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Assessment of Some Haemolysis Biomarkers in Steady State Sickle Cell Anaemia Patients in Kano, Northern Nigeria.

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Abstract
Patients with sickle cell disease (SCD) have been associated with extravascular and intravascular haemolysis. However, scanty information is available on the levels of haemolysis parameters in this condition, hence; the assessment of some of the haemolysis biomarkers in steady state sickle cell anaemia patients was carried out. Forty-five sickle cell anaemia patients (23 males and 22 females) in steady state with age range of 10-24 years from the Sickle Cell Clinic of Murtala Muhammad Specialist Hospital, Kano and 30; age-matched apparently healthy individuals (HbAA subjects) resident in Kano metropolis were recruited for this study between October 2018 and September, 2019. The blood samples collected from the participants were estimated for packed cell volume (PCV), haptoglobin, lactate dehydrogenase (LDH) and total bilirubin using standard techniques. The study showed significantly lower PCV and haptoglobin levels of 0.24±0.04 l/l and 11.99±7.65ng/ml, respectively, and significantly higher LDH and total bilirubin values of 506.82±263.35 U/L and 54.67±27.18 µmol/L, respectively, in steady state SCA patients compared to the values of control group (HbAA subjects) of 0.38±0.03 l/l and 20.01±18.26 ng/ml, respectively, and 329.57±129.33 U/L and 10.27±4.27 µmol/L, respectively ($p<0.05$). There were no significant differences in the values of PCV, haptoglobin, LDH and total bilirubin with respect to age and gender in steady state SCA patients ($p>0.05$). The levels of some haemolysis biomarkers (PCV, haptoglobin, LDH and total bilirubin) were significantly affected in steady state SCA irrespective of age and gender. Therefore, the significant changes of these haemolysis biomarkers can serve as a guide to the clinicians in the management of SCA patients.

Keywords: Assessment, haemolysis biomarkers, sickle cell anaemia.

Introduction

Sickle cell disease (SCD) is one of the most common genetic diseases worldwide but high prevalence rates have been reported in Middle-East, Mediterranean regions, Southeast Asia and Sub-Saharan Africa, especially in Nigeria (WHO, 1989; Serjeant, 1997).

The most frequent phenotype is sickle cell anaemia and it results from the homozygous S mutation (SS), however, sickle cell disease can also result from compound heterozygosity associating HbS and HbC (SC genotype) or HbS and β-thalassaemia (Sβ-thalassaemia genotype). The disease is characterized by haemoglobin polymerization, which includes erythrocyte deformation in various conditions of deoxygenation, and it is responsible for chronic haemolytic anaemia and acute microvascular vaso-occlusions and infarctions (Dubet *et al*., 2017).

Nigeria has the highest birth prevalence of SCD in the world, with an estimation of 150,000 annual births of babies with sickle cell anaemia (SCA) (Piel *et al*., 2013). In Nigeria, SCD affects about 2-3% of the population (Fleming *et al*., 1979) while carrier prevalence is about 20-30% (Fleming *et al*., 1979; Uzoegwu and Onwurah, 2003).

Children with SCD have repeated episodes of painful crises, anaemia and increased susceptibility to infections, with an estimated 50-90% risk of dying before the age of 5 years (Grosse *et al*., 2011; Makani *et al.,* 2011). However, patients with SCA in steady state are free of pains or crisis in the preceding 4 weeks as well as symptoms or signs attributable to acute illnesses (Akinola *et al.*, 1992; Awotua-Efebo *et al*., 2004).

SCD patients are associated with both extravascular and intravascular haemolysis, and it has been estimated that ⅓ of the haemolytic process in SCD is intravascular. Haemoglobin (Hb) released from haemolyssed red blood cells is bound by haptoglobin (Hp), which is then taken up macrophages through the scavenger receptor (CD163). If the binding capacity of haptoglobin is exceeded, concentration of cell free Hb in plasma increases, and the excess cell free Hb is a nitric oxide (NO) scavenger which leads to a nitric-oxide depletion state (Brown, 2011).

According to Kato *et al.* (2006), lactate dehydrogenase (LDH) is a marker of intravascular haemolysis and its elevation in plasma is associated with clinical phenotype of pulmonary hypertension, priapism and leg ulcers or severity of the disease.

