

Full Length Research Paper

Biochemical and hematological diagnostic indices of homozygous sickle cell anemia patients in the steady state

^{1*}Obimba Kelechukwu Clarence, ²Eziuzor, Samuel Chukwunyelum, ³Ozougwu, Jervas Chibuike, ⁴Ihedimbu, Chiamaka Perpetua

^{1,4}Department of Biochemistry, School of Biological Sciences, Federal University of Technology, Owerri, Nigeria.

^{2,3}Department of Biological Sciences, College of Basic and Applied Sciences, Rhema University Aba, Abia State, Nigeria.

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The aim of the study was to investigate the efficacy of the use of some biochemical and hematological clinical indices as diagnostic parameters essential to the treatment and management of homozygous sickle cell anemia in the steady state. Twenty (20) healthy, homozygous AA human subjects and twenty (20) homozygous sickle cell anemia patients in the steady state were subjected to Hemoglobin genotype, packed cell volume (PCV%), white blood cell total (WBC_{Total}), aspartate amino transferase (AST), serum alkaline phosphatase (AP), serum triglyceride (TG), serum total cholesterol (C), Total bilirubin (Tb), and Random blood glucose (RBG) analyses. The experimental design is a single factor completely randomized design (CRD). Results recorded of the healthy human subjects and sickle cell anemia patients, expressed as mean \pm standard error (S.E) (unit) were as follows : (PCV%) 45 ± 0.1 and 23.87 ± 0.2 , (WBC_{Total}) 5.17 ± 3.94 and 11.27 ± 3.94 ($\times 10^3/\mu\text{l}$), (AST) 9.18 ± 1.16 and 19.5 ± 2.16 (U/l), (AP) 140 ± 1.2 and 215.2 ± 1.3 (IU/L), (TG) 75 ± 1 and 117.87 ± 1.2 (mg/dl), (C) 185 ± 2.3 and 140.5 ± 1.2 (mg/dl), (Tb) 0.7 ± 0.1 and 2.8 ± 0.2 (mg%), (RBG) 82.18 ± 4.16 and 90.87 ± 4.23 (mg/dl), respectively. The mean values of WBC_{Total}, AST, AP, TG and RBG were significantly higher ($p < 0.05$) : but those of PCV% and C were significantly lower ($p < 0.05$) in sickle cell anemia patients compared with healthy human subjects. Incidence of homozygous sickle cell anemia in the steady state correlated positively and significantly ($p < 0.05$) with significant increase ($p < 0.05$) in WBC_{Total}, AST, AP, TG and RBG; and significant decrease ($p < 0.05$) in PCV% and C. The statistical regression and correlation between serum triglyceride and PCV% of the patients were significant ($p < 0.05$), $r = 0.983$. Observed values of PCV% could be used with high precision to predict serum triglyceride levels of homozygous sickle cell anemia patients in the steady state.

Key words: Homozygous, hemoglobin genotype, random blood glucose.

INTRODUCTION

Sickle-cell disease (SCD), also known as sickle-cell anaemia (SCA) and drepanocytosis, is a hereditary blood disorder, characterized by an abnormality in the oxygen-

carrying haemoglobin molecule in red blood cells. The cells assume an abnormal, rigid, sickle-like shape under certain circumstances. Sickle-cell disease is associated with severe infections, attacks of severe pain ("sickle-cell crisis"), and stroke, and there is an increased risk of death (Yawn *et al.*, 2014).

Almost 300,000 children are born with a form of sickle-cell disease every year, mostly in sub-Saharan Africa, but also in other parts of the world such as the West Indies and in people of African origin elsewhere in the world. In 2013 it resulted in 176,000 deaths up from 113,000 deaths in 1990 [Roberts and De Montalembert (2007); Wellem's *et al.* (2009)].

