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Status of Some Hematological and Antioxidant Parameters in SCA Patients of Chhattishgarh Region

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Abstracts

In India, haemoglobinopathies, especially sickle hemoglobin 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 country. 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 120 human subjects. out of which 40 were suffering from Sickle cell disease , 40 were Sickle Cell Trait and 40 were taken as control group. Hematological parameter & antioxidants parameters were estimated. Hb, RBC, decreased significantly (P<0.001) while HCT (P<0.05) in Group III and in Group II also decreased significantly (P<0.05) while there is no significant change were found in the level of MCV in both group as compare to control. The level of antioxidants significant (P<0.001) decrease except SOD in group II as compare to controls. In group III the level of antioxidant decreased significantly (P<0.05) except SOD as compare to control. In subjects with homozygous sickle cell anemia, level of Plasma Malondialdehyde (MDA) were significantly (P<0.001) increased as compare to controls. Thus our study shows elevated plasma MDA and depleted antioxidant vitamins indicate oxidative stress in SCD. Therefore supplementation with antioxidants vitamin may ameliorate some of the sickle cell symptoms and improve quality of life.

Introduction

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 country. The prevalence of sickle haemoglobin from various parts of Madhya Pradesh and Chhattisgarh varied from 15 to 30 percent[1]. Sickle cell spontaneously generates approximately two times more amount of reactive oxygen species. A high production rate of reactive oxygen species in Sickle cell disease caused by several factors such

as chronic inflammation, intravascular hemolysis, ischemia reperfusion injury and decreased level of antioxidants[2]. Since the various membrane abnormalities of sickle erythrocytes might result from excessive accumulation of oxidant damage and decrease activity of antioxidant defense in sickle cell patients, hence we planned our study to know the status of haematological and antioxidants in Sickle cell disorder (Heterozygous HbAS and Homozygous HbSS).

Material and methods

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 120 human subjects. out of which 40 were suffering from Sickle cell disease , 40 were Sickle Cell Trait and 40 were taken as control group (Healthy age matched ,having no blood disorder). we have selected those SCD patients who were not taking medicine for at least a month before taking blood sample i.e. free from acute illness. we have excluded SCD patients having history of recent blood transfusion, on medication severe illness and were suffering from other diseases like HTN, Diabetes, CVD, Thelasmia , HIV etc . The family history, clinical manifestations pertaining to disease was collected in the study Performa. The written consent were also obtained before starting the study. The study was approved by the Institutional Ethical committee of CIMS, Bilaspur (C.G.). A Blood sample for the estimation of all the parameters was collected from the subjects after overnight fasting. Eight ml (08 ml) Fasting blood samples were freshly withdrawn from the anticubital vein and collected. one part is distributed into anticoagulant (EDTA) tube and other part was kept for clotting from both patients and volunteer and immediately transferred to laboratory. Each sample was centrifuged at 3000 rpm and the serum was kept at -20^{o}C for further estimation but hemoglobin indices were taken to measure immediately. Hematological parameter, hemoglobin indices were estimated by Hematological using hematology analyzer make Orphee, France and solubility test by commercially available kit (HIMEDIA). Hemoglobin Electrophoresis (Alkaline) by GENIO S, The assay was performed exactly as recommended by the manufacturer. Antioxidants profile by manual method using double beam spectrophotometer Systronic 2202. Data analyses were performed with the Statistical Package for the Social Sciences, version 16.0 (SPSS, Chicago, Illinois, USA).

Observation and results

Table no. 1 & 2 Shows the level of Hb, RBC decreased significantly (P<0.001) while HCT (P<0.05) in Group III and in Group II also decreased significantly (P<0.05) while there is no significant change were found in the level of MCV in both group as compare to control.

table no. 3 shows the level of antioxidants significant (P<0.05) decrease except SOD in group II as compare to controls. In group III the level of antioxidant decreased significantly (P<0.001) except SOD as compare to control (table no. 4)

In subjects with homozygous sickle cell anemia, level of Plasma Malondialdehyde (MDA) were significantly (P<0.001) increased as compare to controls (Table no. 4).

Table No. 1: Showing the significant changes of haematological profile in between subjects of Group I and Group II.

