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EVALUATION OF SOME HAEMATOLOGICAL PARAMETERS, LIVER ENZYMES AND BILIRUBIN IN SICKLE CELL DISEASED PATIENTS ATTENDING FEDERAL UNIVERSITY TEACHING HOSPITAL, OWERRI, NIGERIA

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Abstract: Background: Sickle cell disease is a group of haemoglobin-related blood disorders typically inherited. It has remained a clinical burden in the world, particularly in Nigeria. Aim: This study was aimed at evaluating some haematological parameters liver enzymes and bilirubin in sickle cell diseased patients attending Federal University Teaching Hospital, Owerri, Imo State. Method: The study included a total of 60 individuals, 30 of them were sickle cell patients and 30 were apparently healthy individuals who served as controls. 7mls of venous blood was collected from each subject using the standard clean venipuncture technique. Samples for packed cell volume (PCV), haemoglobin (Hb) and total white blood cell (TWBC) estimations were aliquoted into EDTA containers while that of liver enzymes and bilirubin estimations were dispensed into plain tubes. Haematological parameters were determined using a hematological manual methods, while Aspartate transaminase (AST) and Alanine transaminase (ALT) concentrations were determined using Kinetic method and bilirubin was determined using Jendrassic and Grof method. The statistical analysis was done using software statistical package for social sciences (SPSS) version 22.0 and mean, standard deviation, student t-test and correlations were determined. Results: The mean values of PCV (22.17±4.35)% and Haemoglobin concentration (7.61±1.59)g/dl were significantly reduced in sickle cell patients when compared to controls (35.70±2.96)% and (11.92±1.26)g/dl,(p=0.000). The mean value of TWBC (17633.33±9859.99)cells/µl was significantly increased (p=0.000) in sickle cell patients when compared to controls (6765.00±1923.61)cells/µl,(p=0.000).The mean values of AST (17.77±12.97)IU/L, ALT (11.63±6.22)IU/L, Conjugated bilirubin (1.43±1.68)mg/dl and Total bilirubin (5.06±5.13)mg/dl were significantly increased in sickle cell patients when compared to controls (6.05±2.89)IU/L, (5.30±1.42)IU/L, (0.73±0.25)mg/dl and (1.40±0.32)mg/dl (p=0.000, p=0.000, p=0.003 and p=0.003). Conclusion: The findings have shown that sickle cell disease is associated with a decrease in PCV and haemoglobin, an increase in total white blood cell, AST, ALT and total bilirubin levels.
Keywords: Sickle cell; Haemoglobin; Packed cell volume; White blood cell; Bilirubin; Liver enzymes

1 INTRODUCTION

Sickle cell disease (SCD) is a group of blood disorders typically inherited. The most common type is known as sickle cell anaemia. It results in an abnormality in the oxygen-carrying protein haemoglobin found in red blood cells [1]. As of 2015, about 4.4 million people have sickle cell disease, while an additional 43 million have sickle cell trait [2]. About 80% of sickle cell disease cases are believed to occur in Sub-Saharan Africa.

Biochemical abnormalities have been associated with sickle cell disease (SCD). SCD is an hereditary disorder of hemoglobin synthesis that can affect the skeletal system owing to accelerated hematopoiesis and bone infarction [3]. Almost all the blood cells in the human body are red blood cells. PCV blood test or haematocrit test is one of the tests that measure the proportion of RBC in a blood sample. The count of PCV rises due to the increase in the count of red blood cells or when the total volume reduces, as in the case of dehydration. In case the PCV becomes less than normal, it indicates anaemia [4]. While higher counts or values of Hb are linked with higher rates of severe pain in SCD patients, lower steady-state Hb usually accounts for higher risk of stroke in these same patients [5].

Previous reports have demonstrated that high leukocyte count appears to be a risk factor for several severe complications of SCD, such as rates of severe pain, acute chest syndrome, and mortality. A previous report indicated that SCD patients have elevated white blood cell (WBC) counts, activated granulocytes, monocytes, and endothelial cells, enhanced expression of endothelial cell adhesion molecules, elevated cytokine levels and elevated acute-phase reactants. Moreover, another study

has reported that the use of drugs, such as Hydroxyurea, lowers WBC count and thus improves the clinical outcome of SCD patients [6].
Raised bilirubin levels, predominantly unconjugated, are universal in sickle cell patients due to chronic hemolysis. Elevation of the different liver enzymes correlates with the different categories; hemolysis raises plasma aspartate transaminase (AST), while plasma alanine transaminase (ALT) levels more accurately reflects hepatocyte injury (Rasheed *et al.,* 2014).Elevated aminotransferase levels are commonly associated with compromised hepatic integrity from various pathophysiology. In SCD, aspartate transaminase (AST) is released via intravascular hemolysis [7].
There is paucity of information on the levels of haematological parameters, bilirubin and liver enzymes in sickle cell disease patients in Owerri. This study is therefore aimed at evaluating the levels of haemoglobin (Hb), packed cell volume (PCV), White blood cell (WBC), Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Bilirubin in sickle cell diseased patients attending federal university teaching hospital, Owerri Imo State.