Sickle-cell crisis describe several independent acute conditions occurring in patients with SCD, and are : vaso-occlusive crisis, aplastic crisis, sequestration crisis, haemolytic crisis, and others. The vaso-occlusive crisis is caused by sickle-shaped red blood cells that obstruct capillaries and restrict blood flow to an organ resulting in ischaemia, pain, necrosis, and often organ damage. Aplastic crises describe acute worsenings of the patient's baseline anaemia, characterized by pale appearance, fast heart rate, and fatigue. Splenic sequestration describes infarction of the spleen before the end of childhood in individuals suffering from sickle-cell anemia, and increases the risk of infection from encapsulated organisms. Conversely, the steady state in sickle cell anemia disease is defined as that period when the patient with sickle cell anaemia is free of infection, pain, or other disease processes (Juwah *et al.*, 2004). Preventive antibiotics and vaccinations are recommended for those lacking proper spleen function. Haemolytic crises are acute accelerated drops in haemoglobin level resulting from increased rate of apoptosis of red blood cells. Dactylitis, and acute chest syndrome are other manifestations of sickle cell crisis.

Sickle cell red blood cells are less favourable for the growth of malaria than normal red blood cells (Lehninger, 1982). This finding is contrary to the opinion that sickle-cell anemia patients are more vulnerable to malaria for which it has become imperative that people with sickle-cell disease, living in malarial countries, should receive anti-malarial chemoprophylaxis for life (Oniyangi and Omari, 2006). Electron microscope image of sickle red blood cell (left) and normal blood cells is shown in figure 1.

Vessel occlusion and ischaemia caused by decrease in sickle blood cell elasticity and damage to the cell membrane are central to the pathophysiology of sickle cell anemia (Maakaron, 2015).

Point (missense) mutation in the β -chain gene that codes for glutamic acid at the sixth position in each of the two normal β -chains of hemoglobin A, results in the coding of a valine residue at the sixth position in hemoglobin S leading to the loss of one negative charge in each of the two β -chains of hemoglobin S (Lehninger, 1982). Sickle-cell conditions have an autosomal recessive pattern of inheritance from parents (Green *et al.*, 1993).

Children who are sickle-cell disease patients take a 1 mg dose of folic acid daily for life and penicillin daily due to the immature immune system that makes them more prone to early childhood illnesses.

Pain management medications are used as remedies for vaso-occlusive pain crises. Opioids such as NSAIDs (e.g diclofenac or naproxen) are used to manage milder pain crises. More severe pain crises require use of intravenous opioids e.g patient-controlled analgesia (PCA). Diphenhydramine is administered to help control itching associated with the use of opioids (Yawn *et al.*, 2014).

Acute chest crisis is treated in a similar manner as vaso-occlusive pain crises with the addition of antibiotics because cell wall-deficient bacteria are thought to contribute to the sickle-cell disease. Oxygen supplementation is used for the treatment of hypoxia. Simple blood transfusion or exchange transfusion (exchange of a significant portion of the patients red blood cell for normal red cells, which decreases the percent of haemoglobin S in the patient's blood), may also be necessary medications. Hydroxyurea was shown to decrease the number and severity of sickle-cell disease crisis (Charache *et al.*, 1995).

Electrolytes, hepatic enzymes, alkaline phosphatase and glucose were elevated and statistically significant ($P < 0.05$) in patients suffering from sickle cell anemia (Pandey *et al.*, 2012). Whether in steady state or in crisis, provided hepatic and cardiac integrity has not been compromised, Subjects with Sickle cell disease, in steady state or in crisis, would have higher AST levels due to hemolysis (Nsiah *et al.*, 2011).

The short life span red blood cells in sickle cell anemia presents the liver with an augmented load of bilirubin for hepatic clearance (Maddrey *et al.*, 1978). High levels of bilirubin could indicate blood-related diseases such as sickle cell anemia (Hargrove, 1970).

Some of the observed lesions of SCD are due to the alteration of plasma hexose sugar levels (Osugwu and Mbeyi, 2007). Sickle cell disease can affect the skeletal system due to accelerated hematopoiesis and bone infarction (Nelson *et al.*, 2003). The concentration of serum alkaline phosphatase indicates severity of bone damage and could be used in monitoring the management of bone pains in sickle cell anaemia (Afonja and Boyd, 1986).