Increased serum LDH level in steady state sickle cell disease has been linked to damage to cells from several different organs (Kato *et al*., 2013). However, Nduka *et al.* (1995) reported that increase in LDH is an inherent manifestation of SCA due to the continuous haemolytic events and release from infarcted bone marrow.

Haptoglobin and hemopexin levels were found to be depleted in sickle cell anaemia (Muller-Eberhand *et al*., 1968), however, low haptoglobin and increased plasma free Hb levels were associated with increased protein and nitrotyrosine levels in sickle cell anaemia (Kupesiz *et al*., 2012).

Haemolysis has been associated with low levels of haemoglobin and haptoglobin, and high levels of reticulocyte, mean corpuscular haemoglobin (MCH), plasma cell-free haemoglobin, bilirubin, total lactate dehydrogenase and dominance of LDH-1 isoenzyme in the previous study (Kupesiz *et al.*, 2012).

Despite relatively high prevalence of sickle cell anaemia in Nigeria population, limited studies have been carried out on the levels of haemolysis parameters in this condition. Therefore, the aim of the study was to assess some haemolysis biomarkers (PCV, haptoglobin, LDH and total bilirubin) in steady state sickle cell patients in Kano, Northern Nigeria.

Materials and methods

A total of 75 subjects were recruited for this study and this was made up of 45 steady state SCA patients, aged 10-24years, from Sickle Cell Clinic of Murtala Muhammad Specialist Hospital, Kano and 30, age-matched HbAA subjects (control group) resident in Kano metropolis.

The study was carried out between October 2018 and September, 2019 at Aminu Kano Teaching Hospital, Kano after the ethical approval with reference number MOH/OFF/797/T.I/715 was granted by the Ministry of Health Kano Ethical Committee in May, 2018. Informed consent obtained from all the participants and guardians of individuals less than 18 years.

The criteria for the steady state SCA patients considered for the study included no history of crisis in the past 3 months established by careful and complete physical examination and no history of blood transfusion in the past 3 months while the exclusion criteria included SCA patients undergoing crisis and patients with other conditions that may affect haematological indices such as renal failure, pregnancy and recent blood transfusion of about 3 months prior to the study (Omoti, 2005).

Five milliliters of blood sample was collected through antecubital vein of every participant for analysis. Two milliliters (2ml) of the collected blood was dispensed into EDTA tube for the estimation of PCV while the remaining 3ml of blood was put in a plain non-anticoagulated container. The non-anticoagulated blood was allowed to clot and centrifuged to obtain serum,

which was used for the determination of LDH, haptoglobin and total bilirubin. PCV was determined using micro-haematocrit method as described by Bull *et al.* (2000) while LDH and total bilirubin levels were determined by LDH-Liquizyme and Jendrassik Grof kits (Spectrum Diagnostics reagents manufactured by Egyptian Company for Biotechnology (S.A.E.), Cairo, Egypt) according to manufacturers' instructions. However, haptoglobin level was estimated using Haptoglobin detection kit (Melsin Medical Company Limited, Jilin Province, China).

Statistical analysis

This was done using the statistical package for social sciences (SPSS) version 22.0 software. The data were represented as means

Table 1: The levels of haemolysis biomarkers in steady state sickle cell anaemia patients

Parameters	HbAA subjects (n=30)	HbSS patients (n=45)	P-value
PCV (l/l)	0.38 ± 0.03	0.24 ± 0.04	0.0001
Haptoglobin (ng/ml)	20.01 ± 18.26	11.99 ± 7.65	0.0105
LDH (U/L)	329.57 ± 129.33	506.82 ± 263.35	0.001
Total bilirubin (µmol/L)	10.27 ± 4.27	54.67 ± 27.18	0.0001

Table 2: Gender influence on haemolysis parameters in sickle cell anaemia patients in steady state.

Parameters	Males (n=23)	Females (n-22)	P-value
PCV (l/l)	0.24 ± 0.04	0.23 ± 0.04	0.2923
Haptoglobin (ng/ml)	12.9 ± 6.96	11.04 ± 8.36	0.4209
LDH (U/L)	498.47 ± 289.78	515.54 ± 239.13	0.8308
Total bilirubin (µmol/L)	60.23 ± 27.78	48.86 ± 25.88	0.1681

Table 3: Effect of age on haemolysis parameters in sickle cell anaemia patients in steady state.

