Impact Assessment of Foetal Haemoglobin on Biochemical Markers of Liver Function in Sickle Cell Disease Patients

Mathias Abiodun Emokpae, Rossy Jane Umeadi

Department of Medical Laboratory Science, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria

Email address:

biodunemokpae@yahoo.com (M. A. Emokpae)

To cite this article:

Mathias Abiodun Emokpae, Rossy Jane Umeadi. Impact Assessment of Foetal Haemoglobin on Biochemical Markers of Liver Function in Sickle Cell Disease Patients. *American Journal of Biomedical and Life Sciences*. Vol. 3, No. 3, 2015, pp. 61-66.

doi: 10.11648/j.ajbls.20150303.16

Abstract: *Background/objective:* The role of foetal haemoglobin (HbF) on the modulation of clinical manifestations in sickle cell disease is well known but there is inconsistency as to the levels of HbF that may protective against the development of liver disease. This study evaluates the impact of HbF on biochemical markers of liver function in sickle cell disease (SCD) patients in steady clinical state. *Materials and Methods:* Liver function tests: aspartate amino transferase, alanine amino transferase, alkaline phosphatase, bilirubin and proteins as well as HbF were assayed in SCD patients using colorimetric methods. *Results:* Out of 100 SCD patients, 24% had high (>5%) HbF while 76% had low (<4.9%) HbF levels. Those subjects with high HbF had lower ($p < 0.001$) levels of measured variables except albumin which was higher compared to levels in those with low HbF. HbF correlated negatively with the measured variables except albumin. *Conclusion:* The SCD patients with high HbF had lower levels of the measured variables compared to those with low levels of HbF. High HbF levels (>5%) may be protective against the development of liver pathology in SCD patients in steady clinical state.

Keywords: Foetal Haemoglobin, Liver Disease, Liver Function Tests, Sickle Cell Disease, Steady Clinical State

1. Introduction

Sickle cell disease (SCD) is an inherited multisystem disease characterized by chronic haemolytic anaemia and vaso-occlusive crisis which often results in vascular occlusion of micro-vascular vessels by sickled red blood cells leading to multi-organ infarction. It is a condition which has autosomal recessive pattern of inheritance from parents, in which the red blood cells assume an abnormal, rigid, sickle shape in deoxygenated state¹⁻². The degree of HbS polymerization in the circulation determines how likely the individual is to have a vaso-occlusive crisis or other adverse event. This causes it to become trapped, thus blocking small vessels of the microcirculation³⁻⁴.

Liver pathology is a common complication due to the disease itself and its treatment⁵⁻⁸. In addition to the vascular complications from the sickling process, patients with SCD may have received multiple transfusions, placing them at risk for viral hepatitis and iron overload. All these conditions combined with the effects of chronic hemolysis resulting in the development of pigment gallstones which ultimately may

lead to liver disease⁷. In SCD patients, mild to moderate hepatomegaly develops early, followed by shrinkage produced by fibrosis and cirrhosis⁹⁻¹⁰. Hepatic tenderness occurs occasionally, patients with sickle cell anaemia may suffer from a variety of hepatic alterations. The altered shape of red blood cell favours intravascular haemolysis and thus occlusion of the liver vascular bed, leading ultimately to tissue injury which ranges from asymptomatic mild liver function test abnormalities to severe acute damage¹¹.

It is of interest to note that cells are less prone to sickling in individuals who retain a high level of foetal haemoglobin (HbF). Haemoglobin F inhibits the polymerization of the HbS owing to its high oxygen affinity¹². Haemoglobin F dissociates to a dimer, which when combined with HbS, gives a tetramer that does not form a polymer. This is in contrast to HbA, which dissociates to a dimer that combines to give a tetramer that has a 50% chance of polymerizing. It is well recognized that high levels of HbF in SCD patients modulate the clinical manifestations of the disease. The role of HbF on the clinical phenotype is variable and inconsistent; for example HbF levels close to 20% may be found in patients with severe disease^{4,13}. The modulation of symptoms

and complications becomes more consistent when a certain level of HbF threshold has been achieved. The threshold for significant reduction in acute episodes of pain, chest syndromes, and priapism is 20%, and for organ damage 10%³. The symptoms of sickle cell disease (SCD) are almost completely eliminated with HbF levels above 25%; however, studies indicated that any increment in HbF level may improve the overall survival¹³⁻¹⁴. There is however controversy as to the amount of the HbF that may be required to protect the patients from organ damage in our environment and impact of HbF on liver function tests has not been assessed. Therefore, this study evaluates the biochemical markers of liver function in SCD patients in steady clinical state and the protective effect of HbF on the levels of these biochemical markers.