The white blood cells (total) are the lymphocytes, neutrophils, basophils, eosinophils and monocytes, and could because of their sizes (they are larger than the red blood cells), obstruct blood vessels more effectively than red blood cells when attached to the endothelium. Bacterial infection associated with leucocytosis predisposes to sickle cell disease crises [Okpala (1998), Attah and Ekere (1987)]. A high absolute neutrophil count regressed significantly ($p < 0.05$) with clinical severity of sickle cell anaemia (Anyae'gbu *et al.*, 1998). Leucocytosis has been implicated as the causative agent of many complications of sickle cell disease and a risk factor for early sickle cell disease-related death (Platt *et al.*, 1994). Clinically overt stroke, pathogenesis of silent

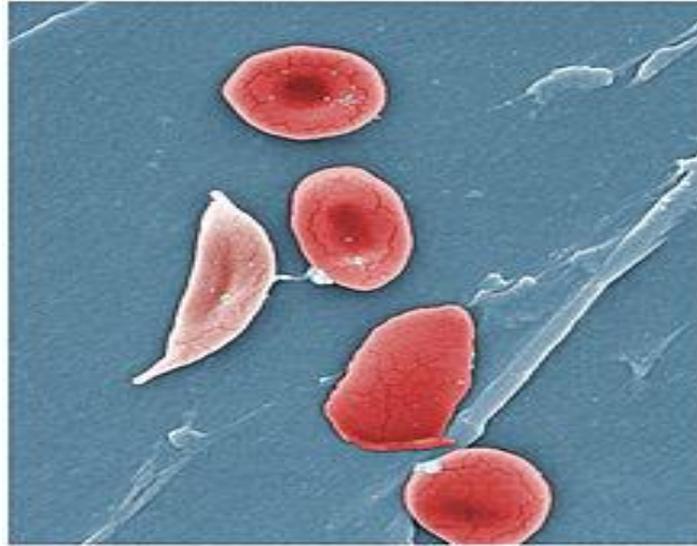


Figure 1. Electron microscope image of sickle red blood cell (left) and normal blood cells (Wikipedia, 2015).

cerebral infarction and acute chest syndrome have been associated with leucocytosis [Powers (2000), Castro *et al.*(1994)]. Sickle cell anaemia (HbSS) patients, particularly children, are vulnerable to infections leading to increased mortality (Falcao and Donadi, 1989). Mean values of white blood cell counts of HbS patients were significantly higher ($p < 0.05$) than those of HbS controls (Ojo and Shokunbi, 2014).

Low serum total cholesterol and triglyceride levels have been associated with incidence of sickle cell anemia disease in adolescents and men, respectively (Shores *et al.*, 2003). In a serum protein profile study of children, it was observed that sicklers have higher levels of serum total proteins than normal children subjects (Isichei, 1979).

A study carried out in the sickle cell clinics of Lagos State University Teaching Hospital Ikeja, Nigeria, revealed that significant decrease ($p < 0.05$) was observed of mean values of hemoglobin concentration and packed cell volume (PCV%) measured of sickle cell disease patients compared with normal human subjects (Akinbami *et al.*, 2012).

Acute chest syndrome (ACS) is the main cause of hospitalization, morbidity, and mortality in sickle cell anemia patients. *Mycoplasma pneumoniae* is commonly associated with the ACS in very young children with sickle cell anemia and can be treated with broad-spectrum antibiotics, including the macrolide class as well as bronchodilator therapy, early transfusion, and respiratory support (Neumayr *et al.*, 2003). *Staphylococcus aureus* was the bacterial pathogen identified in sputum cultures of sickle cell anemia adult patients who had episodes of fever, chest pain, increased leukocytosis, and pulmonary infiltrate ("acute

chest syndrome"). Pneumococcal polysaccharide vaccine has great potential for preventing life-threatening infection in children with sickle cell anemia, but may not change the incidence or severity of the acute chest syndrome in adults (Charache *et al.*, 1979).

