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Haematological indices of Children and Adults suffering from sickle cell disease in Portharcourt Rivers State, Nigeria.

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ABSTRACT

Sickle cell disease is a multi-organ disease characterized by sickled red cells, premature destruction of red cells (haemolysis), a susceptibility to infection and recurrent blood vessel obstruction causing tissue ischaemia with infarction. Inflammatory process in sickle cell disease (SCD) is chronic and progressive, waxing and waning. Clinical episodes such as vaso-occlusive crises (VOC) occur at critical levels of inflammation. The aim of this research was to determine the haematological indices of children and adults suffering from sickle cell disease patients in Nigeria. This research was a case control study carried out in Port Harcourt, Nigeria. There were three groups of 45 subjects each: SCD patients in steady state, SCD in VOC (which constituted the cases) and normal controls with HbAA. These participants had their blood samples analyzed for full blood count. Results were analyzed with the SPSS version 20. The results showed that an increased neutrophil count that correlates with an increased risk of VOC. This study also observed a significantly higher neutrophil count in SCD compared to controls in both children and adults. It's been observed that during acute episodes, neutrophils are massively mobilized from the marginating pool and bone marrow storage. Sickled reticulocytes, and sickled red cells adher readily to neutrophils and cause microvascular trapping and occlusion. These cells adhered to neutrophils, also increase their oxidative activity and therefore tissue damage during these acute episodes. The marked elevation in level of neutrophil count may account for the milder increases in the other white cell compartments. In conclusion an increased neutrophil count has been correlated with an increased risk of vaso-occlusive crisis (VOC).

Keywords: Sickle cell, neutrophils, haematological indices and microvascular trapping.

INTRODUCTION

Sickle cell disease (SCD) is a disorder of great medical importance in Africa because of its endemicity, and associated significant disability, morbidity and mortality in this region of the world [1, 2]. It has also achieved worldwide recognition because of the movement of people from the endemic regions to regions with low prevalence rates. Sickle cell disease is a multi-organ disease characterized by sickled red cells, premature destruction of red cells (haemolysis), a susceptibility to infection and recurrent blood vessel obstruction causing tissue ischaemia with infarction [3]. The latter is the underlying pathology of recurrent acute episodes of pain which is the hallmark of the disease. Although much of the pathophysiology of SCD is related to the polymerization of sickle haemoglobin (Hb) within the red cells, studies have shown that other metabolic processes such as inflammation, haemolysis, nitric oxide (NO) deficiency, ischaemia and reperfusion injury with oxidative stress and cell adhesion, all play key roles in this disorder [4, 5].

MATERIALS AND METHODS

Study Area

This study was carried out in Port Harcourt, using patients recruited from the University of Port Harcourt Teaching Hospital (UPTH), the Braithwaite Memorial Specialist hospital (BMSH), St Martin's Hospital, and the Palmers' Hospital. Port Harcourt is in the South-South zone of Nigeria. These hospitals are major hospitals that render services to patients within the state and neighboring states including Akwa Ibom, Bayelsa, Imo and Abia.

STUDY DESIGN

This was a hospital-based case control study.

Ethical Approval

This study was approved by the UPTH Research and Ethical Board. The approval was presented to the other peripheral hospitals (Appendix I). An informed consent was obtained from all participants of the study.

STUDY POPULATION

The study was composed of a total of 66 children and 69 adults. Study participants were selected in a systematic manner and were divided into 3 subpopulations.

a) SCD patients in steady state who were recruited from the paediatric- (25 from UPTH and 5 from BMH) and adult- (11 from UPTH and 4 from BMH) haematology outpatient clinics of the participating hospitals.
b) SCD patients in Vaso-occlusive crisis recruited through the Accidents and Emergency units and children emergency wards of the participating hospitals. There were 20 children recruited from UPTH and 3 from BMH; 13 adult patients from UPTH, 5 from BMH, 2 from Palmers' and 2 from St. Martin's.
c) Normal healthy adult volunteers attending the blood donor unit in UPTH and healthy children of health care workers.

A questionnaire was filled for participants by the researcher after consent was obtained.

Data in the questionnaire included: participant number, age, gender, Hb phenotype, brief history of any acute illness, the presence of bone pain and characterization (site, severity, character and duration), history of any current medication and the test results of this study.

INCLUSION CRITERIA OF CASES

1. All cases with confirmed SCD by Hb electrophoresis.
   (AND)
2. Consenting SCD child caregiver and adult SCD patients in steady state, (patients with 2 or more month's history of no crisis or blood transfusion)
   (OR)
3. SCD patients in vaso-occlusive crisis (patients admitted for severe bone pain crisis).

