Sickle cell disease (SCD) is clinically one of the most important haemoglobinopathies. It is characterised by haemolytic anaemia, an increased susceptibility to infections and vaso-occlusion that occurs in almost all vascular beds leading to ischaemic tissue injury with organ dysfunction and early death. Outcome is difficult to predict, and few effective therapeutics are available. The most important protein of red blood cells (RBCs) is haemoglobin, which consists of four globin chains, each folded around a haem molecule. Haemoglobin delivers oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs. The predominant haemoglobin in adulthood is HbA (±97%), which consists of two α and two β globin chains (α 2 β 2). Other haemoglobins are HbA2 (2% to 3.5%; α 2 δ2) and HbF (<2%; α2γ2). During intrauterine development, several globin chains are synthesised (α, β, γ, δ, ε, and ζ), with the predominant haemoglobin type during foetal life being HbF. In the first 12 weeks after birth, the HbF% quickly declines, leaving HbA and HbA2 as the remaining haemoglobins. The β globin gene is found on chromosome 11. A single point mutation in the 6th codon leads to substitution of glutamic acid for valine, resulting in an abnormal globin: βS. This results in the formation of ‘sickle haemoglobin’, or HbS (α2βS2). Upon deoxygenation, βS forms hydrophobic interactions with adjacent βS globins, ultimately resulting in the polymerisation of HbS. This is the molecular hallmark of SCD.

Sickle-cell anaemia covers a wide spectrum of illness. Most affected people have chronic anaemia with a haemoglobin concentration of around 8 g/dl. The main problems arise from the tendency of the red blood cells to become sickle-shaped and block capillaries at low oxygen tension. In children, sickle-shaped red blood cells often become trapped in the spleen, leading to a serious risk of death before the age of seven years from a sudden profound anaemia associated with rapid splenic enlargement or because lack of splenic function permits an overwhelming infection. Between 6 and 18 months of age affected children most often present with painful swelling of the hands and/or feet (hand-foot syndrome). Survivors may also suffer
recurrent and unpredictable severe painful crises, as well as "acute chest syndrome" (pneumonia or pulmonary infarction), bone or joint necrosis, priapism or renal failure. For most patients the incidence of complications can be reduced by simple protective measures such as prophylactic administration of penicillin in childhood, avoiding excessive heat or cold and dehydration, and contact as early as possible with a specialist centre. These precautions are most effective if susceptible infants are identified at birth. Some patients have such severe problems that they need regular blood transfusion and iron-chelation therapy.

6.1. Hematological Manifestations of Disease: The newborn with sickle cell anemia has a normal gestational age specific haematological profile. Their red cell resist sickling under prolonged laboratory induced hypoxia because of the high HbF concentration. Hemoglobin level and red cell indices are normal during the first 60 days of life. The hemoglobin concentration then declines steadily until six months of age to plateau at a median hemoglobin concentration of 7.5 g/dL (±2 SD). Increased anisocytosis and poikilocytosis are seen by six month of age, but rarely are irreversibly sickled forms observed until after the first year of life when hyposplenism develops. During the first decade, hemoglobin concentration and mean corpuscular volume are maintained at the constant level without much upward drift. Mean reticulocytes counts are 10 to 20 percent. The total leukocyte count, absolute granulocyte count absolute lymphocyte count and absolute monocyte count are normal in newborns with SCA but increased in patients beyond this age. By six months of age, there is an across the board elevation in all components of the leukocyte count. Median steady-state leukocyte counts are highest during the first 5 years of life and average between 13,000 to 18,000 cells/mm³, declining slightly afterwards. Circulating platelets number are elevated after the onset of splenic hypofunction and autosplenectomy. During the steady state, a consistently elevated platelets count above 350,000/mm³ may mark the patient who has a higher risk of sickle vasoocclusive episodes and, particularly, of childhood stroke.

The rapid postnatal decrease in Hb F coincides with the physiological hypoproduction of erythrocytes that occurs between days 14 and 40 of post natal life. The decline is dependent, in part, on the particular β globin gene cluster.
haplootype inherited. The HbF decent in normal infants is compared with the HbF decline in sickle cell anaemia among Jamaicans. The sickle cell anemia infants continuous to produce more HbF than normal infants. Steady-state HbF is not attained for several years$^{217}$.

In chronic haemolytic anaemias, temporary cessation of erythropoiesis leads to severe anaemia (known as aplastic crises), in which sickle-cell disease is no exception. Although most individuals spontaneously recover in a few days, the anaemia can be so severe that it causes cardiac decompensation, with rare deaths (if anaemia and reticulocytopenia are unrecognised and untreated). Parvovirus B19 infection is responsible for most cases. Cytotoxicity of erythroid precursors by the virus accounts for aplasia, with reticulocytopenia lasting for 7–10 days.

