Original Article

HAEMATOLOGICAL PARAMETERS OF PAEDIATRIC STEADY STATE SICKLE CELL DISEASE IN AZARE, NIGERIA.

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Abstract

Background: *Nigeria has the highest burden of sickle cell disease (SCD) in the world, yet the vast majority of its patients do not have access to evidence-based cure. Haematological parameters in steady-state remain useful as indicators for initiating treatment and monitoring response to therapy as well as for prognostication.*

Objectives:*This study aimed to determine the steady- state haematological parameters of children with SCD at a reference hospital in North-eastern Nigeria.*

Methods: *A prospective observational study of children with steady state sickle cell disease, and aged 2 years – 16 years, was conducted from January, 2019 – October, 2020. The following haematological indices were obtained from participants; total leucocyte count, platelet count, haematocrit and haemoglobin concentration. Analysis of data was done with the statistical package for social sciences (SPSS) version 20.0.*

Results*: One hundred patients were recruited. The mean age was 6.97 ± 3.63 years, with 61(61%) males and 39(39%) females giving a male: female ratio of 1.6:1. Mean total leucocyte count, mean platelet count, mean haemoglobin concentration and mean haematocrit were16.83±7.30 x 10^9/L, 356.66±178.27x10^9/L, 7.65±1.45g/dL and 23.51±3.90% respectively. Males had a significantly higher mean platelet count than females (P= 0.02). However, gender-based differences in the other haematological parameters were not statistically significant.*

Conclusion: *The present study found raised levels of total leucocyte counts and platelet counts but low haematocrit and haemoglobin concentrations in children with steady- state SCD. These findings would be helpful in the management of paediatric SCD in this part of Nigeria.*

KEYWORDS: Sickle cell disease; Total leucocyte count; Platelet count; Haematocrit; Haemoglobin concentration.

Introduction

Every year about 300,000 babies are born worldwide with sickle cell disease (SCD). [1] Nigeria with a prevalence of 20- 30/ 1000 (for the most severe SS variant of the disease) live births annually has the highest global burden of SCD. [2] Indeed, the prevalence of the sickle cell trait is estimated to be 61.8% in the north west of Nigeria alone,[3] with mortality figures indicating that SCD accounts for about a quarter of all under- 5 deaths in Sub-Saharan Africa. [4]

Notwithstanding the high prevalence of SCD in Nigeria, the vast majority of children suffering with these diseases do not have access to haematopoietic stem cell transplantation, presently the only evidence-based cure for the disease. [5]However, the therapeutic methods currently being applied here such as chronic blood transfusions and hydroxyurea among others have been shown to reduce the incidence of complications and improve survival.[6] Haematological parameters namely; total leucocyte count (TLC), haematocrit (HCT), Haemoglobin (Hb) concentration and platelet count (PLT) are often useful as indicators for initiating treatment and monitoring response to these therapies as well as for prognostication.

The haematological profile of paediatric SCD have been described in some parts of Nigeria and beyond.[7,8]However, to the best of our knowledge, no such study has been conducted in this part of North- Eastern Nigeria. Local data on the haematological parameters of paediatric SCD is vital given that variations in severity of clinical presentation are often predetermined by genetic and environmental factors. [7,8] It will also help establish baseline data for our setting. This study thus aimed at outlining the steady- state haematological parameters of children with SCD at a reference hospital in North-eastern Nigeria. Gender-based differences in these values were also explored.

Materials and methods

Ethics

Institutional ethical clearance was obtained from the Ethics and Review Committee with ref. number FMCA/COM/36/Vol.iii. Prior to recruitment, written informed consent was obtained from the parent/caregiver of each participant. Consent was also obtained from some participants after providing necessary details about the research. All the patients' data were handled confidentially.

Study design

This was a prospective observational study of children with steady state SCD aged 2 – 16 years. The subjects were recruited from the Paediatric Haematology and Oncology clinics of a reference hospital in northern Nigeria during routine follow-up care over a 22-month period (January, 2019 – October, 2020). All the patients who met the inclusion criteria and gave consent were consecutively recruited into the study.

Sampling and Sample size determination

Inclusion criteria

1. Children with SCD presenting at steady state to the Paediatric haematology and oncology clinics.
2. Age 2 to 16 years.
3. Children whose parents/ caregivers gave written informed consent.

Exclusion criteria

1. SCD patients with acute illnesses.
2. Children who had at least one blood transfusion in the preceding 3 months.

The sample size was calculated with the formula:[9] $n = z^2pq/d^2$ where; n= desired sample size, z= standard normal deviation, p = prevalence, q= 1-p and d= degree of accuracy. The initial calculated sample size was 322.7. However, the finite population correction (for

populations less than 10,000) was applied and a minimum sample size of 90.3 was determined. Nonetheless, 100 children were recruited.

