Discussion

Sickle cell disease is genetic disorder of hemoglobin (Akinsegun A et al 2012). In India it is more common in central and southern region (Agrawal MB 2005). 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 (Titus J et al 2004). In sickle cell anemia glutamine substitutes valine at position 6th of beta hemoglobin chain. The substitution of glutamine, a positively charged amino acid for a neutral amino acid valine results in the formation of hemoglobin 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. Sickling of red cells leads to major consequences, which include chronic hemolytic anemia, occlusion of the small blood vessels result in ischemic tissue damage. The main clinical manifestation of SCA are infarction, Acute chest syndrome, splenic sequestration pain crisis, renal disorder, cardiac disorder, osteo- articular disorder, neurological disorder, ocular disorder and priapism (Manfredini et al 2008). The disease caused because of point mutation at position 6th in the beta globin gene that give rise to an abnormal hemoglobin molecule called Hb.
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. Hb S is more unstable than normal Hb because the former release high amounts of reactive oxygen species (ROS) ($O_2$, $H_2O_2$, $OH^-$) and has reduced antioxidant capacity. This imbalance leads to oxidative stress. In other words oxidative stress is defined as a result of imbalance between the oxidant and antioxidants in favor of the former. Increased production of oxidants and/or decreased availability of antioxidants triggers a cascade of oxidative reaction damaging lipids, proteins and DNA ultimately leading to (premature) cell death. Under normal condition there is balance between reactive oxygen species and defense system of antioxidants like Superoxide dismutase (SOD), Catalase (CAT), glutathione Peroxidase (GPx), Glutathione, flavonoids and carotenoids, thereby preventing or limiting oxidative damage (Halliwell B et al 1985).

In SCD, oxidative stress is increased and might play a significant role in pathophysiology of macrovascular dysfunction, vaso-occlusion and development of organ damage (Aslan M et al 2001, Klings Es et al 2001, Nath KA et al 2005, Morriis CR et al 5008, Nur E et al 2010). The high production rate of ROS in Sickle cell disease is caused by factor such as increased
in intravascular hemolysis, ischemia and chronic inflammation (Akohoue SA et

Therefore, this study has been aimed to know the status of various kind of
antioxidant system in homozygous and heterozygous group of sickle cell
anemia. The present study has been carried out in 300 subjects out of which
200 were sickle cell disorder and 100 were considered as healthy subjects.
The SCA group is divided into two group, Group II Sickle cell disease
(homozygous) and group III sickle cell trait (Heterozygous). We have selected
the patients in the age group between 10-40 years in both group. The blood
samples of all subjects were analyzed for exogenous and endogenous
antioxidants and hematological profile. The inflammatory marker CRP was
also measured. The blood samples were collected from the area prone for SCD
like Chhattisgarh and south region of Madhya Pradesh. In our study the
statistical data is shown in form of table. Table no. 1 & graph no. 1 showing
the age and BMI criteria of group I, II and III. Table no 2-7 and graph no. 2 - 7
depicting the overall status of hematological and biochemical profile of group
I, II and III. In our study we observed that in female subjects suffering from
sickle cell disease have low hemoglobin and RBC count as compared to male
subjects, although the difference is not statistically significant (p=NS). It may
be due to chronic hemolytic process blood loss due to haematuria, burden of
multiple pregnancies or nutritional deficiencies due to low socioeconomic
status. Akinbani et al (2012) 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 Mohsen A. F. EL-Hazmi et al 1991, A.V. Shrikhande et al 2003, Foluke Fasola et al 2006, Sagir et al 2006, Akinsegun et al 2012, Sangeev Shyam et al 2012, in sickle cell anemia patients. The RBC count is low in our study in female group as compared to male group. There is Rise in the MCV in female between 10-39 years of age but it is not significant. Normally sickle cell disease patients are at a critically balanced status of vit B-12 and folic acid so slight increased in demand due to increased need for erythropoisis because of chronic hemolysis or haematuria and pregnancy can precipitate deficiency state leading to macrocytosis (A. V. Shrikhande 2003).