2. Materials and Methods

This descriptive case control study was conducted at the Department of Medical Laboratory Science, College of Medical sciences, University of Benin. The protocol for the study was reviewed and approved by the Edo state Ministry of Health, Benin City before the commencement of study. Informed consent was obtained from all subjects who participated in the study. The subjects included sickle cell disease patients on routine visit to Sickle Cell Centre, Benin City. A total of 150 samples were analyzed which comprised of 100 SCD patients (HbSS) and 50 controls (HbAA).

Inclusion criteria: All SCD patients on steady clinical state without acute illness such as vaso-occlusive crisis, acute chest pain syndrome or bacterial infection were included in the study. The control subjects were apparently healthy HbAA subjects.

2.1. Exclusion Criteria

All SCD patients with acute illness such as vaso-occlusive crises, acute chest syndrome or bacterial infection were excluded from the study. Subjects with other haemoglobinopathies were also excluded.

2.2. Sample Collection

Five millilitres of blood was collected aseptically and 2mL aliquot was dispensed into EDTA container and 3mL aliquot was emptied into a plain container. The anticoagulated blood was used for HbF estimation using alkaline denaturation method¹⁵. The non anticoagulated blood was spun at 1500rpm for 10minutes and the supernatant serum was separated into a separate tube. The serum was stored at -20⁰c for 2weeks prior to analysis. The biochemical markers of liver function assayed were Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), bilirubin, protein and albumin. They were analyzed with spectrophotometric methods using commercially available reagent kits supplied by Randox laboratories, UK.

2.3. Statistical Analysis

The data obtained were statistically evaluated using Statistical Package for Social Sciences program (SPSS) version 16.0. Values obtained in this research were represented as mean and Standard error of mean (SEM) for both tests and controls. Student's *t* test was used to compare data at 95% confidence intervals ($p < 0.05$). The measured variables were compared in test subjects with high and low HbF concentration and the biochemical markers of liver function were correlated with HbF in test subjects.

3. Results

Table 1 shows the mean levels of HbF, serum AST, ALT, ALP, AST/ALT ratio, total protein, globulin, total bilirubin, conjugated bilirubin in both SCD patients and control subjects. HbF, serum AST, ALT, ALP, AST/ALT ratio, total protein, globulin, conjugated bilirubin, total bilirubin were significantly higher ($p < 0.001$) in SCD patients compared with controls while albumin was lower ($p < 0.001$) in SCD patients compared with controls.

Table 1. Biochemical markers of liver function in SCD patients and control subjects (mean $\pm$ SEM).

Measured variables	SCD patients HbSS	Subject with normal haemoglobin HbAA(controls)	P-Value
Number of subjects	100	50	
Number of males	60	26	
Number of females	40	24	
Age (yrs)	24.25 $\pm$ 0.86	23.28 $\pm$ 0.24	
Foetal haemoglobin(%)	2.38 $\pm$ 0.20	0.29 $\pm$ 0.03	0.001
Alkaline phosphatase(U/L)	169.71 $\pm$ 56.01	83.87 $\pm$ 12.31	0.001
Aspartate amino transferase (U/L)	21.94 $\pm$ 1.30	8.69 $\pm$ 1.91	0.001
Alanine amino transferase (U/L)	15.38 $\pm$ 0.97	10.07 $\pm$ 0.25	0.001
AST : ALT ratio	1.78 $\pm$ 0.13	0.86 $\pm$ 0.02	0.001
Total protein(g/L)	88.34 $\pm$ 1.19	66.86 $\pm$ 0.48	0.001
Albumin(g/L)	33.37 $\pm$ 0.66	40.42 $\pm$ 0.51	0.001
Globulin(g/L)	54.97 $\pm$ 1.56	27.44 $\pm$ 0.50	0.001
Conjugated bilirubin(μ mol/L)	17.13 $\pm$ 1.05	3.42 $\pm$ 0.17	0.001
Total bilirubin(μ mol/L)	55.10 $\pm$ 3.18	10.23 $\pm$ 0.23	0.001

Table shows that ALP, AST, ALT, AST:ALT ratio, total protein, albumin, globulin, CB, and TB in SCD patients with high HbF as compared with those with low HbF. All

measured variables except Albumin were significantly lower (0.001) while albumin is significantly higher ($p < 0.001$) in subjects with high HbF than in those with low HbF levels.

