Investigation of Some Biochemical Parameters in Subject with HbSC and HbSS in Saki West Local Government - a Rural Community in Nigeria

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Abstract

Background: HbSS and HbSC are a group of genetic blood disorders. They are examples of sickle cell diseases.The complication of sickle cell disease could affect spleen, brain, eyes, lungs, liver, heart, kidneys, penis, joints, bones, or skin. **Aim and Objective:** This work was designed to measure plasma ALT, AST, Albumin,Se, Zn and Uric acid in HbSS and HbSC patients in Saki-West Local Government area of Oyo state-Nigeria. **Materials and Methods:** HbSS and HbSC patients in crisis and in non-crisis steady state classified into HbSC (n=25) and HbSS (n=25) aged 10 to 31 years were recruited as test subjects for the study. Apparently healthy 50 HbAA volunteers classified into female (n=25) and male (n=25) aged 10 – 50 years were also studied as control. Subjects residing in Saki-West for not less than 5 years were recruited as test and control subjects. Plasma Albumin, Uric acid, ALT(Alanine Transaminase), AST(Aspartate Trans aminase) were determined biochemically bt spectrophotometry.Plasma Zn (Zinc) and Se (Selenium) were measured by Atomic Absorption Spectrophotometry. **Results:** The result obtained showed a significantly higher mean value of plasma ALT and AST in HbSC and HbSS than HbAA with $p<0.05$. The result also showed a significantly lower plasma selenium mean value in HbSC and HbSS than HbAA with $p<0.05$ This biochemical alterations including plasma Zn was more in crisis than in non-crisis steady state. **Conclusion and Recommendation:** Decreased plasma level of Selenium and increased plasma AST and ALT were found in HbSS and HbSC patients. This biochemical alterations including plasma Zn was more in crisis than in non-crisis steady state.

Keywords: ALT, AST, Sickle cell, Zinc, Selenium, Albumin,Uric acid

1. Introduction

Sickle cell disease (SCD) describes a group of inherited red blood cell disorders. People with SCD have abnormal hemoglobin, called hemoglobin S or sickle hemoglobin, in their red blood cells such as HbSS and HbSC. Problems in sickle cell disease typically begin around 5 to 6 months of age. A number of health problems may develop, such as attacks of pain ("sickle-cell crisis"), anemia, bacterial infections, and stroke.Long term pain may develop as people get older. The average life expectancy in the developed world is 50 years (Rees *et al*., 2010)

The red blood cell sickling and poor oxygen delivery can also cause organ damage. Over a lifetime, SCD can affect spleen, brain, eyes, lungs, liver, heart, kidneys, penis, joints, bones, or skin.Hemoglobin is a protein in red blood cells that carries oxygen throughout the body (Serjeant, 2010). Biochemical alterations have been associated with HbSS and HbSC (Oladipo *et al.,* 2005). HbSS and HbSC is a hereditary disorder of hemoglobin synthesis that can affect the skeletal system owing to accelerated hematopoiesis and bone infarction (Nelson *et al.,* 2003; Nouraie *et al.,* 2011). The level of alkaline phosphatase indicates severity of bone damage and is a useful guide of progress in the management of bone pains in HbSS and HbSC (Afonja and Boyd*.,* 1986; Adewoye *et al.,* 2008). HbSS and HbSC is associated with impaired urinary potassium excretion. Many renal abnormalities have been associated with HbSS and HbSC (Allon, 1990). Total protein concentration is determined by the nutritional state, hepatic function, renal function, and various disease states and hydration. Contribution of bone turnover to the hyper-catabolic state observation in sickle cell anemia is unknown while a study state the increased rates of bone turnover contribute to the increased rates of protein turnover and energy expenditure observed in HbSS (Buchowski., 2001). Hepatic dysfunction is a commonly recognized complication of HbSS and HbSC due to multiple factors such as intra-hepatic sinusoidal sickling, bilirubin gallstones, transfusion-related hepatitis infections or excess iron deposition (Schubert, 1986; Kakarala and Lindberg, 2004; Beutler, 1999). Elevated aminotransferase levels are commonly associated with compromised hepatic integrity from various pathophysiology. In HbSS and HbSC, aspartate transaminase (AST) is released via intravascular hemolysis (Nsiah *et al* 2011). Serum creatinine may be associated with renal insufficiency in sickle cell patients. When serum creatinine is elevated the disease has reached an advanced stage and lead to renal failure. Deoxygenation of sickle cell is known to increase cation permeability of sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) (Vitoux *et al.,* 1989; Rhoda *et al.,* 1990; Gibson *et al.,* 1998). Urea, an end product of protein metabolism, is excreted by the kidney. Blood urea nitrogen directly related to protein intake and nitrogen metabolism and inversely related to the rate of excretion of urea. Urea concentration in glomerular filtrate is the same as in plasma, but its tubular re-absorption is inversely related to the rate of urine formation. Urea may play a significant role in the precipitation of sickling crisis (Vener 1977). AST:ALT (Alanine Aminotransferase) ratio may play role as a hemolytic marker, because it has an inverse association with the hemoglobin level. Whether in steady state or in crisis, provided hepatic and cardiac integrity has not been compromised, subjects with HbSS and HbSC would have higher AST levels due to the hemolytic nature of the condition (Maddrey *et al.,* 1978). In sickle cell anemia the shortened survival of red blood cells presents the liver with an augmented load of bilirubin for hepatic clearance (Conger, 1990). Uric acid is an end product of nucleoprotein metabolism and is excreted by the kidney. An increase in serum uric acid concentration occurs with increased nucleoprotein synthesis or catabolism or decreased renal excretion. It poses a special problem for humans because of its limited solubility, particularly in the acidic environment of the distal nephron of the kidney (Diamond *et al.,* 1979). Hyperuricemia occurs only in patients who develop altered renal tubular function with diminished urate clearance secondary to diminished urate secretion (Diamond *et al.,* 1979).

