickle cell anemia and other sickling syndromes. Wintrobe's clincial hematolgoy. 11th ed. Philadelphia: Lippincott Williams and Wilkins; 2004: 1264-1311.
20. Niki E., Noguchi N., Tsuchihashi H., Gotoh N. Interaction among vitamin C, Vitamin E, and beta carotene Am J Clin Nutr. 1995; 62(suppl6): 1322S-13226S.
21. Chiu D, Vichinsky E, Yee M, Kleman K, Lubin B. Peroxidation of Vit-E and sickle cell Anemia. Ann NyAcadSci 1982;393;323-33.
22. Adelekan D.A.,Thurnham D.I., Adekole A.D. Reduced antioxidant capacity in pediatric patients with homozygous sickle cell disease. Eur J Clin Nutr. 1989; 43: 609-14.
23. Rana M.W. Hasanato. Zinc and antioxidant vitamin deficiency in patients with severe sickle cell anemia, Ann Saudi Med. 2006; 26(1): 17-21.
24. Das, S. K., Nair, R. C. Superoxide dismutase, glutathione peroxidase, Catalase and lipid peroxidation of normal and sickled erythrocytes. Br. J. Haematol. 1980; 44: 87–92.
25. Nwaoguikpe RN, Braide W.The antisickling effects of some micronutrients and antioxidant vitamins in sickle cell disease management.Journal of Medicine and Medical Sciences Vol. 3(5) pp.334-340.