Table 2. Biochemical markers of liver function in SCD patients with high ($\geq 5\%$) and low ($\leq 4.9\%$) HbF (mean $\pm$ SEM).

Measured variables	SCD patients with high HbF ($\geq 5\%$)	SCD patients with low HbF ($\leq 4.9\%$)	P-value
No of subjects	24	76	-
Foetal haemoglobin	5.36 $\pm$ 0.09	1.44 $\pm$ 0.13	0.001
Alkaline phosphatase (U/L)	84.24 $\pm$ 4.06	196.69 $\pm$ 3.52	0.001
Aspartate amino transferase (U/L)	8.53 $\pm$ 0.43	26.18 $\pm$ 1.38	0.001
Alanine amino transferase (U/L)	10.66 $\pm$ 1.29	16.86 $\pm$ 1.15	0.001
AST: ALT ratio	0.90 $\pm$ 0.36	2.05 $\pm$ 1.36	0.001
Total protein (g/L)	73.87 $\pm$ 1.29	92.90 $\pm$ 1.07	0.001
Albumin (g/L)	40.29 $\pm$ 0.82	31.18 $\pm$ 0.63	0.001
Globulin (g/L)	33.58 $\pm$ 1.43	61.72 $\pm$ 1.23	0.001
Conjugated bilirubin (μ mol/L)	5.49 $\pm$ 1.05	20.81 $\pm$ 1.02	0.001
Total bilirubin (μ mol/L)	12.18 $\pm$ 0.45	68.65 $\pm$ 2.70	0.001

Table 3. Correlation of HbF with measured markers of liver function in SCD patients.

Measured variables	r-value	p-value
Alkaline phosphatase (U/L)	-0.77	0.001
Aspartate amino transferase (U/L)	-0.51	0.001
Alanine amino transferase (U/L)	-0.26	0.01
AST: ALT Ratio	-0.33	0.001
Total protein (g/L)	-0.61	0.001
Albumin (g/L)	0.52	0.001
Globulin (g/L)	-0.68	0.001
Conjugated bilirubin (μ mol/L)	-0.62	0.001
Total bilirubin (μ mol/L)	-0.71	0.001

Table 4. Markers of liver function in male and female SCD patients (mean $\pm$ SEM).

Measured variables	Female SCD patients	MALE SCD patients	P-value
No of subjects	60	40	
Foetal Haemoglobin(%)	2.42 $\pm$ 0.25	2.32 $\pm$ 0.31	0.79
ALP(U/L)	168.42 $\pm$ 7.31	171.62 $\pm$ 8.81	0.78
AST(U/L)	22.52 $\pm$ 1.75	21.08 $\pm$ 1.93	0.59
ALT(U/L)	16.18 $\pm$ 1.31	14.18 $\pm$ 1.40	0.31
AST-ALT ratio	1.68 $\pm$ 0.15	1.93 $\pm$ 0.24	0.35
Total Protein (g/L)	87.87 $\pm$ 1.63	89.05 $\pm$ 1.73	0.63
Albumin (g/L)	33.75 $\pm$ 0.82	32.80 $\pm$ 1.09	0.48
Globulin (g/L)	54.12 $\pm$ 2.10	56.25 $\pm$ 2.33	0.51
Conjugated Bilirubin (μ mol/L)	17.47 $\pm$ 1.30	16.63 $\pm$ 1.76	0.69
Total Bilirubin (μ mol/L)	53.69 $\pm$ 4.07	57.20 $\pm$ 5.14	0.59

ALP=Alkaline Phosphatase; AST= Aspartate amino transferase ALT= Alanine amino transferase.

Table 3 shows correlation of HbF with ALP, AST, ALT, AST-ALT ratio, total protein, albumin, globulin, conjugated bilirubin and total bilirubin in SCD patients. HbF levels correlated negatively (0.001) with ALP, AST, AST-ALT ratio, total protein, globulin, conjugated bilirubin, total bilirubin and ALT ($P=0.01$) while HbF correlated positively ($p < 0.001$) with albumin.

The table 4 shows the different levels of measured between male and female SCD patients. The differences were not statistically significant ($p > 0.05$).