Zinc is an essential trace metal, which is second only to Iron. It is present in Zinc metalloenzymes e.g. carbonic anhydrase, alkaline phosphatase, R.N.A and D.N.A polymerases, thimidine kinase, carboxypeptidases and alcohol dehydrogenase. Zinc is an essential element; it is a critical cofactor for carbonic anhydrase, alkalinephosphatase, RNA and DNA polymerases, alcohol dehydrogenase, and many other physiologically important proteins. The peptidases, kinases, and phosphorylases are most sensitive to zinc depletion. Zinc is a key element required for active wound healing (Zorbas *et al.,*2008). Zinc depletion occurs either because it is not absorbed from the diet (excess copper or iron interfere with absorption) or it is lost after absorption. Dietary deficiency may be due to absence (parenteral nutrition) or because the zinc in the diet is bound to phytate (fiber) and not available for absorption. Excess copper and iron in the diet (eg, iron supplements) interfere with zinc uptake (Skelton *et al.,* 2006; Burri *et al.,* 2008).

Selenium is an essential element. It is a cofactor required to maintain activity of glutathione peroxidase (GSH-Px), an enzyme that catalyzes the degradation of organic hydroperoxides. The absence of selenium correlates with loss of GSH-Px activity and is associated with damage to cell membranes due to accumulation of free radicals.The normal daily dietary intake of selenium is 0.01 to 0.04 parts per million (ppm), which is similar to the typical content of soil (0.05 ppm) and sea water (0.09 ppm) (Stadtman and Thressa 2002). In humans, cardiac muscle is the most susceptible to selenium deficiency. With cell membrane damage, normal cells are replaced by fibroblasts. This condition is known as cardiomyopathy and is characterized by an enlarged heart whose muscle is largely replaced by fibrous tissue (Aringer and Graessler, 2008). Selenium is not classified as a human teratogen. Glutathione Peroxidase to test your selenium levels – low selenium levels can cause low immunity, constant fatigue and reproductive problems (Kaneko, 1999).

2. Aim and Objective

This work was designed to determine the plasma level of ALT, AST, Zn,Se, Albumin and Uric acid in patients with HbSC and HbSS in crisis and in non- crisis steady state using HbAA individuals as control subject in a rural community.

3. Materials and Methods

3.1 Materials

3.1.1 Study area
Saki –West local government area which is located at the Nothern-part of Oyo state. It shares border with Benin republic, Kwara State, Saki-East and ATISBO local government areas of Oyo State-Nigeria.