Aplastic crisis is a self limiting condition$^{218,219}$. The timing of haematological investigations is critical in making a diagnosis. Some patients might have been missed due to late presentation to the hospital. In this study, some patients presented as late as eight days after the onset of illness. It is therefore possible that these patients who were severely anaemic at presentation but not significantly jaundiced may have been in the early recovery stage of aplastic crises.

Sickle cell anaemia is one of the variants of disorders of haemoglobin synthesis inherited from both parents in an autosomal recessive fashion. It is characterized by chronic anaemia and/or hand foot syndrome in the first few years of life. This anaemia is exacerbated during periods of rapid red blood cell destruction, failure of the erythroid cell line in the bone marrow, or in acute sequestration episodes. Four major types of crises are recognised in sickle cell anaemia: aplastic, acute sequestration, hyper-haemolytic, and vaso-occlusive crises. Hyper-haemolytic crises are less commonly reported in literature from the temperate climates. This continues to be a major problem among patients with sickle cell anaemia in tribal population of Madhya Pradesh & Chhattisgarh where the natural history of the disease is somewhat complicated with recurrent episodes of malarial infection. Apart from the vaso-occlusive crises, the two listed in this study could lead to severe anaemia in patients with sickle cell anaemia. In Nigeria, the average haemoglobin concentration in patients with sickle cell anaemia is 72 g/l. This study was designed to evaluated the.
difference if any in biochemical indices in anaemic crises in patients with sickle cell anaemia. Despite great studies that have been made in the understanding and management of SCD, the disease and its complications are increasing unmitigated.

Blood biochemistry has significant effect on pathophysiology of human body. Recently few studies found the association of biochemical abnormalities in sickle cell patients. Sickle cell disease showed clinical variability where African ancestors have severe phenotype than Indian sicklers.

6.2. Assessment of Hepatic Biochemical Indices In SCD with various anaemic crises

The clinical manifestation of sickle cell anaemia in India seems to be milder than in Africa and Jamaica\textsuperscript{20}. The clinical spectrum of SCD ranges from mild to severe liver function and clinical crises with marked hyperbilirubinemia and liver failure. Multiple factors may contribute to the aetiology of the liver disease, including ischemia, transfusion related viral hepatitis, iron overload, and gallstones\textsuperscript{207}.

Liver abnormality release aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which makes useful test for detecting liver damage. Hemolysis also raises SGOT and SGPT levels in SCD. Johnson et al\textsuperscript{138} and Nsiah K\textsuperscript{208} reported higher activity of SGOT and SGPT in sickle hepatopathy with various crises associated with sickle cell disease. In the present study levels of SGPT and SGOT were found higher in haemolytic crisis when compared to other three groups (I, III & IV, mean dif. With SGPT 124.12, 67.70,112.54, p<0.001, mean dif. With SGOT 69.23, 26.45, & 59.23, p < 0.001). The value of SGPT and SGOT were also significantly increased in group III when compared with group IV (SGPT mean dif. 44.88, p <0.001, SGOT mean dif. 32.78, p < 0.001). Level of ALP also found highly significant in group – II when compared to other groups except group III (p <0.05). Oparinde DP et al (2006) conducted biochemical assessment regarding severity of sickle cell anemia with reference to role of hepatic enzyme and found that a significant increase in serum ALT, ALP and GGT levels in SCA with persistent hepatomegaly over those without hepatomegaly (p < 0.05, p < 0.05 and p < 0.01 respectively). All the index scores and the final aggregate severity scores were also
significantly higher in SCA subjects with persistent hepatomegaly. Only GGT demonstrated a fairly positive and significant correlation \((r = 0.46, P < 0.05)\) with increased clinical severity among the hepatic enzymes.\(^{154}\) Yahaya et al\(^ {151}\) conducted a study in which activities of alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were significantly higher \((P<0.05)\) in the HbSS patients than the control subjects. This study made a conclusion that level of total protein and albumin is not very much altered in sickle cell crises. Our study is also in agreement with above study although we found statistically significant difference between group I and Group II as the level of total protein and albumin fall higher side of the normal reference interval. However in present study group III shows highly significant mean difference when compared to group I, it may be due to the underlying cause of aplastic crisis in SCD is parvovirus – B19 infection, induced over production of antibody. Statistically insignificant difference of albumin in above group supports this study. Same findings were also reported by U. P. ISICHEF\(^ {206}\). The protein patterns seen in his study are interesting in many respects. A comparison of the serum protein values shows definite evidence of relative hyperproteinaemia as well as hyperglobulinaemia in sickle cell disease. Whereas the albumin levels were almost the same in all groups, the globulin values in sicklers were significantly greater than in normal children of the same age, showing that the globulin fraction is largely accountable for the high total protein.