EXCLUSION CRITERIA OF CASES

1. People with Hb AS phenotype.
2. Subjects with SCD complications, or chronic infections, or chronic inflammatory conditions, (for example, leg ulcers, hypertension).
3. Subjects positive for transfusion-transmissible infections (HIV, hepatitis, and syphilis).
4. Refusal to give consent.
5. Pregnant SCD patients.

CONTROL SELECTION PROCESS

Adult controls who were 18 years and above were recruited from the blood donor clinic. Healthy adults with known HbAA phenotype were selected in a systematic manner. Children used for controls were those less than 18 years who were children of healthcare workers in the participating hospitals. These control individuals were not age- and sex- matched with the SCD patients group, but were healthy individuals with normal haemoglobin profiles and lacked a history of anaemia, inflammatory

conditions, and haematological diseases.

SAMPLE SIZE DETERMINATION

Sample size was calculated using the prevalence of SCA (2.9%) in the University of Port Harcourt Teaching Hospital**Error! Bookmark not defined.** and the formula N= (Z^2pq) /d^2).
N= desired sample size
Z= standard deviation at 1.96 (95% confidence interval)
P= proportion of the target population, estimated to have the particular characteristics (2.9% or 0.029)
q =1-p. d = degree of precision used (0.05)
This gives a minimum sample size of 43.3; approximately 45 for each subgroup of participants.
Each of the subgroups (subjects in VOC, in steady state and normal controls), had a sample size of 45 giving a total of 135.

EQUIPMENT AND MATERIALS

The equipment and materials used were of analytical grade.

METHODOLOGY

Full Blood Count was carried out using a 5 part auto analyzer (BC 6800 Autohaematology analyzer system Mindray® product). The samples were placed on the sample mixing machine. The power supply, connections of the analyzer to the reagents or diluents, to the waste and pneumatic unit were checked. The auto-analyzer, loaded with all its reagents for the analysis, and the PC software were switched on and allowed to boot (analyzer automatically performed a self test procedure, background cycle and initialized the system). The control samples were run first. A clean uncapped EDTA tube was presented to the sample probe, making sure the probe goes deep into the bottom of tube to avoid spills, and bubbles. The aspirate button was pressed to start dispensing the diluents; the tube was removed when buzzer sounds. The machine analyzed a sample in 1.5 minutes; the machine automatically displays the results, which is printed from the printer

STATISTICAL ANALYSIS

Data was entered and analyzed using the IBM statistical package for social sciences software (SPSS) version 20. Descriptive statistics (mean, standard deviation, percentages and charts) were used to summarize the variables and characterize the demographics. Student's t- test and Analysis of variance (ANOVA) were used to compare the differences in means between two and three groups respectively. Post hoc test was performed using Scheffe test to explore significant mean differences across groups. Chi Square or Fishers exact tests were used to compare differences in proportions across groups. Pearson's correlation coefficient was used to examine the correlation between TNF, SAA and the haematological parameters. P-values <0.05 were considered statistically significant.

HAEMATOLOGICAL INDICES IN STUDY PARTICIPANTS

The ranges for Hb concentration in the subgroups were 5.9 to 9.4g/dl (VOC); 5.7 to10.5g/dl (steady state); and 11.3 to 16.2g/dl (control). Two children who came in VOC (8.7%)and 2 in steady state (6.7%) had Hb>9g/dl; 6 in VOC(26.1%) had Hb between 8.1-9g/dl compared to 7(23.3%) in the steady state; 13 in VOC (56.7%) with Hb between 6-8g/dl compared to 18 (60%) of the steady state group; and 2 (8.4%) with< 6g/dl against 3 (10%) in the steady state group. The differences in Hb concentration were not found to be statistically significant. (Tables 1 and 2)
The adult group had 6 cases in VOC (27.3%) and 3 in steady state (20%) having Hb >9g/dl; 3 in VOC (13.6%) had Hb between 8.1-9g/dl compared to 4(26.7%) in the steady state; 13 in VOC (59.1%) with Hb between 6-8g/dl compared to 8 (53.3%) of the steady

state group. No adult case had Hb < 6g/dl in both VOC and steady state. The differences in Hb concentration between the two groups were not found to be statistically significant.

Significantly lower levels of Hb concentration were found in the SCD groups than control group (*p*- value <0.001), in both children and adults.

The children had a mean Hb concentration for the VOC group as 7.88± 1.37g/dl, this value compared statistically to the steady states mean concentration (conc.) of 7.59± 1.09g/d l, and the control mean of 12.21±0.73 g/dl was statistically significant. Similarly, the adult group had a mean Hb concentration for the VOC group as 8.33± 2.57g/dl, this value compared statistically to the steady states mean concentration (conc) of 7.91± 1.39g/d l, and the control mean of 13.84±1.37 g/dl was statistically significant

In the paediatrics group, comparing the mean counts across the three groups showed that the SCD cases had significantly higher reticulocyte count, white cell count, neutrophil count, lymphocyte and eosinophil counts than the control cases (Tables 1 and 2).