Eye problems caused by the sickled blood cells are more common in older children and adults with HbSC and HbSbeta+Thalassemia because patients with these types of sickle cell disease often have a higher concentration of hemoglobin which makes it more difficult for the thick blood to travel through the tiny blood vessels. Sickle cell retinopathy is characterized by decrease in blood flow in blood vessels of the retina, bleeding in the retina, blurred vision, sudden loss of vision, pain in the eyes and subsequent blindness in untreated cases. Early eye checks and treatment with laser can prevent loss of vision/further loss of vision of sickle cell anemia patients (CDC, 2015).

In vitro site-specific correction of the sickle mutation in hematopoietic stem cells (HSCs) would allow permanent production of normal red blood cells, though with a reduction in the *in vivo* correction levels in mice relative to the *in vitro* samples. Zinc-finger nucleases (ZFN)-driven gene correction in CD34+ cells from the bone marrow of sickle patients resulted in the production of wild-type hemoglobin tetramers (Hoban *et al.*, 2015).

Bone marrow transplants have proven effective in children, and is a potential/cure for Sickle cell disease. The estimated mean survival for sickle-cell patients was 53 years old for men and 58 years old for women with homozygous SCD (Walters *et al.*, 1996).

The burning questions are : If sickle cell anemia patients are cured of the disease in their somatic cells, what about the gamete cells ?; If a sickle cell anemia patient is succ-

essfully cured of the disease by bone marrow transplant, is the genetic mutation absolutely reversed? If the sickler procreates with a sickle cell trait carrier, what is the probability that their first filial offspring is a sickler?

The aim of the study was to investigate the efficacy of the use of biochemical, and hematological indices [Hemoglobin genotype, packed cell volume (PCV%), white blood cell total (WBC_{Total}), aspartate amino transferase (AST), serum alkaline phosphatase (AP), serum triglyceride (TG), serum total cholesterol (C), Total bilirubin (Tb), Random blood glucose (RBG)] as diagnostic parameters essential to the treatment and management of sickle cell anemia disease.

MATERIALS AND METHODS

Experimental Design

The experimental design used in the present study is a single factor completely randomized design (CRD) whose linear equation is $\hat{Y} = \mu + T_i + \epsilon_{ij}$

\hat{Y} = individual observation

μ = overall mean

T_i = i th type of infection, and is significant of sickle cell anemia disease.

ϵ_{ij} = error which is independently, randomly and normally distributed with zero mean and constant variance.

SPSS for windows (version 17.0, SPSS, Chicago, IL, USA) was used to perform the statistical analyses. The significance level was p value < 0.05.

Selection of human subjects

Twenty male ($n = 20$) clinically confirmed sickle cell patients (Hemoglobin genotype : HbSS), of age bracket 18-30 years and twenty male ($n=20$) normal or healthy human subjects (Hemoglobin genotype : HbAA) of the same age bracket, voluntarily participated in this study, from the Federal Medical Centre Owerri, Imo State, Nigeria. All patients were on routine vitamin supplements and antibiotics. The subjects were randomly selected between June and August 2015. Exclusion criteria included: gastrointestinal tract infection, protein energy malnutrition, jaundice, cancer, diabetes mellitus, obesity, smoking, alcoholism, persons living with HIV, and malaria patients, previous history of surgery, patients who have received blood transfusion in the past 3 months, sickle cell anemia patients in crisis, heterozygous AS (carriers but normal).

The research was carried out in compliance with the Declaration on the Right of the Patient (WMA, 2000).

Blood was obtained by veni-puncture carried out by a Phlebotomist nurse. The method described by Thavasu *et al.* (1992) was used in obtaining the serum. Whole

blood was collected in a covered test tube, and allowed to clot by leaving it undisturbed for 15-30 minutes at room temperature. The clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes in a refrigerated centrifuge, to obtain the blood serum.