The study subjects were recruited by the investigators, and medical officers posted to the Department of Paediatrics, who then entered the information so obtained, and that derived from the patients' records into a structured questionnaire designed for the study. Subjects were recruited once and seen as many times as necessary to complete the questionnaire within the study period. Care was taken not to negatively impact the patients' waiting time in the clinic. Information obtained included; age in years (at last birthday), sex, and Haemoglobin (Hb) phenotype (obtained from patients' records). Thereafter two milliliters (2 ml) of venous blood was drawninto commercially prepared ethylenediaminetetraacetic acid anticoagulated sample bottles. Each sample was lightly and thoroughly mixed to prevent cell lysis and ensure anticoagulation. They were then immediately transported to the centre's haematology laboratory for analysis by laboratory scientists. Analysis was done with a Sysmex KX 21N haematology analyzer (serial no. 060120920). The following haematological indices were obtained from the samples; TLC, PLT, HCT and Hb concentration. The results were entered into the structured questionnaire on subsequent visits. For the purpose of this study, steady state SCD was defined as the absence of infection or acute clinical symptoms or crisis for at least 3 months prior to recruitment into the study.

Statistical analysis

Analysis of the collected data was done with the statistical package for social sciences (SPSS) version 20.0. Presentation of data was done in prose, tables and figures. Means ± standard deviations were computed for the haematological parameters. The means were compared with the t-test and ANOVA test as appropriate. A P value < 0.05 was regarded as statistically significant.

Results

One hundred SCD patients with ages ranging from 2 to 16 years were recruited. The mean age of the study subjects was 6.97 ± 3.63 years. Thirty- seven (37%) of the subjects were within the ages of 5- 8 years, while 11(11%) fell within the range of 13-16 years. There were 61(61%) males and 39(39%) females giving a male: female ratio of 1.6:1. Table 1 also outlines the haemoglobin (Hb) phenotype pattern of the study participants.

Table 2 shows the mean haematological parameters of the study participants by gender.The mean HCT for males was 23.23%± 4.19% that for females was 23.97%± 3.38%. However, this difference did not reach statistical significance (P = 0.24). Mean PLT for females was 348.55± 217.57 x 10^9/L that for males was 361.23± 153.45 x 10^9/L the difference was statistically significant (P = 0.02).

The association between the mean haemoglobin parameters by age is displayed on Table 3. It shows that the mean TLC decreased with increasing age, however, this did not reach statistical significance (P = 0.77).

Figure 1shows that the TLC of the study participants ranged from 2.8 x 10^9/L to 51.6 x 10^9/L. The range of PLT is displayed in Figure 2 as 58 x 10^9/L to 883 x 10^9/L, while Figure 3 shows Hb concentrations ranging from 3.3 – 11.5g/L. Figure 4 outlines the HCT of the patients ranging from 13% to 37%.

Discussion

This study was designed to evaluate the steady- state haematological parameters of children with SCD at a reference hospital, and the mean values for TLC, PLT, HCT and Hb

concentration for this cohort of patients were thus established.

Steady-state TLC in SCD has been shown by various investigators to be relatively high.[10-13]This steady- state leucocytosis is thought to be due to the influence of elevated acute phase proteins on bone marrow production of leucocytes. It is postulated that the steady-state of SCD is characterized by minimal levels of vaso-occlusion that may result in increased amounts of acute phase proteins and cytokine mediators with subsequent increased production of leucocytes.[14]The current study also found a high mean TLC among this cohort of patients. There was no statistically significant gender-based difference in the mean TLC which is also consistent with most reports. [10-13] However, Rasaki et al[15]reported a statistically significant difference between the TLC of both genders (P= 0.006) in Gombe, North-eastern Nigeria. The reason for the disparity in both studies in this regard is not clear. Nonetheless, the male: female ratio in the present study is significantly higher than that from Gombe, hence the higher number of females in their study may have accounted for the difference. Additionally, and in keeping with previous findings, the mean TLCs of this group of patients decreased with increasing age,[15] which may be indicative of the level of contact with foreign antigens by the various age groups.[16]

