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HAEMATOLOGICAL VALUES IN SICKLE CELL ANAEMIA IN STEADY STATE AND DURING VASO-OCCLUSIVE CRISIS IN BENIN CITY, NIGERIA

C. E. Omoti

Department of Haematology, University of Benin Teaching Hospital, Benin City, Nigeria
Reprint requests to: Dr. C. E. Omoti, Department of Haematology, University of Benin Teaching Hospital, P.M.B. 1111, Benin City, Nigeria. E-mail: ediomoti@yahoo.com

Key words: Haematological values, sickle cell anaemia

Abstract

Background: Sickle cell anaemia (SCA) is a major cause of morbidity and mortality in Africa where there is no readily available effective treatment. This study was designed to determine the haematological values that can be used in monitoring the status and management of SCA patients.

Method: A prospective study of 200 patients (81.3%) in steady state, 46 patients (18.7%) during vaso-occlusive crisis (VOC) and 84 control subjects seen between August 2001 and July 2002 in 3 centers in Benin City, Nigeria had their blood samples analyzed within two hours of collection. Automated Coulter Counter was used to determine the complete blood counts while the foetal haemoglobin (HbF) was estimated by the modified Betke method and haemoglobin A_2 by HbS-free microcolumn chromatography.

Results: The mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in steady state were 79.38fl ± 22.41, 28.31pg ± 3.58 and 32.56g/dl ± 2.27 while in VOC they were 85.50fl ± 8.14, 28.79pg ± 2.78 and 33.76g/dl±3.44 respectively. The red cell distribution width (RDW), haemoglobin A_2 and F in steady state were 23.76% ± 6.49, 4.52% ±1.16 and 2.17% ±1.81 while during VOC they were 21.62% ±5.11, 3.82%±1.27 and 2.05%±1.19 respectively. The neutrophil count ($P<0.01$), MCV ($P<0.01$) and MCHC ($P<0.05$) were significantly higher during VOC than steady state while the RDW ($P<0.05$) and haemoglobin A_2 were significantly higher in steady state than during VOC.

Conclusion: Parameters, which are not usually reported in previous studies, have been shown in steady state and VOC.

Mots clés : Valuers hématologiques, la drépanocytose

Résumé

Introduction : La drépanocytose est la cause principale de la morbidité et mortalité en Afrique où il n' y a aucun traitement efficace facilement dispobible. L'objet de cette étude est de déterminer des valuers hématologiques qu'on pourrait utiliser dans la surveillance du statut et la prise en charge des patients atteints de la drépanocytose.

Méthode : Une étude potentielle composée de 200 patients soit 81,3% dans un état normal, 46 patients soit 18,7% pendant la crise vasco-occlusive (VOC) et 84 sujets contôles vus entre août 2001 et juillet 2002 dans 3 centres à la ville de Benin, Nigéria avaient eu leur prise du sang analysé en moins de deux heures de la collection Décompte Coulter automatisé était utilié pour déterminer le décompte du sang total tandis que l'hémoglobine foetale (hbF) était évaluée à travers la chromatographie microcolumn libre – HbS.

Résultats : Le moyen du volume de la cellule (MVC), d'hémoglobine cellulaire moyenne (HCM) et la concentration hèmoglobine cellulaire moyenne (CHCM) dans un état stable étaient 79,38fl+- 22,41, 28,31pg +- 3,58 et 32,56/dl+- 2,27 tandis que dans le VOC ils étaient 85,50fl+- 8;14,28,79pg +- 33,76g/dl+- 3,44 respectivment. La largeur de la distribution du globule rouge (LDGR), hémoglobine A2 et F dans un état stable étaient 23,76 %+-6.49,4,52%+-1,16 et 2,17%+-1,81 tandis que pendant le VOC

ils étaient 21,62%%+- 5,11, 3,82%+- 1,27 et 2,05%+-1,19 respectivement, le décompte neutrophil, (P<0,01), MVC (P<0,01) et CHCM (P<0,05) étaient sensiblement très élevé pendant VOC que était stable tandis que la LDGR (P<0,05) et hémoglobine A2 étaient sensiblement plus élevée dans l'état stable plus que pendant VOC.

Conclusion : Des paramètres, qu'on n'avait pas rapporté normalement dans des études précédentes, ont été montrés dans l'état stable et VOC.

Introduction

Patients with sickle cell disease (SCD) have varying amounts of abnormal haemoglobin called the sickle cell or "S" haemoglobin in their erythrocytes.[1] Sickle cell anaemia (SCA) is due to the substitution of adenine with thymine in the glutamic DNA codon (GAG→GTG), which results in turn, in substitution of β_6 valine for glutamic acid.

The disease accounts for over 60% of the world's major haemoglobinopathies with an estimated 2-3 million Nigerians affected by the "S" gene.[2] The extent of the problems of SCD in Nigerian cannot therefore be overemphasized because of the "S" gene said to be between 25-30%.[3] The majority of the patients born to rural dwellers do not usually survive childhood.[4] In Nigeria, two out of every one hundred have SCA.[4] Furthermore, there is no widely acceptable and readily available cure for patients with SCA at present. Curable methods such as gene therapy and bone marrow transplantation, which may be associated with several complications, are not readily available in developing nations. It is therefore imperative on us to be well acquainted with routine haematological parameters that may assist in managing these patients adequately in developing countries.