Determination of Haemoglobin Genotype

The technique for haemoglobin electrophoresis described by John and Lewis (1986) and Tidi *et al.* (2013) was employed. Fifty micro-liters of washed cells were added into khan tubes containing 50 micro-liter of 0.1% white saponin and were mixed thoroughly (haemolysate). The haemolysate was centrifuged to remove any debris. The supernatant was used for the test. Cellulose acetate papers were soaked and blotted. Haemoglobin genotype controls used include: HbA, HbF, HbS and HbC. One hundred ml of the Tris-EDTA and boric acid buffer was introduced into each of the outer section of the electrophoresis chamber. One micro-liter of each haemolysate sample (tests and controls) was transferred into the well plate. Using an applicator, 0.5 micro-liter of the haemolysate (samples and controls) was applied onto the cellulose acetate paper leaving about 0.5 cm gap for each sample. The cellulose paper was placed on a cathode bridge of the electrophoresis chamber containing Tris-EDTA and boric acid buffer. Two hundred voltages were applied for 15 minutes, and the results recorded.

Packed Cell Volume (PCV%)

Analysis of packed cell volume (PCV%) was carried out according to the method described by Ovuakporaye (2011). A plain capillary tube was filled with whole blood in an EDTA container by capillary action. It was sealed using plasticine or bunsen burner flame and placed in the haematocrit centrifuge for 10mins and the value of PCV% was obtained using haematocrit reader.

Lipid Profile Assays

Serum total cholesterol (C), and serum triacylglycerol (TG) were determined using commercial kits (Randox Laboratory Ltd., UK), in conformity with the methods employed by Ibegbulem and Chikezie (2012); Chikezie and Okpara (2013).

White Blood Cell total (WBC_{Total}) Assay

The white blood cell total count (mcl) was determined according to the method described by Annan and Plahar (1995). Blood samples (0.02 ml) were mixed with sequesterine and diluted in 0.38 ml diluting fluid (1.5 ml glacial acetic acid, 0.5 ml malachite green, 98.0 ml water). The diluted blood was mounted on a counting chamber, and white blood cells were counted.

Total Bilirubin Assay

Total bilirubin assay was carried out consistent with the methods described by Simmons (1968). In the determination of total bilirubin, the serum was added to a caffeine reagent, which acted as an accelerator, and then mixed with combined diazo reagent. The diazo reaction was terminated by the addition of ascorbic acid which destroyed the excess diazo reagent, the azo bilirubin was made alkaline by the addition of a tartrate buffer, and the intensity of the colour (absorbance) was read at 600 nm.

In Vitro Quantitative Analysis of Aspartate Amino Transferase (AST)

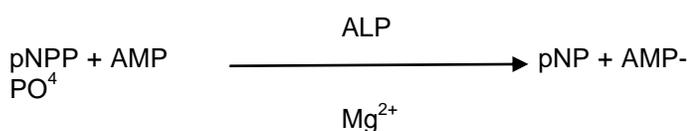
Quantitative *in vitro* determination of serum aspartate amino transferase (AST) was carried out using the method employed by Reitman and Frankel (1957). The test based on the reaction in which L-aspartate and α -ketoglutarate are converted to L-glutamate and oxaloacetate by the catalytic activity of AST. The oxaloacetate so formed, forms a complex known as oxaloacetate hydrazone with 2,4-dinitrophenyl hydrazine. The intensity of the colour of the hydrazone, which is measurable with a colorimeter at 578nm is directly proportional to the AST enzyme activity.

In Vitro Quantitative Determination Random Blood Glucose (RBG)

The RBG assay was carried out according to the method described by Pandey *et al.* (2012), in which Randox diagnostic kit, Beckman-CX-4 and CX-9 auto analyzers were used.

In Vitro Quantitative Analysis of alkaline phosphatase (ALP)

Alkaline phosphatase activity is determined by measuring the rate of conversion of p-nitro-phenylphosphate (pNPP) in the presence of 2-amino-2-methyl-1-propanol (AMP) at pH 10.4.



The rate of change in absorbance due to the formation of pNP is measured colorimetrically at 480 nm and is directly proportional to the ALP activity in the sample (Bowers and McComb, 1975).