The mean PLT of this group of patients was high, as demonstrated by previous studies.[10-13,15]It is however slightly lower than the 409.22± 145.54 $x10^9$/L reported by Abubakar et al in Zaria, North-west Nigeria,[10]but lower than the 301.5±173.2 $x10^9$/L reported in Enugu, South-east Nigeria.[17]Thrombocytosis is known to be associated with steady-state SCD, yet the pathogenesis is not clear. Nevertheless, it is being hypothesized that it may be a consequence of high erythropoietin levels induced by the anaemic state. Erythropoietin is structurally similar to thrombopoietin and hence may play a role in development of reactive thrombocytosis. [18,19] There is also evidence that the high levels of antioxidants that persist in steady-state SCD may contribute to the preservation of high PLT and TLC. [20] In concordance with other studies, there was no statistically significant association between mean PLT and age in this study.[10-12,15,21]However, we observed a gender-based difference in mean PLT, males had a higher mean PLT than females. This finding is at odds with the widely held view. [22] The reasons for this finding in this cohort is not evident, nonetheless it may be due to the fact that the majority of our female participants were pre-pubertal, limiting the effects of variations in hormonal profiles, as well as that of menstruation induced compensatory haematopoiesis on PLT. Our findings in this contextwere however, similar to that of Iheanacho[21]in Benin City, Nigeria.

The mean HCT and Hb concentration values were low as reported in other studies. [10-12,15,21] The chronic haemolysis as well as reduced red blood cell life span associated with SCD readily explains the low values of these parameters. In addition, SCD patients have been shown to have a relatively low erythropoietin response when compared to individuals with normal haemoglobin.[23]Age and gender were also not significantly associated with mean HCT and Hb concentration in this study. This is inconsistent with findings from Gombe, Nigeria where the investigators demonstrated a significantly higher mean Hb concentration value in females. The basis for this discrepancy is not obvious but may be a product of the higher number of female subjects recruited into the aforementioned study.

This study has provided baseline value ranges for TLC, PLT, HCT and Hb concentration in

Paediatric steady- state SCD in the context of a reference hospital setting in North-eastern Nigeria. These values would be of use to paediatric clinicians practicing in this part of Nigeria. However, our results may have been limited by the unintended skewedness of participants towards the male gender. Also, a comparison with haematological parameters of age and gender- matched controls with normal haemoglobin as well as SCD patients in various crises states, would have increased the strength of our findings. Future studies in addition to taking the above limitations into account, may benefit from a much larger sample size and a look at the impact of hydroxyurea therapy on these haematological parameters.

Conclusion

The current study found raised levels of TLC and PLT as well as low values for HCT and Hb concentration in children with steady- state SCD. Mean PLT was also significantly higher in males than in females. These data are illustrative of the haematological profile of SCD in steady- state amongst children in this part of North-eastern Nigeria and would be of help to clinicians in the management of these patients.

Acknowledgement

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Conflicts of interest: None

Author contributions

Imoudu IA conceptualized and designed the study, participated in data collection, analyzed and interpreted the data and drafted the manuscript. Yusuf MO participated in study design, data collection, and data analysis. Both authors reviewed and approved the final manuscript.

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Tables and Figure legends

Table 1; Age and haemoglobin phenotype distribution by gender of sickle cell disease patients

Characteristics	Gender		Total (%)
	Male (%)	Female (%)	
Age (years)			
2-4	20.0(20.0)	12.0(12.0)	32.0(32.0)
5-8	21.0(21.0)	16.0(16.0)	37.0(37.0)
9-12	11.0(11.0)	9.0(9.0)	20.0(20.0)
13-16	9.0(9.0)	2.0(2.0)	11.0(11.0)
Haemoglobin phenotype			
SS	56.0(56.0)	36.0(36.0)	92.0(92.0)
SS+F	4.0(4.0)	2.0(2.0)	6.0(6.0)
SC	1.0(1.0)	1.0(1.0)	2.0(2.0)

Table 2; Mean haematological parameters by gender

Haematological parameters	Population mean	Sample mean		T	P-value
		Males	Females		
Total leucocyte count (x 10^9/L)	16.83± 7.30	16.93±6.13	16.64±9.07	0.18	0.22
Haematocrit (%)	23.51±3.90	23.23±4.19	23.97±3.38	-0.91	0.24
Haemoglobin concentration (g/dl)	7.65±1.45	7.66±1.50	7.62±1.38	0.13	0.38
Platelet (x 10^9/L)	356.66±178.27	361.23±153.45	348.55±217.57	0.33	0.02