4. Discussion

Hepatic disorders in SCD may be due to sickling process, acute hepatitis or hepatic sequestration crisis. The frequency and Pathophysiology of liver disease in SCD is under debate⁷. Even though the etiology of liver disease is multifactorial, available evidence suggest the importance of vascular changes and the participation of sickling process was the major cause of chronic hepatic changes observed⁷. The levels of measured biochemical variables in this study were higher

($p < 0.001$) in SCD than control subjects except albumin which was lower ($p < 0.001$) in SCD than controls. Twenty four percent (24%) of the study subject had persistent high ($>5\%$) HbF while 76% had low ($<4.9\%$) levels of HbF. The levels of the biochemical markers of liver function were lower in SCD patients with high HbF than those with low HbF ($p < 0.001$). Haemoglobin F correlated negatively with ALP, AST, ALT, AST:ALT ratio, total protein, globulin, conjugated bilirubin and total bilirubin but correlated positively with albumin. There was also no significant difference in the levels of HbF and the other measured variables in female SCD patients compared to their male counterpart.

Raised levels of HbF were observed to increase survival in SCD patients¹ and therefore therapeutic induction of HbF (r-gene, HbG1, HbG2) expression is used in the management of SCD patients. However raised HbF only may not be sufficient to forestall complications associated with SCD, as some patients with increased HbF (upto 20%) were observed to have severe SCD manifestations¹. A study in Jamaica, revealed that patients with high HbF has better haematological indices compared to those with low HbF¹⁶. Earlier studies did not however observe any association between HbF and disease severity¹⁷⁻¹⁸. It was suggested that the level of HbF needed to prevent acute clinical event was about 20% while levels of 10% was needed to prevent organ damage¹⁸. Twenty four (24%) of the study subjects had high HbF and is consistent with 29% reported previously in Sokoto, northern Nigeria¹⁹. High level of HbF in the red blood cell of an individual tends to impair sickling at physiologic oxygen tension and may clinically improve the general well-being of the subject. Most of the SCD patients with high HbF are reported to be free from most of the severe clinical manifestations associated with SCD¹⁹. This may probably account for the lower levels of biochemical markers of liver function observed in this study.

The gender of the patients did not significantly affect the levels of the HbF but mean values was higher in female (2.42 ± 0.25) than males (2.32 ± 0.31), this agreed with that reported by Adekile and Huisman²⁰. They reported that female sickle cell patients with haplotypes 19/19 had higher HbF levels than their male counterparts, the same was observed for the patients with the 19/3 haplotype combination but not for those with the 20/3 haplotype combination. Gladwin et al²¹ also reported that female patients have slightly greater HbF levels, which may be protective; and concluded that the basis for these differences could lie in the observation that nitric oxide bioavailability and responsiveness are reduced in males but not females with sickle cell disease. Nitric oxide is thought to be important in maintaining vasomotor tone, limiting platelet aggregation, inhibiting ischemia-reperfusion injury, and modulating endothelial adhesion molecule expression. Estrogens facilitate nitric oxide production and limit its consumption. In the same vein, Ikuta et al²² linked nitric oxide to transcriptional control of HbF and could therefore contribute to gender differences in HbF expression.

The mean HbF levels (2.38 ± 0.20) observed in this study is

consistent with (2.99 ± 5.16) reported in Sokoto²³ and (3.05 ± 1.61) reported in Calabar²⁴, it is however lower than (5.9 ± 3.8) reported in Ibadan²⁵.

There was significant increase in the mean values of ALP, AST, ALT and bilirubin in SCD patients when compared with control subjects. This observation is in agreement with previous reports that there is liver dysfunction in steady state sickle cell disease²⁶. According to Kotila et al¹⁸, acute intrahepatic cholestasis or the extreme haemolysis which causes hyperbilirubinaemia, a common event in sickle cell disease is the cause of the increased ALP in these patients. Hemolysis also raises AST and ALT levels in SCD²⁷⁻²⁹. There was significant increase in the mean values of AST:ALT ratio in SCD patients when compared with control subjects, this is in agreement with Nsiahet al²⁷, they reported that there is increased AST:ALT ratio greater than 1 owing to the fact that there is presence of intravascular haemolysis in SCD. Abnormalities in liver function tests were reported in 27% of a group of 48 subjects¹¹. Others had reported that abnormal liver function tests may be common in the absence of significant liver pathology³⁰, but recent studies have reported the presence of hepatomegaly in 40-80% of living SCD patients and 100% of cases in autopsy study^{11,31}. The elevated levels of liver enzymes may be related to haemolysis and/or ineffective erythropoiesis. Serum ALT levels may accurately reflect hepatocyte injury while ALP which is of bone origin may also be elevated³¹.