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 (Akinbani et al 2012) (table no. 8, 9 & graph no. 8, 9). The reduction in values are more significant in females as compared to male subjects (table no. 8 & graph no. 8).
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 (Milhorat AT et al 1942). Leucocytosis in sickle cell anemia patients may also be due to splenectomy resulting from recurrent splenic vessels, occlusion which make patients more vulnerable to overwhelming infection, particularly of encapsulated organism like streptococcus pneumonia and homophiles influenza (Oloopoena L et al 1990). High leucocytes count is observed in sickle cell anemia patients as compared to control. Same is reported by Wood & Granger (2007). Increase leukocyte, which are potentially important source of ROS produces flux of superoxide anion in SCD. The contributions of leucocyte-derived ROS to the haemolysis associated with infections suggests that, like sickle RBC, leucocytes may serve both as targets of lipid peroxidation as well as haemoglobin oxidation and the oxidative environment in SCD (Agil A et al 2000, Benkerrou M et al 2002, Jison et al 2004) NADPH oxidase, the major superoxide producing enzyme in leucocytes, is a potentially noteworthy source of ROS in SCD (Haynes J et al 2002).

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) (table no. 10 & 11 graph no. 10, 11). The RBC breakdown in this group
is more in female as compared to male subjects (table no. 11 & graph no. 11). The pathology of sickle cell hemoglobin is considerably variable. In the heterozygous state, the presence of HbS in low concentration in red cells does not result in the sickling phenomenon under normal conditions and so the carriers of HbS are generally asymptomatic with normal hematological parameters values. The homozygous state has been defined as an incapacitating disease in which the hematological and several biochemical parameter values are abnormal. The clinical expression of HbS is reported to be variable even within the same population. It ranges from a severe disease with painful crisis and life threatening complications to a disease with mild symptoms. The effect on hematological and biochemical parameter values is also variable and is influenced by the severity of the disease and the occurrence of the crisis (Weatherall et al 1969, Serjeant GR 1974, Afonja OA 1982).

In Sickle cell disease (SCD) Chronic activation and damage of endothelial cells by sickle erythrocytes, heme, polymorphonuclear neutrophils (PMNs), and inflammatory mediators contribute to progressive microvascular damage in all organs, including the brain, lungs, and kidneys. Chronic oxidative stress constitutes a critical factor in endothelial dysfunction, inflammation, and multiple organ damage in SCD (Nur et al 2011). There are several causes of oxidative stress in SCD. Major sources of ROS in SCD are thought to be the (i)
enhanced rate of HbS auto-oxidation (Coletta M et al 1982, Habble et al 1988, sheng K et al 1998, Akohoue SA et al 2007) (ii) increased xanthine oxidase activity in SCD aortic endothelium, and (iii) higher number of leucocytes, which produce twice the fluxes of superoxide in SCD (Wood & Granger, 2007). 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.

The oxidative stress in the cell could be measured by knowing the status of Superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathione reductase (GR) and Glutathione (GSH) as endogenous antioxidants defense while Vitamin C, Vitamin E and Vitamin A (β Carotene) exogenous defense system. The lipid peroxidation is assessed by the end product of lipid peroxidation i.e. Malondialdehyde (MDA).