The adult study group showed significantly higher values in the SCD when compared to the controls in all the parameters, (reticulocyte, white cell, neutrophil, lymphocyte, eosinophil and basophil counts).

Table 1: Haematological indices of children compared across the study groups

Variables	VOC Mean ± SD	Steady State Mean ± SD	Control Mean ± SD	ANOVA	P-value
Hb (g/dl)	7.88±1.37	7.59±1.09	12.21±0.73	81.193	*0.0001**
Reticulocyte (x10^9/L)	235.8±11.90	265.3±8.73	38.0±1.73	28.897	*0.0001**
White blood cell (x10^9/L)	25.64±14.21	14.84±5.58	6.14±2.58	19.686	*0.0001**
Platelet (x 10^9/L)	363.43±262.00	365.27±156.44	322.31±99.30	0.251	*0.779*
Neutrophil (x 10^9/L)	15.41±2.9	7.70±0.7	2.70±0.3	4.439	*0.016**
Lymphocyte(x 10^9/L)	6.56±2.3	5.32±0.7	2.74±0.3	8.394	*0.001**
Monocyte(x 10^9/L)	1.46±0.7	1.16±0.2	0.50±0.06	2.849	*0.065*
Eosinophil(x 10^9/L)	0.10±0.1	0.54±0.2	0.18±0.07	7.755	*0.001**
Basophil (x 10^9/L)	0.25±0.2	0.13±0.07	0.02±0.004	1.614	*0.207*

*Statistically significant SD-Standard deviation

Table 2: Haematological indices of adults compared across the study groups

Haematological indices	VOC Mean ± SD	Steady State Mean ± SD	Control Mean ± SD	ANOVA	P-value
Hb (g/dl)	8.33±2.57	7.91±1.39	13.84±1.37	82.332	*0.0001**
Reticulocyte (x10^9/L)	236.1±12.11	194.4±5.88	42.5±1.95	49.443	*0.0001**
White blood cell(x10^9/L)	20.07±11.45	12.39±3.92	4.67±1.09	3.804	*0.027**
Platelet (x 10^9/L)	388.32±168.34	435.33±177.12	227.47±80.33	15.375	*0.0001**
Neutrophil (x 10^9/L)	13.57±1.6	6.49±0.3	2.0±0.1	39.863	*0.0001**
Lymphocyte(x 10^9/L)	5.06±1.4	4.57±0.3	2.11±0.1	33.807	*0.0001**
Monocyte(x 10^9/L)	1.14±0.3	0.95±0.1	0.36±0.02	5.265	*0.008**
Eosinophil(x 10^9/L)	0.12±0.1	0.32±0.1	0.22±0.03	19.348	*0.0001**
Basophil (x 10^9/L)	0.17±0.1	0.07±0.01	0.02±0.003	3.770	*0.028**

*Statistically significant SD-Standard deviation

DISCUSSION

The pathogenesis of sickle cell disease (SCD) vaso-occlusive crisis involves significant subclinical microvascular occlusion, and ischaemia in the steady state [2]. The process of vaso-occlusion is initiated by the adhesiveness of sickled reticulocytes and irreversibly sickled cells to the vascular endothelium and other cellular elements especially the neutrophils. The endothelial cell and blood cellular components are in a state of constant activation by this interaction with HbSS red cells. When activated, they produce inflammatory cytokines which also induce the liver to produce acute phase reactants, at levels above normal but below that of clinical inflammation thereby inducing a state of subclinical inflammation in the steady state individual [5].

Sickle cell disease is a chronic inflammatory disorder, confirmed in this study by significantly elevated levels of white cell count, neutrophils, lymphocytes, platelets and reticulocyte counts observed in SCD compared with controls in both children and adults. This is similar to other studies in Nigeria, and other countries. In these studies an increased neutrophil count has been correlated with an increased risk of VOC. This study also observed a significantly higher neutrophil count in SCD compared to controls in both children and adults. It's been observed that during acute episodes neutrophils are massively mobilized from the marginating pool and bone marrow storage. Sickled reticulocytes, and sickled red cells adher readily to neutrophils and cause microvascular trapping and occlusion. These cells adhered to neutrophils, also increase their oxidative activity and therefore tissue damage during these acute episodes. The marked elevation in level of neutrophil count may account for the milder increases in the other white cell compartments. This appears like a homeostatic attempt at stable cellular equilibrium or balance. The higher levels in the steady state are therefore indicative of chronicity.

CONCLUSION

In conclusion an increased neutrophil count has been correlated with an increased risk of vaso-occlusive crisis (VOC).

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