2 MATERIALS AND METHODS

2.1 Study Area

The study was carried out at Federal University Teaching Hospital, Owerri, Nigeria.

2.2 Study Design

A cross-sectional study were used. The period of subjects enrollment, classification, administration of questionnaires, sample collection, determination of hematological parameters and biochemical parameters and data generation in this study lasted from June to August 2023. Additional demographic data were obtained using the study questionnaires. These included basic and socioeconomic information, medical health history etc. About 80 subjects were given questionnaires; out of which 30 gave there informed consent. Blood samples were collected and analyzed in the laboratory using standard operating procedure. The results of the tests were analyzed using SPSS version 22.0.

2.3 Study population

The present study included a total of 60 individuals; 30 out of them were sickle cell patients and 30 were healthy individuals. All were of average age 10-40 years. All subjects were investigated for PCV, Hb, total WBC, liver enzymes and bilirubin.

2.4 Ethical Approval

The research study was approved by the ethics committee of the federal university teaching hospital, Owerri, Nigeria. A written informed consent was obtained from the subjects before sample collection.

2.5 Sample Collection

Seven milliliters (7mls) of venous blood was collected from each subject using the standard clean venipuncture technique and 2 mls was dispensed into labeled EDTA bottles for Total WBC, PCV, and Hb estimations, while 5mls was aliquoted into plain bottles for AST, ALT, and bilirubin estimations.

2.6 Laboratory Analysis

Hemoglobin estimation was done (Hb) using cyanmethemoglobin method. Total white blood cell count was determined using manual cell count method, AST and ALT estimations were performed using the kinetic method (Randox reagent kits), while bilirubin estimation was done using the Jendrassik and Grof Method.

2.7 Statistical Analysis

The statistical analysis was done using software statistical package for social sciences (SPSS) version 22.0. The results were expressed as mean and standard deviation (mean ±SD). Difference in mean values between groups was assessed by student t-test. Tests with a probability value of $p< 0.05$ was considered statistically significant.

3 RESULTS

The mean values of PCV (22.17±4.35)% and Haemoglobin concentration (7.61±1.59)g/dl were significantly reduced in sickle cell diseased patients when compared to controls (35.70±2.96)% and (11.92±1.26)g/dl.(t=12.15, p=0.000 and t=10.16, p=0.000). The mean values of TWBC (17633.33±9859.99)cells/µl, AST (17.77±12.97)IU/L, ALT (11.63±6.22)IU/L, Conjugated bilirubin (1.43±1.68)mg/dl and Total bilirubin (5.06±5.13)mg/dl were significantly increased

(6765.00±1823.61)cells/ul in sickle cell diseased patients when compared to controls (6.05±2.89)IU/L, (5.30±1.42)IU/L, (0.73±0.25)mg/dl and (1.40±0.32)mg/dl (t= 4.85,p=0.000; t=3.96, p=0.000; t=4.46, p=0.000; t=1.83, p=0.003; and t=3.18, p=0.003) (Table 1)

Table 1 Mean Values of PCV, Haemoglobin, TWBC, AST, ALT, Bilirubin (Conjugated Bilirubin, Total in Sickle Cell Patients Versus Controls (Mean ±SD)

Parameter	Test N=30	Control N=30	t-value	p-value
PCV (%)	22.17±4.35	35.70±2.96	12.15	0.000*
Hb (g/dl)	7.61±1.59	11.92±1.26	10.16	0.000*
TWBC (cells/µl)	17633.33±9859.99	6765.00±1923.61	4.85	0.000*
AST (IU/L)	17.77±12.97	6.05±2.89	3.96	0.000*
ALT (IU/L)	11.63±6.22	5.30±1.42	4.46	0.000*
CB (mg/dl)	1.43±1.68	0.73±0.25	1.83	0.003*
TB (mg/dl)	5.06±5.13	1.40±0.32	3.18	0.003*

KEY: PCV= Packed cell volume; HB= Haemoglobin; TWBC= Total white blood cell; AST= Asparlate transaminase; ALT= Alumine transaminase; CB= Conjugated Bilirubin; TB= TotalBilirubin

There were no significant difference in the mean values of PCV, Haemoglobin, TWBC, AST, ALT, Conjugated and Total bilirubin in male sickle cell patients (23.41±4.17)%, (8.05±1.52)g/dl, (15105.88±7000.76) cells/µl, (17.12±9.86) IU/L, (11.82±5.79) IU/L, (1.27±1.53)mg/dl and (5.82±5.49)mg/dl when compared to female sickle cell patients (20.54±4.18)%, (7.02±1.54)g/dl, (20938.46±12194.91) cells/µl, (18.62±16.59)IU/L, (11.38±6.98)IU/L, (1.63±1.91)mg/dl and (4.08±4.65)mg/dl.(t= 1.87, p= 0.072; t= 1.83, p= 0.079; t= 1.65, p= 0.110; t=0.31, p= 0.760; t= 0.09, p= 0.852; t= 0.57, p= 0.571 and t= 0.92, p= 0.366) (Table 2).