In our study we found that exogenous and endogenous both antioxidant defensive system i.e. Catalase, Glutathione Peroxidase, Glutathione reductase and Glutathione, vitamin E , vitamin C , vitamin A level decreased significantly except Superoxide dismutase in both sex (table no. 12,13,14 &15 , graph no. 12 (a,b,c)13 (a,b,C) 14 (a,b,c) 15 (a, b, c)), as compared to control group. The decrease is more in females as compared to male subjects of
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) (p<.001) and heterozygous group (Hb AS) (p<.05) subjects as compared to control group (table no. 12,13,14 & 15 graph no. 12 (a,b,c)13 (a,b,C) 14 (a,b,c) 15 (a, b, c)). 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 (Wu et al 2004). 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. Measurement of glutathione may provide a valuable marker of in vivo oxidative stress (Jones et al 2002, Asfaq et al 2006). A strong inverse correlation between plasma AGEs and GSH level in
sickle erythrocyte has been reported by Somjee et al (2005). This is consistent with the studies of Neil A et al 1983, Raj N et al 1983, Morris et al 2008, Gizi A wt al 2011, E, Stephan C et al 2013, Adelakun A 2014. Glutathione serves as an antioxidant by direct radical scavenging or participating in enzymatic reactions. Consequently, GSH is oxidized to GSSG, which can be converted back to GSH in a reaction catalyzed by NADPH-dependent GR (Kalpravidh et al 2013).

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 (Kassab et 2003, Bastawy et al 2013.). Under normal condition low rate of production of H$_2$O$_2$ in RBC seems to be mainly GPx, but Catalase does make some contribution if intracellular concentration of H$_2$O$_2$ is raised. GPx activity is decreased in our study in both Hb S and Hb AS group (table no. 12,13,14,&15 graph no. 12 (a,b,c)13 (a,b,C) 14 (a,b,c) 15 (a, b, c)). This is same as quoted by Natta et al 1990, Ren H et al 2008, Abiodum et al 2010, Suman et al 2013. Alsultan et al 2010 . The major
function of GPx may be the disposal of organic peroxidase rather than H$_2$O$_2$. GPx may protect both membrane and Hb from peroxidative damage. Human erythrocytes contain large amounts of Catalase enzyme. The Catalase and NADPH/GSH/GPx system is very important for disposal of H$_2$O$_2$ in human erythrocytes (Yoshihito Iuchi et al 2012 ). Catalase also regulate the intracellular reactive oxygen species by converting H$_2$O$_2$ to H$_2$O and O$_2$ in peroxisome (Amstad P et 1991). In our study we found in both group (II & III) the significant decrease in Catalase level (table no.12,13,14,&15 graph no. 12 (a,b,c)13 (a,b,C) 14 (a,b,c) 15 (a, b, c)). The SCA group showed more significant decrease (P<.001) as compared to the group III i.e. heterozygous (Hb AS) group (table no. 14 & 15 graph no. 14 &15 (a, b, c). A significant low Catalase also is reported by Das et al 1980, Aslan, M et al 2000, Morris et al 2008, Venusa et 2008, Goswami K et al 2011,. The H$_2$O$_2$ is produced either via two electron transfer or a result of sickling and is removed by GPx and Catalase, catalase is usually more important than GPx because of its ability to degrade H$_2$O$_2$ by consuming cellular reducing equivalent. There is a discrepancy in the value of CAT in SCD. Some studies have shown decrease CAT activity in SCD while Manfredini et al (2008) Das & Nair (1980) observed increase CAT level . Increase CAT level is protective effect while decrease levels might be due to overwhelming and chronic oxidative stress (Chirico et al 2012) in homozygous group.
Erythrocyte glutathione reductase is key enzyme in RBC metabolism. It is a flavonoids protein and its activity relies on sufficient flavin intake. Alleged GR deficiency are usually due to shortage of this cofactor and are easily corrected by supplementation of riboflavin in food (Schulz GT et al 1982, Z Wieten R.V. 2014). GR activity in SCA patients is also reduced (Verma et al 1983). This is consistent with our study. In our study we have also observed the decreased level in both Hb S and Hb AS group (table no. 12,13,14 & 15) but decreased is more significant in SCA subjects (group II) (P<.001) (table no 12, & 13). GR reduced the oxidized glutathione in RBC to reduced glutathione with concomitant oxidation of NADPH to NADP.