Parameters	**Group I Mean ± SD**	**Group II Mean ± SD**
Hb g/dl	14.10 ± .90	13.19±0.70*
RBC 10^6/µl	4.67 ± .45	4.10± .54*
HCT %	42.67±3.53	40.66±3.94^{NS}
MCV µm^3	85.51±4.66	87.69±7.77^{NS}
WBC 10^3/µl	7000±1700	8900±2300*

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

Table no 2: Showing the significant changes of haematological profile in between subjects of Group I and Group III.

Parameters	**Group I Mean ± SD**	**Group III Mean ± SD**
Hb g/dl	14.10 ± .90	8.57±1.15**
RBC 10^6/µl	4.67 ± .45	3.30±0.43**
HCT %	42.67±3.53	33.19±4.77*
MCV μm^3	85.51±4.66	88.74±9.57NS
WBC 10^3/µl	7000±1700	11300±3200**

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

Table No. 3: Showing the significant changes of endogenous & exogenous antioxidants in between subjects of Group I and Group II.

Parameters	Group I Mean ± SD	Group II Mean ± SD
SOD U/gm of Hb	5.74± 00.62	6.75±0.83*
CAT U/gm of Hb	6.47± 00.89	6.10±0.73*
GPx U/mg of Hb	8.79± 00.83	7.12±01.52*
GSH mg %	24.86±02.72	19.79±02.95*
MDA nmol/ml	2.32±00.44	3.08±01.15*

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

Table No. 4: Showing the significant changes of endogenous & exogenous antioxidants in between subjects of Group I and Group III.

Parameters	**Group I Mean ± SD**	**Group III Mean ± SD**
SOD U/gm of Hb	5.74± 00.62	9.78± 2.91**
CAT U/gm of Hb	6.47± 00.89	4.11±1.07**
GPx U/mg of Hb	8.79± 00.83	4.23±1.29**
GSH mg %	24.86±02.72	13.21±3.51**
MDA nmol/ml	2.32±00.44	7.42±02.57**

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

Discussion

Sickle cell disease is genetic disorder of haemoglobin. In India it is more common in central and southern region[3]. Due to autosomal inheritance it may present itself either in milder heterozygous or in severe homozygous form. In homozygous (Hb SS) form all hemoglobin present is sickle hemoglobin, in heterozygous (Hb AS) form up to 40% of Hb is sickle hemoglobin[4]. In sickle cell anemia glutamine substitutes valine at position 6^{th} of beta hemoglobin chain. The substitution of glutamine, a positively charged amino acid for a neutral amino acid valine results in the formation of haemoglobin S and causes a variety of pathological condition that affects the hemoglobin concentration and the packed cell volume of the individual. Substitution of normal hemoglobin with the Hb S, interferes with the oxygenation of HB and subsequently sickling of red blood cells (Holly leaf 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[5].

In our study we observed that in group III subjects suffering from sickle cell disease have low hemoglobin and RBC count as compared to healthy subjects, although. It may be due to chronic hemolytic process blood loss due to haematuria, Akinbani et al (2012)[6] reported that the rate of chronic hemolysis associated with sickle cell anemia patients could account for these decreased values, there is a also a blunted response to erythropoietin secretion in SCA, the rate of its increased secretion is proportional to the degree of anemia . This may be due to right shifted hemoglobin dissociation curve seen in sickle cell disease, the same result is reported by Akinsegun et al 2012[6], Sangeev Shyam et al 2012[7], The mean cell volume (MCV) and Packed cell volume /HCT are reduced in sickle cell anemia as compared to control subjects. This may

be due to effect of chronic anemia disease infection and hemolysis[6] (table no. 2).

The overall mean WBC count in our study ranges from 4600-12000. Low WBC count in some patients due to pain, nausea , vomiting which are reported to cause leucocytosis in the absence of infection. Leucocytosis in sickle cell anemia patients may also be due to splenectomy resulting from recurrent spleenic vessels, occlusion which make patients more vulnerable to overwhelming infection, particularly of encapsulated organism like streptococcus pneumonia and homophiles influenza . High leucocytes count is observed in sickle cell anemia patients as compared to control. Same is reported by Wood & Granger (2007)[8].