There was significant increase in the mean values of total protein, globulin in SCD patients when compared with control subjects, while albumin, was reduce in SCD patients with respect to control subjects, this is in accordance with Adu et al³² who reported hyperproteinaemia in the sickle cell individuals as a result of hyperglobulinaemia which occur in these individuals due to the gamma (γ) globulin fraction. The increase gamma (γ) globulin is due to the extent of antigenic stimulation coming from the environment³³, also in another study, a high level of globulin in sickle cell homozygous individuals was reported³⁴. Adenike et al³⁵ attributed this hyperglobulinaemia to increased erythrocyte destruction during sickling. This is in contrast to Famodu et al³⁶ who reported high level of albumin in sickle cell patients especially during vaso-occlusive crisis and Tripathi et al³⁷ who observed low levels of total protein and albumin in SCD patients.

HbF correlated negatively with serum ALP, AST, ALT, AST-ALT ratio, total protein, albumin, globulin, conjugated bilirubin and total bilirubin, in SCD patients. This negative correlation may indicate that high HbF may be protective against increased liver enzymes in patients with steady clinical state.

In conclusion, the measured biochemical markers of liver function were higher in SCD patients than controls except albumin which was higher in controls than SCD patients. The SCD patients with high HbF had lower levels of the measured variables compared to those with low levels of HbF. High HbF levels ($>5\%$) may be protective against the development of liver pathology in SCD patients.