Fig 1: Role of EGR in red cell metabolism. (source: Verma et al 1983)
A decrease in GSH level in RBC resulting in decreased GR activity may impaired this process resulting in accumulation of NADPH which may bechanneled into superoxide pathway leading to deoxygenation of cell and increase H$_2$O$_2$ production. GSH/GSSG reaction may enhance the formation of Heinz bodies, which are also detected in sickle cell anemia. GR activity thus effect on polymerization of deoxy Hb S molecule or an inhibitory effect on mechanism that protect the cell from oxidative stress (Kalpravidh et al 2013).

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. 12,13,14 & 15 graph no. 12 (a,b,c)13 (a,b,C) 14 (a,b,c) 15 (a, b, c)). This is same as quoted by Das et al 1980, Beretta L 1983, Titus J et al 2004, Menfridini et al 2008, Gizi et al 2011, shiva DGH et al 2013. This may be due to defence mechanism in response to oxidative stress Schacter et al (1982) reported a decrease SOD related to disease severity in SCD patients. Titus J et al (2004) 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$_2$O$_2$. Further we have not observed any significant increase and decrease in antioxidant enzymes according to age.
and sex (table no. 17-23 graph no. 17-23 (a,b,c).

Apart from endogenous antioxidants vitamins A, C, E are known to be naturally occurring antioxidant that prevent peroxidation of lipid exposed to oxygen. Sickle cell disease is known to be associated with membrane lipid abnormally long chain unsaturated fatty acid are known to increase membrane fluidity, decrease adhesion and aggregation of RBC. They are mainly derived from food and other dietary source. Both Vitamin E and C have protective role against oxidative membrane attack, while caroteniods act at low oxygen tension (Mayes et al 2000).

In the present study we have considered role of vitamin E, C, A in sickle cell anemia and observed that all these vitamins levels decreased significantly in Group II and group III as compared to control group (table no. 12, 13, 14 & 15 graph no. 12 (a,b,c)13 (a,b,C) 14 (a,b,c) 15 (a, b, c)). This is same as quoted by Adelki D. A. et al 1989, Stone et 1990, Essein EU 1995, Charache et 1992, N.H. Jiya et al 2005, , Rana H.W. Hasanato 2006, Deves Ray et al 2006, Rain H et al 2008, Wali U et al 2013, John kennedy 2014, sumitra et al 2014.

Vitamin E is lipid soluble chain breaking antioxidant, which has protective role in almost all cells of the body. It scavenges free radical by its ability to transfer phenolic hydrogen to a peroxyl free radical of per oxidized polyunsaturated fatty acid. Thus, in view of the evidence of multiple membrane defects in this condition (Hebbel et al 1991), 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 (Wang et al 2004). Low hemoglobin level as found in the SCA subjects as a result of intravascular hemolysis is known to be a potent catalyst of lipid peroxidation. The plasma hemoglobin might, therefore catalyze the oxidative destruction of Tocopherol and account for the lower plasma Tocopherol levels found in these subjects (table no. 12,13,14 & 15 graph no. 12 (a,b,c)13 (a,b,C) 14 (a,b,c) 15 (a, b, c) (Natta et al 1979). The vitamin E level in homozygous cases is significantly decreased as compared to control while the heterozygous group shows less significant change as compared to control group (table no. 14 &15). It is observed that decrease in vitamin E level is not affected by age of the subjects (Debes ray et al 2006) (table no. 17-23 graph no. 17-23 (a,b,c)). Therefore, we can say that in presence of oxidative stress vitamin E get depleted in proportion to the severity of oxidative stress. We observed in our study that vitamin C level change was almost similar to that of vitamin E. This suggest that vitamin C is not involve in first line of antioxidant system but it might play a role as a replenishing agent for vitamin E (Packer et al 1979, Niki et al 1995).(Table no. 12, 13, 14,& 15 graph no. 12 (a,b,c)13 (a,b,C) 14 (a,b,c) 15 (a, b, c )). Vitamin C, a free radical scavenger, directly accepts electron from superoxide
hydroxyl anion as well as from various lipid hydroxyl peroxides. The lowered vitamin C level in sickle cell anemia patients indicates their exhausted status in an attempt to quench increased free radicals. Ascorbic acid (Vitamin C), an aqueous phase antioxidant, has excellent protective role in regeneration of the reduced form of other powerful antioxidants namely glutathione peroxide and Vitamin E and thus stops free radical chain reaction (Chiu et al 1982). As sickle cell anemia (SS) patients are more prone to haemolysis and have a greater propensity for the formation of superoxide and hydrogen peroxide, the vitamin C levels decreases and show a negative correlation with serum MDA level.