Table 2 Mean Value of PCV, Haemoglobin, TWBC, AST, ALT, Bilirubin, (Total and Conjugated) in Males Sickle Cell Patient Vs Females (Mean ± SD)

Parameter	Male N= 17	Female N= 13	t-value	p-value
PCV (%)	23.41±4.17	20.54±4.18	1.87	0.072
Hb (g/dl)	8.05±1.52	7.02±1.54	1.83	0.079
TWBC (cells/µl)	15105.88±7000.76	20938.46±12194.91	1.65	0.110
AST (IU/L)	17.12±9.86	18.62±16.59	0.31	0.760
ALT (IU/L)	11.82±5.79	11.38±6.98	0.19	0.852
CB (mg/dl)	1.27±1.53	1.63±1.91	0.57	0.571
TB (mg/dl)	5.82±5.49	4.08±4.65	0.92	0.366

There was a non-significant positive association of PCV with AST, ALT, Conjugated and Total bilirubin in sickle cell diseased patients (r=0.04, p=0.837; r=0.05, p=0.800; r=0.949 and r=0.08, p=0.675) (Table 3).

Table 3 Correlation of PCV with AST, ALT, Bilirubin and (Total and Conjugated) in Sickle Cell Diseased Patients

Variable	n	r	p-value
AST(IU/L)	30	0.04	0.837
ALT (IU/L)	30	0.05	0.800
CB (mg/dl)	30	0.01	0.949
TB (mg/dl)	30	0.08	0.675

There was a non-significant positive association of TWBC with AST, ALT, Conjugated bilirubin and Total bilirubin in sickle cell diseased patients. (r=0.06, p=0.742; r=0.29, p=0.118; r=0.01, p=0.942 and r=0.25, p=0.189) (Table 4).

Table 4 Correlation of TWBC with AST, ALT, CB and TB in Sickle Cell Diseased Patients

Variable	n	r	p-value
AST (IU/L)	30	0.06	0.742
ALT (IU/L	30	0.29	0.118
CB (mg/dl)	30	0.01	0.942
TB (mg/dl)	30	0.25	0.189

KEYS: AST= Aspartate Transaminase; ALT= Alanine Transaminase; CB= Conjugated Bilirubin; TB= Total Bilirubin

4 DISCUSSION

Sickle cell disease (SCD) is an inherited autosomal recessive disease, which manifests as chronic haemolyticanaemia, painful episodes of vaso-occlusive crisis and polysystemic organic damage [8].

In the present study, the mean values of Hb and PCV were significantly reduced in sickle cell patients when compared to controls. This could be due to the haemolysis that occurs in sickle cell anaemic patients and accounts for the reduction in PCV and haemoglobin level. The reduction in haemoglobin and PCV levels in sickle cell patients could also be due to nutritional deficiency. The finding of this study is similar to the report by Ngasia et al. (2017) [9], who reported that sickle cell patients suffer from anaemia due to increase in haemolysis.

The mean values of TWBC was significantly increased in sickle cell patients when compared to controls. This is keeping with previous studies which showed that a moderate leukocytosis is a common feature of sickle cell anaemia which is thought to be due to redistribution of leucocyte from a marginal pool of circulating granulocyte [10].

In this study, the mean values of ALT and AST were significantly increased in sickle cell patients when compared to controls. These transaminases (AST and ALT) are marker enzymes for liver toxicity especially ALT which is more specific to the liver. Elevated aminotransferases (AST and ALT) levels are commonly associated with compromised hepatic integrity where increase in ALT is far higher than AST while in intravascular haemolysis of the red blood cells, AST levels is higher than ALT as previously reported by Nsiah et al. (2021) [7].

The mean value of total bilirubin was significantly increased in sickle cell patients when compared to controls. Hyperbilirubinemia is common in patients with sickle cell disease and is mainly unconjugated and derives from chronic RBC hemolysis. The result of this study is in consistent with the report by Johnson et al. (2015) [11], who reported that total bilirubin is usually above 6 mg/dl and the unconjugated bilirubin is mainly raised.

Total bilirubin comprises of unconjugated bilirubin and conjugated bilirubin, the conjugated bilirubin is mainly elevated in cases of liver disease such as hepatobillary disease, while the unconjugated is mainly elevated in cases of hemolysis [11]. There was a significant negative correlation of total bilirubin with Hb and PCV. This explains that as haemolysis increases the level of PCV and Hb reduces. This is similar to the report by Ngasia et al., (2017) [9].

There was a non-significant positive correlation of PCV, TWBC with AST, ALT, conjugated and total bilirubin. This result contradicts the findings of Harkness (2018) [10], who reported that haemolysis brings about an increase in total bilirubin, and there is an increase in the marginal pool of distribution of leucocyte. This is also in agreement with the study carried out Viktória et al. (2018) [12], who reported a similar finding. Though factors such as sample size, treatment regimen may have influenced the result.

5 CONCLUSION

The findings have shown that sickle cell disease is associated with a decrease in PCV, haemoglobin and an increase in total white blood cell, AST, ALT and total bilirubin levels. But there is no significant relationship between sickle cell anaemia, and liver disease.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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