3.1.2 Study population and criteria
HbSS and HbSC patients in crisis and in non-crisis steady state classified into HbSC (n=25) and HbSS(n=25) aged 10 to 31 years attending Baptist Medical Centre, Saki – Nigeria were recruited as test subjects for the study. Apparently healthy 50 HbAA volunteers classified into female(n=25) and male(n=25) aged 10 – 50years were also studied as control.

Five (5) mililitres of venous blood was obtained into each of the subjects into Lithium heparinized tube for haemoglobin electrophoresis and estimation of ALT,AST, Albumin, Selenium, Zinc and Uric acid.

Only HbSS and HbSC in crisis and non-crisis steady state were recruited.

Subjects with evidence of liver disease were excluded.

Subjects who are alcoholics and cigarette smokers were excluded.

3.2 Methods

3.2.1 BromoCresolGreen method
Serum albumin estimation was carried out on all the volunteers [test and control] by BromoCresolGreen method.

Principle of the test: Bromocresol green is an indicator which is yellow between P^H 3.5 – 4.2.

When it binds to albumin the colour of the indicator changes from yellow to blue – green. The absorbance of the colour produced is measured in a colorimeter using an orange filter in a spectrophotometer at 632 nm wavelengths.

3.2.2 Determination of serum levels of Zn and Se.

Serum levels of these elements were determined with flame atomic absorption spectrophotometer (AAS) using a direct method described by Kaneko (Kaneko, 1999).

Principle of the test: the method is based on the principle that atoms of the element when aspirated into AAS vaporized and absorbed light of the same wavelength as emitted by the element when in the excited state.

Procedure:

1. Atomic Absorption Spectrophotometric measurement of the micronutrients concentrations were performed on a BECK 200 atomic absorption spectrophotometer.

2. Flame was ignited and lamp switched on.

3. The instrument was allowed to warm up while aspirating de-ionized water into the flame and then adjusted to zero absorbance.

4 Standards of corresponding analyte was aspirated to standardize the machine. This standardization was repeated periodically to asses any drift of sensitivity.

5. The smples kept frozen were thawed and 1:25 dilution was made for Mg while samples for other micronutrients under investigation were aspirated directly into AAS for analysis.

6. The results of the assays were displayed digitally on the instrument.

Calculation: Meter reading multiply by dilution factor multiplies by PPM of standard.

3.2.3 Aspartate aminotransferase

Aspartate aminotransferase (AST/SGOT) was determined in all the sera of the volunteers spectrophotometrically by using the reagent kit of; Cromatest Linear Chemicals S.L, Joaquim Costa,18, 2^a Planta ,08390 Montgat– Barcelona(Spain). http://www.linear.es

Priciple of test: Aspatate aminotransferase (AST/SGOT) catalyses the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate and oxaloacetate.The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD^+, proportional to the activity of AST present in the sample.

L-aspartate + 2-oxoglutarate AST/SGOT L-glutamate + oxaloacetate

Oxaloacetate + NADH + H^+ MDH L-malate + NAD^+

3.2.4 Alanine aminotransferase

Alanine aminotransferase (ALT/SGPT) was determined in all the sera of the volunteers spectrophotometrically by using the reagent kit of; Cromatest Linear Chemicals S.L, Joaquim Costa,18, 2^a Planta, 08390 Montgat-Barcelona(Spain). http://www.linear.es

Priciple of test: Alanine aminotransferase (ALT/SGPT) reversibly transfers the amino group from alanine to alpha – ketoglutarate, forming pyruvate and glutarmate. The rate of formation of pyruvate is determined by coupling the ALT reaction with that of lactate dehydrogenase (LDH), which converts the pyruvate to lactate; the decrease in absorbance at 340nm is measured as NADH is oxidized to NAD^+.

3.2.5 Uric acid

Uric acid was determined using RANDOX reagent kit.

3.2.6 Haemoglobin electrophoresis

Haemoglobin electrophoresis was carried on each of the subjects as described by Chessbrough (Cheesbrough, 2002).

3.3 Ethical Consideration

The proposal of this work was submitted to the Research and Ethical committee of Baptist Medical Centre, Saki-Nigeria for consideration. The proposal was approved before the commencement of this work.

3.4 Method of Data analysis

The raw data was subjected to statistical analysis using SPSS 18.0 to determine the mean, standard deviation, probability, 't' test value and level of significance at 0.05.