RESULTS

Table 1 shows the results on the hematological indices of

the sickle cell anemia patients, and healthy human subjects. The method of hemoglobin electrophoresis was used in identifying and labeling twenty (20) homozygous (SS) sickle cell anemia patients in the steady state, and twenty (20) homozygous (AA) normal, healthy human subjects. The mean value of the PCV% of the normal, healthy human subjects was significantly higher ($p < 0.05$) than that value of the sickle cell anemia patients.

The results shown in Table 2 reveal that the mean values of Random blood glucose, serum aspartate amino transferase, $\text{WBC}_{\text{total}}$, serum alkaline phosphatase listed in order of consecutive significant decrease ($p < 0.05$) are as follows : sickle cell anemia patients, normal, healthy human subjects. The mean value of total bilirubin was numerically, but not significantly higher ($p < 0.05$) in sickle cell anemia patients compared with normal, healthy human subjects.

The mean value of serum total cholesterol was significantly higher ($p < 0.05$) in normal, healthy human subjects in comparison with the sickle cell anemia patients. Conversely, the mean value of serum triglyceride was significantly lower ($p < 0.05$) in normal, healthy human subjects compared with the corresponding value of the sickle cell anemia patients (Figure 2).

DISCUSSION

Hemolysis occurs in sickle cell anemia disease due to phagocytosis of red cells that have undergone sickling and lysis of complement-sensitive red cells [Allan *et al.* (1982), Test *et al.* (1991)]. These events are largely responsible for the significant reduction ($p < 0.05$) of PCV% observed of sickle cell anaemia patients, in the present study (Table 1). Homozygous sickle cell disease patients have lower values of hemoglobin parameters, but higher values of white cell and platelets counts compared with haemoglobin phenotype AA controls (Akinbami *et al.*, 2012).

Pain, nausea, vomiting and anxiety associated with redistribution of the white blood cells between the marginal and circulating pools cause leucocytosis in the absence of infection, and is responsible for the significant difference ($p < 0.05$) in white blood cell (total) reported in the present study (Table 2), and given credence by the corroborative findings of Ojo and Shokunbi (2014) and Akinbami *et al.* (2012).

Mean values of AST were significantly higher ($p < 0.05$) in sickle cell anemia patients compared with healthy human subjects (controls) (Table 2), and is in keeping with the finding that compromised hepatic integrity leading to release of aspartate amino transferase (AST) via intravascular hemolysis accounts for elevated activity of

Table 1. Results on the Hematological indices: Hemoglobin genotype, and PCV% sickle cell anemia patients, and healthy human subjects.

	Hemoglobin genotype	PCV%
Healthy human subjects	AA (X 20)	45 ± 0.1 ^a
Sickle cell anemia patients	SS (X 20)	23.87 ± 0.2 ^b

Results on the PCV% are expressed as mean ± standard error (S.E) (%) (n = 20). Values that are labeled, in the PCV% column, with the same superscripts, are not significantly different (p<0.05).

Table 2. Results on the biochemical indices: Random blood glucose, Total bilirubin, serum aspartate aminotransferase (AST), Total white blood cell (WBC_{total}), serum alkaline phosphatase, and serum albumin of the sickle cell anemia patients, and healthy human subjects.

	Random blood glucose (RBG) (mg/dl)	Total bilirubin (mg%)	Serum aspartate aminotransferase (U/l)	WBC _{total} x 10 ³ /μl	Serum alkaline phosphatase (I.U/L)
Healthy human subjects	82.18 ± 4.16 ^a	0.7 ± 0.1 ^a	9.18 ± 1.16 ^a	5.17 ± 3.94 ^a	140 ± 1.2 ^a
Sickle cell anemia patients	90.87 ± 4.23 ^b	2.8 ± 0.2 ^a	19.5 ± 2.16 ^b	11.27 ± 3.94 ^b	215.2 ± 1.3 ^b

Results are expressed as mean ± standard error (S.E) (unit) (n = 20). Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

liver enzymes in sickle cell anaemia disease [Kehinde *et al.* (2010), Nsiah *et al.* (2011), Pandey *et al.* (2012)].