Marked hyperbilirubinemia of up to 57 mg/dL, in most cases predominantly conjugated with only a mild elevation in ALT levels, has been described by Buchanan et al.\(^ {209}\) in 6 children with minimal or no symptoms. In the present study we found highly significant Bilirubin mean differences among all the groups. Similar pattern was also observed by Ebert EC et al\(^ {153}\) reported the most common laboratory abnormality is an elevation of unconjugated bilirubin level. Bilirubin and lactate dehydrogenase levels correlate with one another, suggesting that chronic hemolysis and ineffective erythropoiesis, rather than liver disease, are the sources of hyperbilirubinemia. Abdominal pain is very common in SCD and is usually due to sickling Even in the steady state Bilirubin is significantly high with the asymptomatic jaundice. In the present study
The total serum bilirubin concentration was also significantly increased in haemolytic and aplastic crisis (mean 18.95, 2.51) above the steady state level. Bilirubin concentration in Haemolytic crisis is much more higher when compare to other groups, is due to access hemolysis of RBC in the crisis which is not present up to that extent in aplastic and steady state SCD. Ojuwa et al\textsuperscript{156} studied thirty children with SCA and assayed serum alanine aminotransferase, alkaline phosphatase, total protein, albumin and bilirubin, during vaso-occlusive crisis and at recovery. Alanine aminotransferase, alkaline phosphatase and bilirubin levels were significantly higher during crisis than at recovery, (p < 0.005) especially in the young patient. However, the total protein and albumin levels were not significantly different in crisis and at recovery. A transient hepatic functional derangement during vaso-occlusive crisis is a probable explanation for the reported changes.\textsuperscript{156} In aplastic crisis no any significantly change was observed in Unconjugated bilirubin level.

Bone changes are common in SCD but the pathogenesis is not fully understood\textsuperscript{210}. The level of alkaline phosphatase indicates severity of bone damage and is a useful guide of progress in the management of bone pains in sickle cell anaemia\textsuperscript{211}. Bone disease with osteoporosis and osteomalacia are common in SCD. Some patients have vitamin D deficiency and low bone mineral density. Delayed growth and bone destruction may contribute to the elevated levels of alkaline phosphatase. Higher levels of alkaline phosphatase may be due to associated vasoocclusive crises involving the bones rather than pathology of the liver\textsuperscript{155, 212}. The level of the heat-labile alkaline phosphatase indicates severity of bone damage and is a useful guide of progress in the management of bone pains in sickle cell anaemia\textsuperscript{211, 210}. In the present study we found highly significant level of alkaline phosphatase in all crises present in SCD when compare with normal (p <0.001), which is similar to a study conducted by Isichei UP\textsuperscript{157}. Brody JI et al\textsuperscript{158} studied behavior of ALP in sickle cell anemia patients and found Physical and biochemical criteria identified bone alkaline phosphatase as the principal, although not necessarily the sole, enzyme fraction that increases during symptomatic sickle cell crises. Moreover, there appeared to be concordance between crisis severity, serum levels of alkaline phosphatase, and
isoenzyme patterns; electrophoretic and biochemical abnormalities could be detected even when the patients were asymptomatic. GGT is another useful enzyme to assess hepatic function in SCD. In our study we found statistically highly significant raised level of GGT among all the group when compared to normal (p <0.001, Group IV/I p <0.05). similar findings were obtained by Oparinde DP et al[154] concluded Only GGT demonstrated a fairly positive and significant correlation (r = 0.46, P < 0.05) with increased clinical severity among the hepatic enzymes.

The present data suggest that the serum alkaline phosphatase level may be an additional indicator of the degree, frequency, and persistence of tissue injuries that occur in sickle cell anemia.

6.3. Assessment of renal status: Patients with sickle cell anemia or sickle cell trait may present several types of renal dysfunction[220]. The GFR in homozygous SCD is supra-normal in childhood but falls steeply with age, often culminating in renal failure[221]. In our study blood urea was low in sickle cell patient in compression to controls. The mean value of urea was 28.43 ± 9.33, 21.16 ± 5.37 and 28.65 ± 7.27 in Group – II, Group – III and Group IV respectively. Mean difference between Group II Vs III, Group – III Vs I and Group III Vs IV were highly significant. Creatinine levels were similar in the sickle homozygous with crises patients as well as controls . The creatinine level was not statistically significant (P value > 0.05). The value of creatinine was 0.95 ± 0.27, 0.96 ± 0.23 and 1.01 ± 0.22 is Group II, III and IV respectively. Similarly few studies eg. Vernar KJ[294], Marouf R et al[259] Al Naama LM et al[189] report the considerably lower urea and creatinine in the SCD patients. These observations suggest the renal insufficiency is uncommon in Indian sicklers.