Vitamin A or the carotenoids also play a vital role as antioxidant. In our study we observed significantly low vitamin A (β Carotene) level in SCA group as compared to the sickle cell trait and control group (table no. 12-15). In SCA group due to redox imbalance the vitamin A level decrease because the sickle erythrocyte have been shown to susceptible to lipid peroxidation (Adelekan et al 1989).

The result of Vitamin A (β Carotene) almost same in homozygous , In heterozygous did not show any significant decrease. Sickling percentage is inversely correlated with plasma retinol level, resulting sickling influences the plasma retinol concentration in the body. This may be due to the fact that sickling cell more susceptible to oxidative stress, that require more amount of
vitamin A as an antioxidant to neutralize reactive oxygen species. (Schallji et al 2004. Deves et al 2007). Wali U et al reported significant lower level of vitamin A, E, C in sickle cell anemic subject than non sickling anemic subject. The decreased level of antioxidants vitamins are connected to increased oxidative stress in sickle cell anemic patients, resulting in higher utilization of these vitamins and consequently leading to their deficiency as sickle cell anemia is characterized by elevated level of oxidative stress via increased generation of reactive oxygen species (Ayub et al 2003). This result is also consistent with Rana et al (2006) and he suggested that deficiency of these antioxidants vitamin could for some of the observed manifestation of sickle cell disease such as increased susceptibility to infection and hemolysis (Esseien et al 1995) Nwaoguikpe et al 2012 assayed the antioxidant vitamin A, E & C and found these vitamin are potent inhibitor of sickle cell hemoglobin polymerization and equally improved the oxidant status of sickle erythrocytes.

So it is clearly evident that both homozygous as well as heterozygous are exposed to oxidative stress and this stress could be, to some extent, be relieved by supplementation these antioxidant vitamin in the dietary source.

In our study we also measured Total antioxidant capacity (TAC) in both cases i.e. homozygous (Hb SS) and heterozygous (Hb AS) and found significantly reduced level antioxidant in SCA patients as compared to control subjects.
This study consistent with the studies of Faluka et al 2007, Hundekar et al 2010 (table no. 12,13,14 &15 graph no. 12 (a,b,c)13 (a,b,C) 14 (a,b,c) 15 (a, b, c)). Total antioxidant capacity reflects the collective contribution to the reducing property of non protein antioxidant or electron donating component. Total antioxidant capacity (TAC) values are more informative than the knowledge of individual antioxidants. Reduced antioxidant defense in SCA both the enzymatic and low molecular weight antioxidant have been shown to have decrease activity and concentration in plasma (Das et al 1980, Jain et al 1990).

The Sickle cell anemia, produces greater amount of reactive oxygen species and has reduced antioxidant capacity this imbalance lead to oxidative stress. The increased production of ROS in SCD can be grossly amplified in response to a variety of path physiological condition including renal disorder, inflammation, immunologic disorder, hypoxia and deficiency of antioxidant enzyme (Klings et al 2001, Emokpae et al 2010).