Blood glucose concentration alteration could be employed as a possible biomarker for sickle-cell anemia intensity (Osuagwu and Mbeyi, 2007). Observed lesions in sickle cell anemia patients could be as a result of altered blood glucose levels. The significant increase (p<0.05) in the mean value of random blood glucose of sickle cell anemia patients in comparison with normal healthy human subjects (controls), reported in Table 2, is consistent with the postulates of Lachant *et al.* (1983), and Pandey *et al.* (2012).

Abnormally elevated levels of bilirubin (a compound produced by the bio-degradation of hemoglobin from red blood cells), indicates liver and/or renal dysfunction or blood disease such as sickle cell anemia. Hemolysis, therefore, is a likely source of the observed elevated levels in mean values of total bilirubin, in sickle cell anemia patients (Table 2), and conforms to the findings of Hargrove (1970), who observed marked increase in serum bilirubin in sickle cell anemia patients.

Accelerated hematopoiesis, osteoporosis, osteomalacia and bone infarction are characteristic of sickle cell anemia disease (Nourai *et al.*, 2011). A bio-indicator of the severity of bone damage is the level of alkaline phosphatase (Afonja and Boyd, 1986). Mean value of alkaline phosphatase of the sickle cell anemia patients observed in the present study, was significantly higher (p<0.05) than the corresponding value in normal healthy subjects (Table 2), and is consistent with the findings that

liver dysfunction and elevated alkaline phosphate activity are associated with sickle cell anemia disease in steady state (Kotila *et al.*, 2005).

Pulmonary hypertension in sickle cell disease is characterized by oxidant stress caused by intravascular hemolysis and might be due to low levels of plasma total cholesterol and low-density lipoprotein cholesterol (LDL-C) in sickle cell disease patients [Buchowski *et al.* (2007), Marzouki and Khoja (2003)].

Mean values of serum total cholesterol were significantly higher (p< 0.05) in the controls (normal healthy human subjects) compared with the sickle cell anemia patients. Conversely, mean values of serum triglyceride were significantly lower (p< 0.05) in the controls compared with the sickle cell anemia patients (Figure 2). These findings are given credence by the postulates of Zorca *et al.* (2010), who reported hypocholesterolemia and hypertriglyceridemia in sickle-cell disease associated with hemolytic severity, vascular dysfunction and pulmonary hypertension. The rate of triglyceride synthesis from glycerol, in sickle cell anemia disease is elevated up to 4-fold in sickled reticulocytes (Lane *et al.*, 1976).

Multiple regression studies revealed that PCV% regressed significantly (p<0.05) with WBC_{Total}, AST, AP, TG and RBG of homozygous sickle cell anemia patients in the steady state. The correlation statistical analysis of serum triglyceride (a biochemical index) and PCV% (a hematological index) of the patients was significant (p<0.05) with a Pearson's product moment correlation coefficient of 0.983. The concentration of serum tri-