6.4. Trace Elements :- Trace elements are essential inorganic molecules found in minute quantities of milligram or microgram per kilogram of body weight. Trace elements include zinc, copper, selenium, manganese, chromium, magnesium, fluorine, cobalt, iron and iodine. People with sickle cell disease suffer from many micronutrient deficiency but preliminary research on dietary habits show that food and nutrient intake by sickle cell patients meet or exceeds recommendation and is not significantly
different from healthy controls. This suggests that higher rates of nutrient deficiency may be due to increased needs of many nutrients in sickle cell patients. The global use of micronutrients in health care delivery system has taken central stage due to the realization of their importance in disease management. Free radicals are generated in sickle cell disease, hence a balance between minerals and antioxidants is imperative in maintaining red cell membrane integrity and function. Protection of red cell membrane from free radical mediated oxidative stress is crucial to the management of sickle cell disease. Minerals such as copper, zinc, iron, chromium, magnesium, selenium, vanadium as well as vitamins like vitamin A, C, E, folate and vitamin B complex have been found to relieve oxidative stress associated with red cell membranes. Attempts have been made over a number of years, to treat the disease by modifying the hemoglobin S molecule so as to suppress the sickling process, but in recent years, trace element metabolism have been receiving increasing attention in sickle cell anemia research and attempts have been made to relate the concentration of the elements to the sickling process.

A significantly low concentration of serum zinc were obtained in the present study from the comparison of SCD with various anemic crises and normal control subjects (P < 0.05). In a study conducted by Keen and Gershwin, low concentration of red blood cell Zinc has been recorded in patients with sickle cell disease. This in turn is likely to contribute to recurrent infection resulting from poor immune system, growth retardation, hypogonadism in males, hyperammonemia, abnormal dark adaptation and a concomitant increase in other symptoms of sickle cell disease. Significantly low serum Zinc obtained in this study may also contribute to the low red cell Zinc level which is in agreement with the study of Keen and Gershwin (1990).

Zinc deficiency can also be the result of the adverse effect of hydroxyurea which increases Zinc excretion as reported by Sindel et al. A statistically significant decrease in the concentration of Zn was found in the whole blood, erythrocytes and plasma of the sickle cell anemia subjects as compared with the controls. Prasad, et al. also found that the excretion of Zn in the urine of the sickle cell anemia subjects was higher than that of the controls suggesting that increased loss of Zn in hyperzincuria may be one of the mechanisms by which the
sickle cell anemia subjects become Zn deficient. Ojo and Oluwole\textsuperscript{227} also observed that there was mild Zn deficiency, more seriously in males, in the study when they determined the trace elements in whole blood, erythrocytes, plasma, head, hair, and nail of sickle cell anemia patients and compared them with identical samples from normal control subjects. A significantly low concentration of serum selenium and zinc were also obtained from the comparison of sickle cell homozygous subjects and normal control subjects (P < 0.05), but no significant difference was observed in the serum copper and magnesium concentrations in both groups (P > 0.05).

In the present study we found low iron concentration in blood. (p < 0.001) which is in positive agreement with other studies Rao NJ et al\textsuperscript{233} & Mohanty D\textsuperscript{234} et al. This difference in iron level could be attributed to the difference in environment, ie developing versus developed country and one could deduce that lower socioeconomic status in developing countries may be associated with a lower dietary iron intake. Indeed, iron deficiency is less of a problem in sickle cell disease in the United States; iron excess is more often observed as many more patients receive chronic blood transfusion here than in other countries.\textsuperscript{235} However, there is concern about the finding that high transfusion volume is more significantly associated with hepatic iron overload than serum iron markers\textsuperscript{235}.

Several studies were reported increased copper level in sickle cell disease, similarly in the present study we found statistically higher serum copper level (mean 71.65, 73.13 & 66.58 Group II, III and IV respectively) when compared to control subjects (mean 65.63, p < 0.001 when compared with II & III group, p > 0.05 when compared with group IV). Behera SK et al\textsuperscript{236}, Pellegrini BJA et al\textsuperscript{237} and Akenami FO et al\textsuperscript{238} also reported similar findings.