Table 3; Mean haematological parameters by age

Haematological parameters	**Age (Years)**				**P- value**
	2-4	**5-8**	**9-12**	**13-16**	
Mean total leucocyte counts ($x10^9/L$)	17.69±7.22	16.95±6.39	16.19±10.10	15.04±4.84	0.77
Mean platelet counts ($x10^9/L$)	301.03±118.18	394.44±189.93	372.83±234.83	360.40±148.40	0.21
Mean haemoglobin concentration (g/dl)	7.66±1.38	7.59±1.55	7.60±1.43	7.87±1.51	0.95
Mean haematocrit (%)	23.24±3.11	23.68±4.12	23.24±4.11	24.245.11	0.88

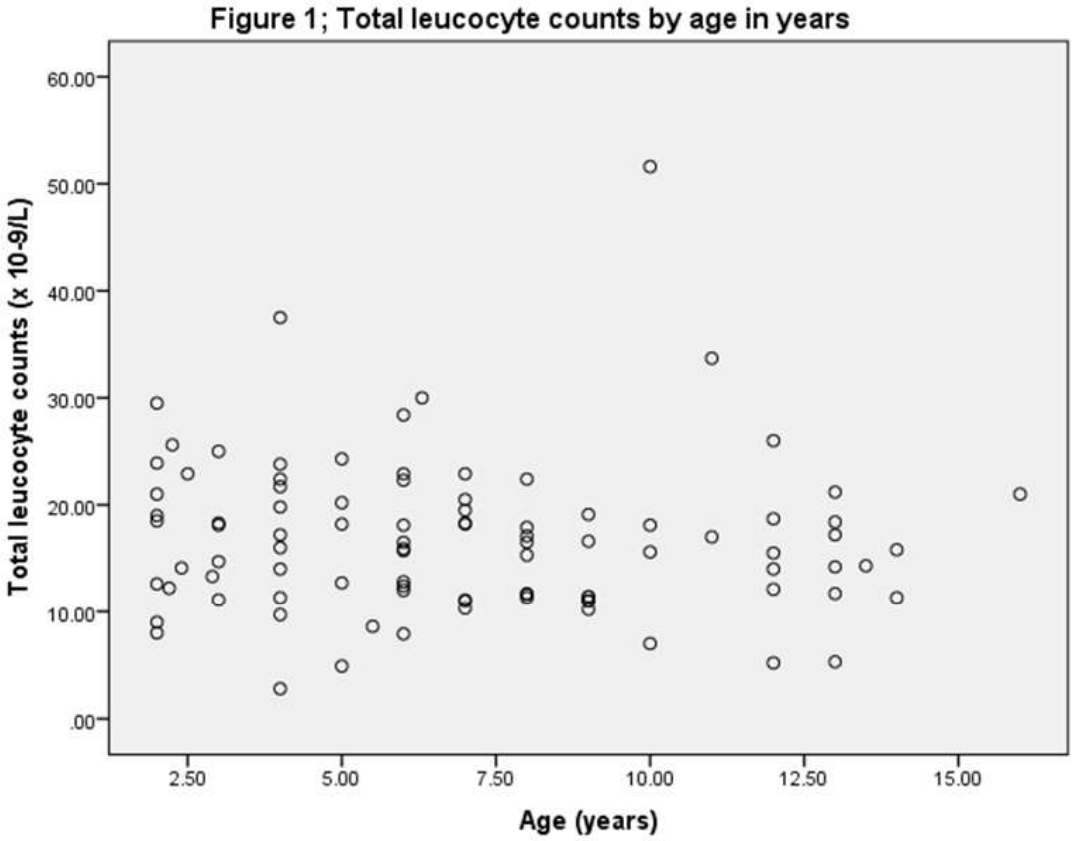

Figure 2; Platelet counts by age of sickle cell disease patients

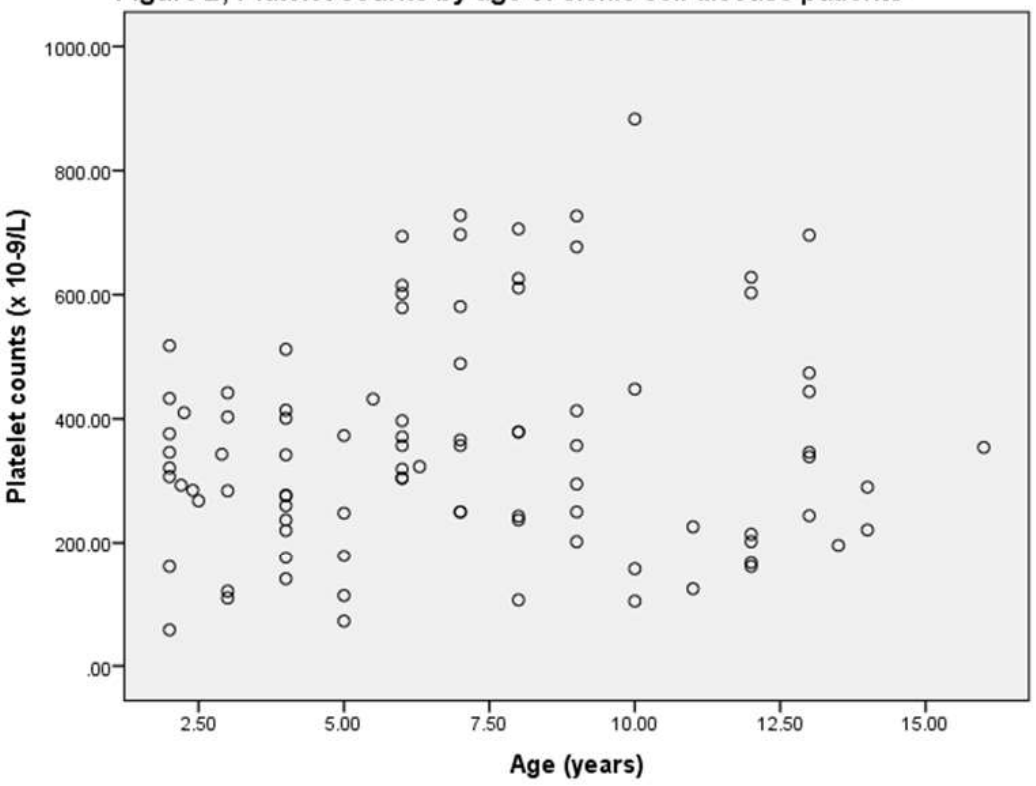

Figure 3; Haematocrit of sickle cell disease patients by age

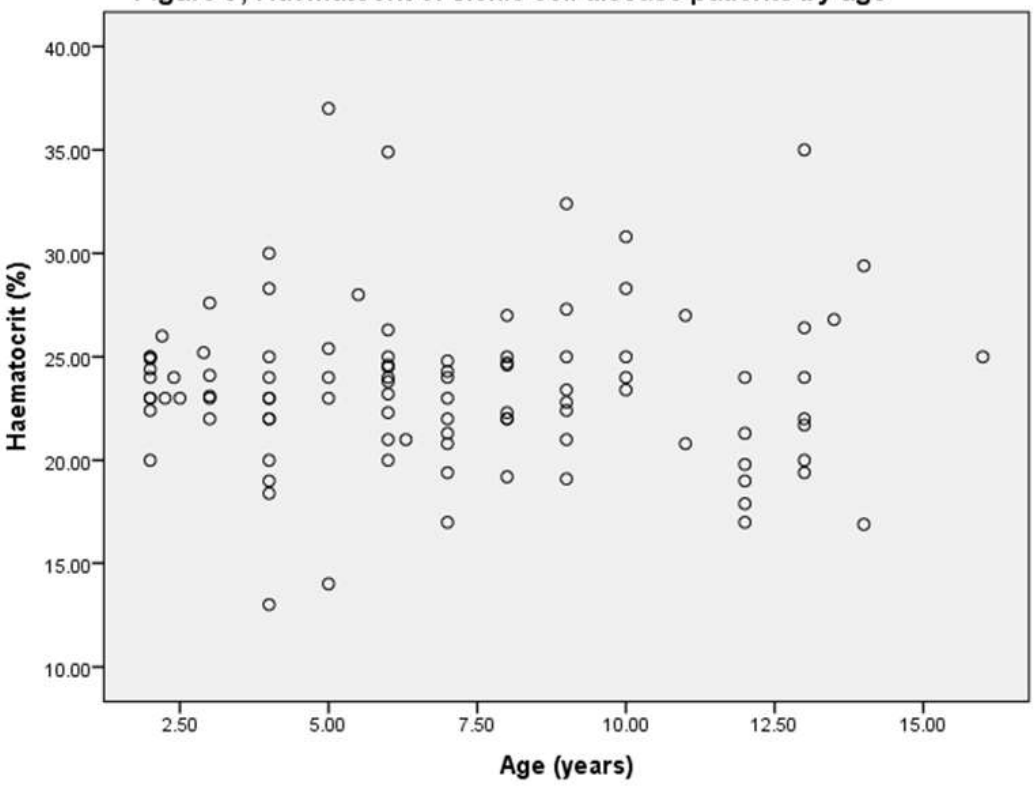

Figure 4; Haemoglobin concentrations of sickle cell disease patients by age