4. Results

The results obtained showed that there was no significant difference in the mean values of ALT, AST, albumin, Zn, Se and Uric acid obtained in HbSC and HbSS with p>0.05(Tables 1 &4; figure 1). However, there was a significantly higher mean value of plasma ALT and AST in HbSC and HbSS than HbAA with p<0.05(Tables 1 &4). The result also showed a significantly lower plasma selenium mean value in HbSC and HbSS than HbAA with p<0.05(Tables 1 &4). There was no significant difference in the mean values of plasma albumin and uric acid in HbSS and HbSC in crisis compared to when they were in non-crisis steady date(p>0.05)(Tables 1,2,3; figures 1,2).This difference was also found in the plasma value of ALT and Se in HbSS patient in crisis and in non-crisis steady state. There was a significantly higher plasma value of ALT, AST and a lower plasma Zn and Se in HbSS patients in crisis than in non-crisis steady state with p<0.05. There was a significantly higher plasma value of AST and a lower plasma Zn in HbSS patients in crisis than in non-crisis steady state with p<0.05 (Tables 1,2,3 ; figures 1,2).

Table 1. The mean and standard deviation of the values of ALT,AST,albumin, Zn, Se and Uric acid obtained in HbSC, HbSS in non-crisis steady state and HbAA subjects.

	HbSC(n=25)	HbSS(n=25)	HbAA(n=50)
ALT(U/L)	64±5.0	68±5.0	17±3.0
AST(U/L)	51±4.0	58±2	10±2;0
Albumin(g/dl)	3.0±0.4	2.8±0.5	4.1±0.6
Zn(mcg/ml)	0.41±0.01	0.38±0.01	0.9±0.03
Se(ng/ml)	58±3.0	55±4.0	86±3.0
Uric acid(mg/dl)	3.0±0.4	2.7±0.4	3.9±0.5

Table 2. The mean and standard deviation of the values of ALT,AST,albumin, Zn, Se and Uric acid obtained in HbSC, HbSS in crisis and HbAA subjects.

	HbSC(n=25)	HbSS(n=25)	HbAA(n=50)
ALT(U/L)	69±2.0	86±3.0	17±3.0
AST(U/L)	68±3.0	79±2.0	10±2;0
Albumin(g/dl)	3.2±0.3	3.0±0.3	4.1±0.6
Zn(mcg/ml)	0.3±0.01	0.29±0.02	0.9±0.03
Se(ng/ml)	51±2.0	49±3.0	86±3.0
Uric acid(mg/dl)	3.9±0.3	4.5±0.2	3.9±0.5

Table3. Comparative analysis of the biochemical alterations in HbSS and HbSC in crisis and in non-crisis steady state.

		ALT	AST	Albumin	Zn	Se	Uric acid
HbSS in crisis and in non-crisis steady state	't'	3.09	7.43	0.343.	-4.03	-3.2	1.88
	'p'	0.045	0.009	0.38	0.028	0.043	0.10
	comment	p<0.05**	p<0.05**	p>0.05*	p<0.05**	p<0.05**	p>0.05*
HbSS in crisis and in non-crisis steady state	't'	0.93	3.4	0.99	-7.78	-1.94.	1.8.
	'p'	0.23	0.038	0 .21	0.008	0.096	0.11
	comment	p>0.05*	p<0.05**	p>0.05*	p<0.05**	p>0.05*	p>0.05*

Table 4. Shows the level of significance of the difference in the mean value of the biochemical parameters obtained in HbSS, HbSC and HbAA.

|		HbSC/HbSS	HbSC/HbAA	HbSS/HbAA
ALT(U/L)	't' value	-0.56569.	-3.48526.	8.74643
	'p' value	0.314305*	0.036689**	0.006411**
AST(U/L)	't' value	-1.56525.	9.16788	16.97056
	'p' value	0.129001*	0.005845**	0.001727**
Albumin(g/dl)	't' value	0.31235.	-1.52543	-1.66448
	'p' value	0.392167*	0.133333*	0.118963*
Zn(mcg/ml)	't' value	2.12132	-1.63243	-1.73237
	'p' value	0.083975*	0.122092*	.112673*
Se(ng/ml)	't' value	0.6	-6.59966	-6.2
	'p' value	0.304717*	0.011099**	0.012521**
Uric acid(mg/dl)	't' value	0.53033	-1.40556	-1.87409
	'p' value	0.324438*	0.147533*	0.100886*