In erythrocyte copper is either normal\textsuperscript{239} or increased.\textsuperscript{240} The clinical significance of this elevation in plasma copper is unclear, but it has been reported to occur in the event of decreased plasma zinc levels.\textsuperscript{237-238} Prasad et al observed decreased plasma copper levels in a patient who was receiving zinc as an anti-sickling agent,\textsuperscript{311} albeit with some hematologic consequences (microcytosis, and relative neutropenia) which were easily corrected with copper supplementation. In addition they reported decreased ceruloplasmin levels, also reversed by the copper
supplementation. The findings of low circulating zinc and concomitant high circulating copper levels have been consistent in patients with severe HbSS\textsuperscript{241}. Copper excess may be contributing to free radical production and oxidative damage in HbSS\textsuperscript{242}. These data suggest the need for a delicate balance between zinc and copper supplementation in general and for patients with severe HbSS in particular.\textsuperscript{241}

6.5. Inflammatory Markers: In the present study Proinflammatory cytokine levels in serum, namely, IL-6, TNF-α, and CRP as a inflammatory marker measured in normal and sickle cell disease patients. We found significantly increased mean level of all three parameters when compared to control with the sub groups (IL-6 Group I V/s Group II p < 0.001, Group I V/s Group III p < 0.001, Group I v/s Group IV p < 0.05, TNF-α Group I V/s Group II p < 0.001, Group I V/s Group III p < 0.05, CRP Group I V/s Group II p < 0.001, Group I V/s Group III p < 0.001).

Crpziat et al\textsuperscript{243}, Taylor SC et al\textsuperscript{244}, Bourantas KL\textsuperscript{245} Raghupathy R et al\textsuperscript{246} and many other studies have shown increased levels of cytokines in serum even during the steady state of SCD. It is believed that there are significant subclinical microvascular occlusions in steady state due to ongoing local tissue ischemia with necrosis. These subclinical micro-infarctions are induced by the enhanced adhesiveness of sickle reticulocytes and reversibly sickled erythrocytes to the vascular endothelium\textsuperscript{247}, along with the associated chronic endothelial activation and injury and by the production of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α) by activated endothelial cells. The degree of stimulation and production of cytokines is not high enough to trigger clinically evident vaso-occlusions in the steady state. However, this balance can be very easily tilted and additional small insults may be enough to precipitate a crisis.

It is now clear that local patterns of cytokine and hormone expression regulate lymphocyte effector mechanisms\textsuperscript{248}. Th2 clones typically produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. They provide optimal help for humoral immune responses, including IgG1 and IgE switching and mucosal immunity, stimulation of mast cells, eosinophil growth and differentiation, and IgA synthesis. Thus, Th1 cytokines are associated with cell-mediated inflammatory responses, whereas Th2 cytokines are associated with strong antibody and allergic responses. Cytokines from Th1 cells
inhibit the actions of Th2 cells and vice-versa, thus an immune response becomes polarized to a predominant Th1 or Th2 type.

Cytokines like IL-6 and TNF-α liberated from the vaso-occluded area stimulate production of acute-phase proteins by the liver. It is postulated that the rise in the acute-phase proteins minimizes tissue damage caused by the microvascular infarctions. Increased levels of acute-phase proteins in plasma have been reported even during the steady state of SCD. In our study, we observed that CRP was high in steady state and showed a further considerable increase during various crises state. Serum levels of such proteins increase when the inflammatory stimulus is of low-grade intensity and of short duration, such as the subclinical microvascular occlusions in steady state. This process is further augmented in crisis.

Cytokines, such as IL-1β, IL-6, IL-8, and TNF-α, produced during infection or any other inflammatory stress can also stimulate vascular endothelium, induce the adhesion of red cells to endothelium, and lead to painful vaso-occlusive crises. In one of our study group - III, acute crisis was associated with a viral infection. Thus, elevated levels of proinflammatory cytokines like TNF-α, IL-6, and IL-8 may be related, in this group of patients, to an anti-infectious response.

In the positive agreement with Lowenthal et al, the present study we also found significantly increased level of homocystein when compared to control subject. It is known that patients with sickle cell disease present activation of the blood coagulation and fibrinolytic systems, especially during vaso-occlusive crises, but also during the steady state of the disease. Due to decreased erythrocyte half-life, individuals with homozygous sickle cell disease have increased erythropoietic demands for folate. Subjects with inadequate folate status might be expected to manifest higher blood levels of homocysteine. It is possible therefore that raised homocysteine levels in SS disease predispose to the development of thrombosis through inhibition of the protein C anticoagulant pathway. The significance, interactions, and sources of coagulation abnormalities and their relationship to clinical severity and painful episodes in sickle cell disease are not clear.