In herterozygous group hematological profile showed significant change in all parameters as compared to control group ($p<0.05$) but all parameter were within normal range in both sex this is same as reported by Mohsen et al (2000)[9] (table no.1).

Endothelial dysfunction in patients with SCD has been related to inflammation, high levels of production of ROS and reactive nitrogen species, and erythrocyte adhesion to blood vessel walls. There have been several studies showing that patients with SCD have a high level of oxidative damage, assessed through lipid peroxidation.

Contradictory reports are available regarding the levels of antioxidant enzyme in sickle cell disease. Our findings show significant impairment of glutathione system in homozygous (Hb S) and heterozygous group (Hb AS) subjects as compared to control group (table no. 3 & 4). Reduced glutathione (γ-L-glutamyl-L-cysteinyl glycine, GSH) is the most abundant thiol-containing peptide existing in most cell types, especially liver, spleen, kidneys, erythrocytes, and lens of the eye. It is the key molecule for maintenance of cellular redox because of its strong electron-donation potential via the sulfhydryl groups of cysteine residues[10]. Under oxidative stress, GSH donates reducing equivalents to free-radical scavenging enzymes, including glutathione peroxidase (GPx) and glutathione-S-transferase (GST), and converts to its oxidized form (GSSG). This GSSG can be reconverted to GSH by a reaction catalyzed by glutathione reductase (GR). Therefore, a lower ratio of reduced to oxidized glutathione (GSH/GSSG) may indicate higher oxidative stress in cells. In SCD, both group total glutathione levels and GSH/GSSH ratio are decreased.[11]

Glutathione Peroxidase levels in the body are in close relation with the glutathione which is the most important antioxidant present in the cytoplasm of the cells. The stability of the cellular and sub cellular membranes depends mainly on glutathione peroxidase and the protective antioxidant effect of glutathione peroxidase depends on the presence of selenium. Glutathione peroxidase (GPX) also protects the heart from damage by oxidative stress due to free radicals through its antioxidant effect[12]. Under normal condition low rate of production of H_2O_2 in RBC seems to be mainly GPx, but Catalase does make some contribution if intracellular concentration of H_2O_2 is raised. GPx activity is decreased in our study in both Hb S and Hb AS group (table no. 3 & 4). This is same as quoted by Alsultan et al 2010[13].

Human erythrocytes contain large amounts of Catalase enzyme. The Catalase and NADPH/GSH/GPx system is very important for disposal of H_2O_2 in human erythrocytes[14]. Catalase also regulate the intracellular reactive oxygen species by converting H_2O_2 to H_2O and O_2 in peroxisome. In our study we found in both group (II & III) the significant decrease in Catalase level (table no. 3 & 4).

Among the known antioxidative proteins, superoxide dismutase (SOD) is thought to play a central role because of its ability to scavenge superoxide anions. In homozygous group (group II) we observed significant increased of SOD as compared to control group (table no. 4). This is same as quoted by Gizi et al 2011[12]. This may be due to defence mechanism in response to oxidative stress. Schacter et al (1982)[15] reported a decrease SOD related to disease severity in SCD

patients. Titus J et al (2004)[4] observed increased SOD level in homozygous and heterozygous group of SCA. Increased level of SOD could be able to destimulate the increase flux of superoxide ions, exposing the sickle erythrocyte to high level of H_2O_2.

Oxidative stress is usually assessed in human by measuring Malondialdehyde (MDA) which is one of the end products of lipid peroxidation and is form by fatty acids with two or more double bond. MDA is a biomarker of damage caused by ROS derived from lipid peroxidation of membrane, its accumulation change the organization of membrane phospholipids contributing to the process of cellular degeneration[16]. In our study we measured lipid peroxidation end product i.e. Malondialdehyde (MDA) and found elevated level of MDA in both group i. e. Homozygous (Hb SS) and heterozygous (Hb AS) as compared to control subjects (table no.3 & 4), but in the Homozygous (Hb SS) group is more significant than heterozygous (Hb AS) group . These result are consistent with the study of Goswami et al 2011[17], Emokpae et al 2012[18].

Thus our study shows elevated plasma MDA and depleted antioxidant vitamins indicate oxidative stress in SCD. Therefore supplementation with antioxidants vitamin may ameliorate some of the sickle cell symptoms and improve quality of life.

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