 *Not Significant   **Significant
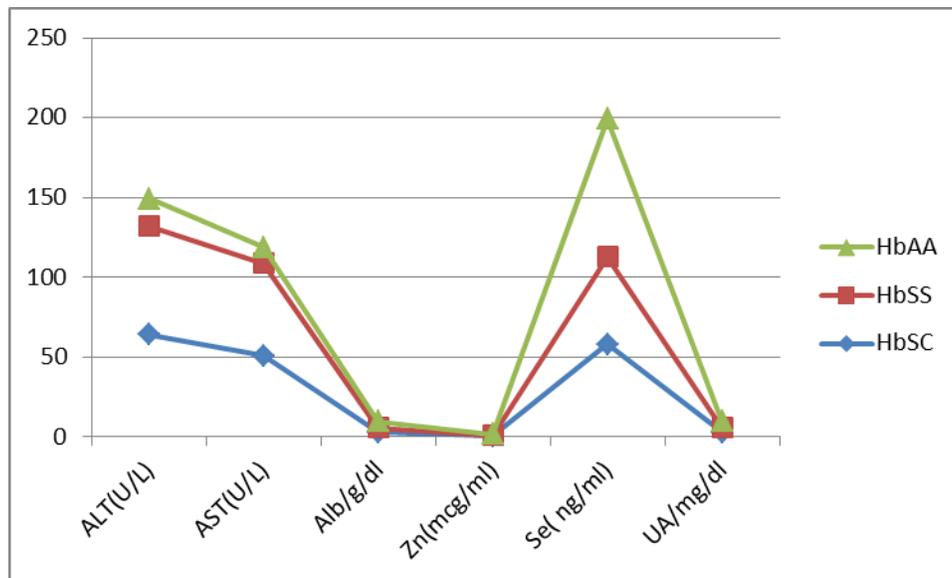
Figure 1. The mean and standard deviation of the values of ALT, AST, albumin, Zn, Se and Uric acid obtained in HbSC, HbSS in non-crisis steady state and HbAA subjects.
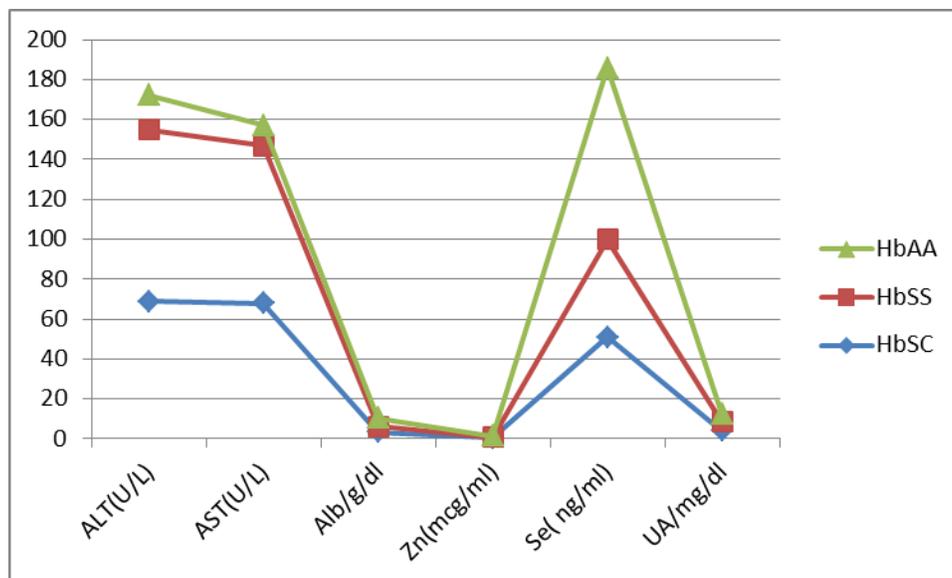

Figure 2. The mean and standard deviation of the values of ALT, AST, albumin, Zn, Se and Uric acid obtained in HbSC, HbSS in crisis and HbAA subjects.