Parameters	10-24years (n=8)	15-19years (n=29)	20-24years (n=8)	P-value
PCV (l/l)	0.23 ± 0.03	0.24 ± 0.04	0.24 ± 0.05	0.818
Haptoglobin (ng/ml)	8.36 ± 1.55	13.41 ± 8.86	10.48 ± 4.99	0.214
LDH (U/L)	512.0 ± 76.55	486.55 ± 298.44	575.13 ± 258.37	0.710
Total bilirubin (µmol/L)	51.75 ± 14.64	54.0 ± 29.77	60.0 ± 28.99	0.819

Discussion

This study has revealed significantly lower levels of PCV in steady state SCA patients compared to the control group (HbAA subjects). This is in agreement with the previous reports on the same subjects (Moreira *et al.,* 2015; Antwi-Boasiako *et al.*, 2018). However, the reduced PCV level in SCD has been associated with chronic haemolysis (Ashutosh and Elliot, 2005), shortened red cell survival (Iheanacho, 2015) and reduced erythropoietin response (Sherwood *et al.*, 1986).

The study showed that there was no significant difference between the PCV values in males and females with SCA in steady state. These findings are comparable to the earlier reports on sickle cell anaemia patients with respect to gender (Iheanacho, 2015; Abubakar *et al.*, 2019).

There is limited information on the effect of age on SCA patients in steady state, however, this study showed no influence of age on the PCV value of SCA patients.

Our study has further shown that haptoglobin level is significantly lower in SCA patients in steady state compared to control group (HbAA subjects). This finding agrees with the previous studies (Muller-Eberhand, 1968; Satiago *et al*., 2018). However, the reduced haptoglobin level has been associated with chronic intravascular haemolysis (Muller-Eberhand, 1968; Satiago *et al.*, 2018). The study further revealed that age and gender had no effects on the haptoglobin levels.

This study has further supported the significantly high value of LDH observed in SCA patients in steady state in previous studies (Adefehinti *et al*., 2015; Mikobi *et al.*, 2015). However, elevated LDH values in sickle cell disease has been associated with haemolysis (Ballas and Marcolina, 2006; Kato *et al.*, 2013), pulmonary hypertension, priapism and leg ulcers (Kato *et al.*, 2006). Influences of age and gender were not revealed in previous studies, however, this study showed that age and gender had no significant effects on LDH levels.

Elevated total bilirubin level in SCA patients in steady state in our study is in conformity with the reports of earlier researchers (Pandey *et al.*, 2012; Akuyam *et al.*, 2014; Adefehinti *et al.,* 2015; Satiago *et al*., 2018). Hyperbilirubinaemia in patients with SCD has been associated with chronic red cells haemolysis (Johnson *et al.*, 1985; Papafragkakis *et al.*, 2014).

Our study also showed that gender does not influence the total bilirubin level of patients with sickle cell anaemia in steady state. This is in agreement with the report of Alkindi *et al.* (2015) but at variance with the findings of Adekile *et al.* (2005), which showed that gender significantly, influenced serum bilirubin level in SCA patients in steady state. However, variation in HbF level has been considered to possibly influence the serum bilirubin levels in males and females with SCA (Alkindi *et al.*, 2015).

This study has shown that age had no effect on total bilirubin level in SCA patients in steady state. This is in conformity with the study from the earlier researcher but at variance with the findings of McKerrell *et al.* (2004), who reported significantly lower level of indirect bilirubin amongst older population (41-67 years) compared to the younger group (12-29 years). However, the contradicting report of Mckerrell *et al*. (2004) could be associated with the older age and inconsistent age groups of SCA patients considered in their study.

In conclusion, the study has shown that sickle cell anaemia patients in steady state are associated with significantly low levels of PCV and haptoglobin, and significantly high values of LDH and total bilirubin irrespective of the age and gender. The alteration of these haemolysis biomarkers can serve as guide to the physicians in the management of SCA patients in steady state.

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