References

- [1] Steinberg MH, Forget BG, Higgs DR, Weatherall DJ. (2009). Disorders of Hemoglobin: Genetics, Pathophysiology, Clinical Management (2nd ed). Cambridge, United Kingdom: Cambridge University Press. 2009,p 209 – 213.
- [2] Ballas SK. Sick cell anaemia; progress in pathogenesis and treatment. *Drugs* 2002; 62(8):1143-1172.
- [3] Bactor FN, Uehlinger J. Malaria and Sick cell Disease. *N Engl J Med* 2002; 347(1):35-36.
- [4] Oneal PA, Gantt NM, Schwartz JD, Bhanu NV, Lee YT, Moroney JW et al. Fetal haemoglobin silencing in humans. *Blood* 2006; 108(6):2081-2086.
- [5] Banerjee S, Owen C, Chopra S. Sick cell hepatopathy. *Hepatology* 2001; 33: 1021.
- [6] Berry P, Cross T, Thein S. Hepatic dysfunction in sick cell disease: a new system of classification based on global assessment. *Clin. Gastroenterol Hepatol* 2007; 5: 1469.
- [7] Maher MM, Mansour AH. Study of chronic hepatopathy in patients with sick cell disease. *Gastroenterol Res* 2009; 2(6):338-343.
- [8] Ebert E, Nagar M, Hagspiel K. Gastrointestinal and hepatic complications of sick cell disease. *Clin Gastroenterol Hepatol* 2010;8: 483.
- [9] Sheeley TW. Sick cell hepatopathy. *South Med J* 1977;70(5):533-538.
- [10] O'Callaghan A, O'Brien SG, Ninkovic M, Butcher GP, Foster CS, Walters JR, Roberts IA. Chronic intrahepatic cholestasis in sick cell disease requiring exchange transfusion. *Gut* 1995;37(1):144-147.
- [11] Gurkan E, Ergun Y, Zorludemir S, Baslamisli F, Kocak R. Liver involvement in sick cell disease. *Turk J Gastroenterol* 2005;16(4):194-198.
- [12] Akinsheye I, Alsultan A, Solovieff N, Ngo D, Baldwin CT, Sebastina P et al. Fetal haemoglobin in sick cell anemia. 2011;118:19-27.
- [13] Atweh GF, Loukopoulos D. Pharmacological induction of fetal haemoglobin in sick cell disease and beta-thalassemia. *Semin Hematol* 2001;38(4):367-373.
- [14] Mehta SR, Afenyi-Annan A, Byrns PJ, Lottenberg R. Opportunities to improve outcomes in sick cell disease. *Am Fam Physician* 2006;74(2):303-310.
- [15] Betke K, Marti HR, Schlicht I. Estimation of small percentages of foetal haemoglobin. *Nature (Lond)*, 1959;184:1877-1878.
- [16] Donaldson A, Thomas P, Serjeant BE, Serjeant GR. Foetal haemoglobin in homozygous sick cell disease: a study of patients with low HbF levels. *Clin Lab Haematol* 2001; 23(5):285-289.
- [17] Powars D, Weiss N, Chan S, Schroeder A.. Is there a threshold level of fetal hemoglobin that ameliorates morbidity in sick cell anemia? *Blood*. 1984;63(4):921-926.
- [18] Kotila TR, Fawole OI, Shokunbi WA. Haemoglobin F and clinical severity of sick cell anaemia among Nigerian adults. *Afr J Med Sci* 2000;29(3-4):229-231.
- [19] Jiya NM, Matazu IK, Bilbis LS, Shehu RA. Serum levels of antioxidant vitamins in foetal haemoglobin (HbF) persistent sick cell anaemia children in Sokoto, Nigeria. *Annals Afr Med* 2005;4(4):168-171.
- [20] Adekile A, Huisman T. Level of fetal hemoglobin in children with sick cell anemia: influence of gender, haplotype and alpha-thalassemia-2 trait. *Acta Haematol* 1993; 90(1): 34.
- [21] Gladwin M., Schechter A., Coles W., Reiter c. and Ognibene. Divergent nitric oxide bioavailability in men and women with sick cell disease. *Circulation*. 2003;107: 271-278.
- [22] Ikuta T, Ausenda S, Cappellini M. Mechanism for fetal globin gene expression: role of the soluble guanylate cyclase-cGMP-dependent protein kinase pathway. *Proc. Natl. Acad. Sci. USA*. 2001;98:1847-1852.
- [23] Isah I, Udomah F, Erhabor O, Aghedo F, Uko E, Okwubili A. Fetal Haemoglobin levels in SCD patients in Sokoto, Nigeria. *Brit. J. Med. Health Sci*. 2013;1 (6): 36-47.
- [24] Uko E, Ibeh M, Gwanmesia F. Frequency of foetal haemoglobin and haemoglobin values in various haemoglobin genotype in Calabar, Nigeria. *East Afr. Med. J*. 1997;74: 809-811.
- [25] Falusi A, Esan G. Foetal haemoglobin levels in sick cell anaemia in Nigeria. *Afr J Med Med Sci* 1989;18:145-149.
- [26] Taiwo K, Kayode A, Aduragbenro A, Olayiwola O, Eytayo F, Biobele B. Liver dysfunction in steady state sick cell disease. *Annals of Hepatol*. 2005; 4(4): 261-263.
- [27] Nsiah K, Dzogbefia V, Ansong D, Osei AA, Boateng H, Ocloo D. Pattern of AST and ALT changes in relation to hemolysis in sick cell disease. *Clin Med Insight Blood Disord* 2011;4:1-9.
- [28] Pandey S, Sharma A, Dahia S, Shah V R. Biochemical Indicator of Sick Cell Disease: Preliminary Report from India. *Ind. J. Clin Biochem* 2012;27(2): 191-195.
- [29] Schubert TT. Hepatobiliary system in Sick cell disease. *Gastroenterology* 1986;90(6):2013-2021.
- [30] Molavi MA, Sajjadi HS, Nazemi A, Ghahramani F, Molavi K, Amernia B, Doozandeh H. The determination of hepatic enzymes levels in Children with sick cell anaemia and correlation with age, gender, HbS and HbF variables. *Asian J Med Pharm Res* 2012;2(2):43-46.
- [31] Murakami J, Shimuzu Y. Hepatic manifestations in haematological disorders. *Int J Hepatol* 2013(2013):1-13.
- [32] Adu EM, Okosun RE, Bini EN, Ophori EA. Effects of the sick cell (s) gene on serum protein profile. *Continental J Biomed Sci* 2012;6(2):1-5.
- [33] Johnson S, Omata M, Tong J, Simmons F, Weiner J, Tatter D. (1985). Liver involvement in sick cell disease. *Med. (Baltimore)* 1985;64:349-356.
- [34] Isichie P. Serum proteins profile in sick cell disease. *J. Clin Path* 1979; 32: 171-121.
- [35] Adenike A, Bakare R, Fajimi L, Ogunyemi O, Obisesan A, Sokoya G. Serum protein levels in sick cell disease. *Afr. J. Med. Pharm. Sci*. 1998;1: 40-43.

- [36] Famodu A, Omodiale P, Adedeji O, Reid L. Serum protein study in adult Nigerians with sickle cell anaemia. Med. Sci. Res. 1987;15: 193-194.
- [37] Tripathi S, Dadsena R, Kumar A. Study of Certain Biochemical Parameters in Patients of Sickle Cell Anemia. AdvBiores 2011; 2 (2):79-81.