5. Discussion

Significantly higher mean value of plasma ALT and AST found in HbSC and HbSS than HbAA is consistent with the report of Chuku *et al.*, (Chuku *et al.,* 2012). ALT is found in the liver and could be released to the blood circulation when the liver is injured or disturbed.

This will therefore result into plasma elevation of ALT. This finding could also be associated with the fact that the red blood cell sickling and poor oxygen delivery can cause organ damage. Over a lifetime, SCD can affect spleen, brain, eyes, lungs, liver, heart, kidneys, penis, joints, bones, or skin (Banerjee *et al*., 2001) AST is found in the liver as well as other tissue including red

blood cells. Sickle cell disease is characterized by haemolysis and liver is also involved in the pathophysiology. These could cause leakage of AST into the blood circulation. Patients with SCD commonly present with one of several acute hepatic syndromes that manifest clinically as fever, right upper quadrant pain, and jaundice. Frequent haemolytic crisis leading to anaemia and shorter life span of sickle cells than normal could be contributory to increased AST leaked into the plasma as a result of massive red blood cell damage (Diggs and Bell, 1965. Patients with acute sickle hepatic cri- sis typically present with in- creased serum AST and ALT levels up to 300 U/L and an increased serum bilirubin level up to 15 mg/dL. (Sheehy , 1977; Schubert , 1986; Susan *et al.,* 2009).

Significantly lower mean value of plasma selenium was found in HbSC and HbSS than HbAA. One of the hallmarks of both sickle cell disease (SCD) and thalassemia major (TM) is accelerated oxidative damage. Decreased antioxidant levels and increased oxidant stress biomarkers are found in both diseases (Susan *et al.,* 2009).. Susan *et al.,* 2009 reported that 40–75% of the patients were deficient in A, C, D and selenium and 28–38% of the patients had low levels of B vitamins and folate which is consistent with the findings of this study. Selenium is an antioxidant that prevents cellular damage which is common in sickle cell disease that may warrant excessive use of Selenium leading to decresed concentration as found in this study. Mona *et al.,* 2015 also found a depleted antioxidants level in the studied group of Egyptian children with TM and SCD relative to healthy controls.

There was a significantly higher plasma value of AST and a lower plasma Zn in HbSS patients in crisis than in non-crisis steady state. There was also a significantly higher plasma value of ALT, AST and a lower plasma Zn and Se in HbSS patients in crisis than in non-crisis steady state. This finding is consistent with the report of Oladipo *et al.,* 2005 that associated biochemical alterations with HbSS and HbSC which is more exacerbated in sickle cell crisis. Elevated aminotransferase levels are commonly associated with compromised hepatic integrity

from various pathophysiology (Banerjee *et al.,* 2001). Elevated AST and ALT in this study could be due to the liver involvement in the pathophysiology of sickle cell diseases/crisis due to the fact that AST and ALT are liver enzymes that could leak to the plasma in hepatic disorder This finding is also consistent with the report of Nsiah *et al.,*2011 that in HbSS and HbSC, aspartate transaminase (AST) is released via intravascular hemolysis. In addition there used to be massive haemolysis in sickle cell crisis making the liver to metabolise RBC excessively which may bring about liver disorder hence the biochemical alterations in the value AST and ALT (Banerjee *et al.,* 2001; Nsiah *et al.,*2011)

Zn and Se are antioxidants and acute phase metals. In crisis they are more utilized to prevent or scavenge oxidation which could be responsible for the decreased concentration in HbSS and HbSC patients crisis compared with those in non-crisis steady state. As acute phase metals they have their level decreased on infection and inflammation which was found in this study (Banerjee *et al.,* 2001; Mona *et al.,* 2015).

6. Conclusion

The biochemical profile of sickle cell patients has been investigated to reveal Significantly lower mean value of plasma selenium in HbSC and HbSS than HbAA and a Significantly higher mean value of plasma ALT and AST in HbSC and HbSS than HbAA. There was an increase in the plasma activities of ALT and AST and a decrese in the plasma value of Zn and Se in HbSS and HbSC in crisis than in non-crisis steady state.

Recommendation

It is therefore suggested that the routine Laboratory investigation of HbSS and HbSC should include measurement of AST,ALT, Zn and Se in crisis and in non-crisis steady state for effective management.

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