Reactive oxygen species can cause significant damage to biomolecules since membrane lipids readily reacts with ROS resulting in lipid peroxidation. Lipid peroxidation is chain reaction producing a continuous supply of free radicals that initiate further peroxidation. It 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 (Hundekar et al 2010). 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.12,13,14 & 15 graph no. 12 (a,b,c) 13 (a,b,C) 14 (a,b,c) 15 (a, b, c)), but in the Homozygous (Hb SS) group is more significant (p<.001) than heterozygous (Hb AS) group (p<.05). These result are consistent with the study of Vanusa et al 2008, Abiodum et al 2010, Emokpae et al 2010, Hundekar et al 2010, Goswami et al 2011, Emokpae et al 2012, Sumitra et al 2014.

The Sickle cell disease is characterized by chronic inflammatory state (Yung et al 1998, Hebble et al 2004), patients exhibit elevated level of leucocyte count, abnormal activation of granulocytes, monocyte, endothelial cell (Solovey et al 1997, Inwald et al 2000, Belchel et al 2000) and increased level of multiple inflammatory mediators. Therefore in our study we analyze C-reactive protein, a inflammatory marker. C – reactive protein (CRP) is an important acute phase protein which increase in various inflammatory and neoplastic condition. In this our study we observed that elevated level of CRP in sickle cell anemia subjects as compared to control group. The CRP
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level was more significantly higher in the Homozygous (Hb SS) group than heterozygous (Hb AS) group. This is consistent with the studies of result Supported by Schnog, et al. 2004, Kenneth et al. 2008, Subha et al. 2009, Abiodum et al. 2010, Sanjana Bhagat et al. 2012, Kibileri et al. 2012 (table no. 12, 13, 14 & 15 graph no. 12 (a, b, c) 13 (a, b, c) 14 (a, b, c) 15 (a, b, c)). It is an abnormal specific glycoprotein produced by liver and excreted into blood stream during the acute phase inflammation (Dehgher et al. 2008). The determination of CRP is clinically useful for screening for organic disease, assessment of activity of inflammatory disease. Circulating CRP levels are determined solely by its rate of synthesis and thus reflect the presence of and strength of pathological stimuli that are present in the individual (Vigushin et al. 1993). Elevation of CRP that are associated with acute infection and inflammation. CRP concentration generally stable falling within a characteristic range for each individual (Macy et al. 1997, Horschfield 2003). Elevated level of CRP as a general marker of inflammation have been previously reported in patients with SCD (Platt et al. 2000, Schnog et al. 2004, Mohammedd et al. 2010). Higher level of CRP in SCA indicate a covert inflammatory response, despite the absence of crisis. Kibileri et al. (2012) also reported the relationship between systemic inflammation and sickle cell disease in children and aldoscent.

Further, to confirm the study on the role of exogenous and endogenous antioxidant on homozygous and heterozygous sickle cell anemia we made
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some correlative study. Very few scientists have been quoted correlated studies in SCA group. Total antioxidant capacity (TAC), which is reflection of all antioxidants activity showed positive correlation with all antioxidants except SOD, elevation of SOD may increase in response to oxidative stress or its more expression due to $\text{H}_2\text{O}_2$ production in cell (table no. 24a & 24b). Similarly CRP is correlated positively with neutrophil and WBC count (Table no. 26). It is also related with low hemoglobin level, high CRP suggesting systemic inflammation may play important role in pathophysiology of lung disease in SCA group subjects. This relationship support the hypothesis that the steady state Hemoglobin may be considered a surrogate marker of severity of sickle cell disease in population (table no. 26) (Willium K et al 2012).

Exogenous and Endogenous antioxidants are also positively correlated with MDA, suggesting of increased lipid peroxidation and increase oxidative stress in SCA subjects Kalyan et al 2010, Sumitra et al 2014) (Table no. 25a & 25 b). The role of these antioxidants is more suggestive of oxidative stress in SCA group than trait sickle group (table no. 28 & 29).

It is therefore, concluded that SCA is also a chronic inflammatory disorder and decrease exogenous and endogenous antioxidant are suggestive of oxidative stress in sickle cell disorder.