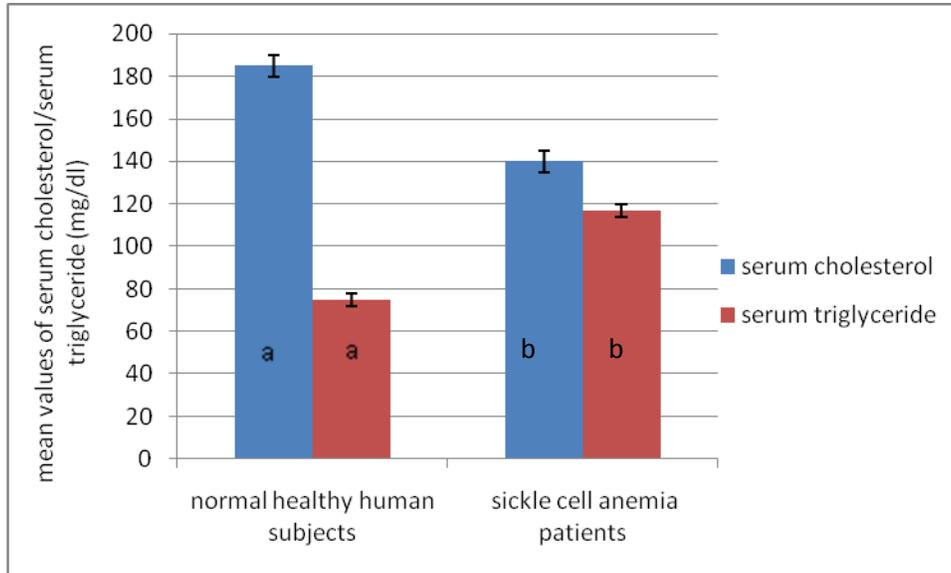


Figure 2. Graphical results on the biochemical indices : serum total cholesterol and serum triglyceride of the sickle cell anemia patients, and normal healthy human subjects (homozygous AA, controls). Statistical results are expressed as mean \pm standard error (mg/dl) (n = 20). Error bars represent values of standard error (1.0 – 2.3 mg/dl). Corresponding bars labeled with the same letters represent mean values of serum cholesterol or serum triglyceride which are not significantly different ($p < 0.05$).

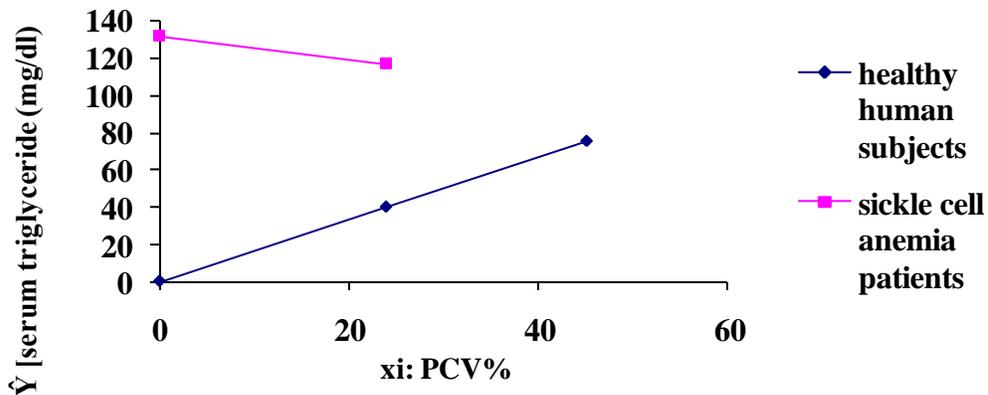


Figure 3. Regression curve of Serum triglyceride (mg/dl) and PCV%: (sickle cell anemia patients) : \hat{Y} (mg/dl) = 131.375 - 14.375xi (%). (Healthy human subjects) : \hat{Y} (mg/dl) = 0.025 + 1.67xi (%).

glyceride could be predicted from the regression curve [\hat{Y} (predicted value of serum triglyceride) = \hat{Y} (mg/dl) = 131.375 - 14.375xi (%).], xi is the observed value of PCV% (figure 3).

CONCLUSION

Incidence of homozygous sickle cell anemia in the steady state correlated positively and significantly ($p < 0.05$) with significant increase ($p < 0.05$) in the diagnostic indices: WBC_{Total} , AST, AP, TG and RBG; and significant decrease ($p < 0.05$) in PCV% and C. These indices are thus effective diagnostic parameters of sickle cell anemia disease that could be used in the management, and monitoring of recovery from the disease (treatment). There was a significant, positive association between serum triglyceride and PCV% of homozygous sickle cell anemia patients in the steady state. Observed values of PCV% could be used with high precision to predict serum triglyceride levels of homozygous sickle cell anemia patients in the steady state. It is suggested that hemolysis (a function of significant decrease in PCV%), resulting in the release of membrane lipids is the cause of elevated serum triglyceride and concomitant decrease in serum total cholesterol, in sickle cell anemia patients.

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