Many patients with SCA are in reasonably good health most of the time and achieving a steady state level of fitness. This state of relative well-being is periodically interrupted by crisis of which the vaso-occlusive crisis (VOC) is the most common and hallmark of patients with SCD.[5] The importance of early recognition and subsequent clinical and haematological assessment of the disease are greatly facilitated by familiarity with the patient's steady state. A patient with SCA is said to be in steady state when there is absence of infection, acute complicating factors or acute clinical symptoms or crisis for at least three months.[6] Crisis refers to episodes of acute illness attributable to the sickling phenomenon in which there is a sudden deviation for the worse or a sudden exacerbation of symptoms and signs of patients with SCA who had hitherto been in stable condition.[7] The occurrence of crisis makes the disease incapacitating to the patient and frustrating to parents and physicians.

Many previous studies have lumped the haematological variables in steady state and crisis together. This study will determine if there is a difference in the haematological variables between steady state period and VOC. Furthermore, many of the established values were determined over a decade ago. Various changes in therapeutic, economic, social, technological measures have taken place since then. This study will therefore determine if these changes have affected what we accept as the 'normal' values in these patients. Less commonly determined variables such as erythrocyte indices, red cell distribution width, haemoglobin A_2 and haemoglobin F will also be determined along with the routine haematological tests.

Materials and Method

Patients

Two hundred patients with a diagnosis of SCA attending the consultant outpatient clinic at the University of Benin Teaching Hospital, Central Hospital and Sickle Cell Center in Benin City, Nigeria between August 2001 and July 2002 in their steady state were recruited into the study after obtaining informed consent. Forty-six patients in VOC presenting to the consultant outpatient and casualty departments were also recruited. Their blood samples were analyzed within two hours of collection.

Inclusion criteria

1. Known patients with SCA (HbSS as diagnosed by cellulose acetate electrophoresis at pH 8.6) in the steady state i.e. established by a steady haematocrit and haemoglobin values over a given period of 2-3 clinic visits at 4-6 weeks intervals and a state of well being without any symptoms or signs suggestive of crisis established by a careful history and complete physical examination.[8]
2. Known patients with SCA considered clinically to be in VOC by the following criteria: Bone and joint pains or multiple sites of pain, requirement for analgesics and patients considering the episode as typical of crisis which necessitates hospital admission.

Exclusion criteria

1. Any patient with disorders that may affect the haematological values such as renal disease.
2. Pregnancy
3. Any patient with recent blood transfusion during the preceding three months.

Sample collection

5ml of venous blood was collected by clean venepuncture from each patient via the antecubital vein using a plastic syringe with minimum stasis, into commercially prepared concentrations of sequestrene Ethylene Di-amine Tetracetic Acid (EDTA) bottles.

Each sample was mixed gently and thoroughly to prevent cell lysis and ensure anticoagulation. An aliquot was used to determine complete blood counts (CBC) within 2 hours of collection while the remainder was used to prepare haemolysate for haemoglobin A_2 and foetal haemoglobin estimation. For patients in VOC, samples were collected and analyzed within 24 hours of onset of pain.

Methodology

1. The complete blood counts (CBC) were analyzed using the automated COULTER^R A^C T diff^{TM} Analyzer (1997 model). The CBC includes haemoglobin, haematocrit, total white blood cell count and differential, platelet count, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration and red cell distribution width.
2. Measurement of HbA_2 was done by the chromatographic-spectrophotometric ion exchange-HbS interference free technique using the Biosystem^R kit with ISO 9001 certificate reg: 091006696.The procedure recommended by the manufacturer was applied. HbA_2 percentage was calculated using the formula (A^{415}=Absorbance at 415nm, HbA_O=Total haemoglobin):

$$\%HbA_2 = \frac{A^{415}\ HbA_2}{A^{415}HbA_2 + 3 \times A^{415}HbA_0} \times 100$$

3. Measurement of HbF was done by the modified Betke method[9] because it is reliable and designed for quantitation of small levels of HbF (below 10-15%). HbF percentage was calculated using the formula:

$$\%HbF = \frac{A413\ filtrate \times 100}{A^{413}\ standard \times 20}$$

Precautions taken to ensure reliable results included:

1. All samples were subjected to the same analytical procedure e.g. haemolysate preparation for the estimation of HbA_2 and HbF.
2. It was ensured that Betke's solution was not frothy (indicating contamination by detergent), as this will reduce the resistance of haemoglobin F alkaline denaturation leading to falsely low result.
3. Reagents and columns used for HbA_2 were allowed to warm to room temperature (failure to do this will give results up to 25% lower than expected).
4. The sample used for HbA_2 was allowed to be completely absorbed into the resin. The haemolysate will have a glossy appearance on complete absorption by the resin. Upon complete absorption, but without drying out, the top of the resin will have a dull malte-like appearance.

Data analysis

Data was analyzed by SPSS version 3.0. An initial frequency count of all variables was done. The means ranges and standard deviations (S.D) of the haematological values in the steady state, during VOC and in controls were calculated. Values in the steady state were compared with values in VOC using the student't' test. Values in steady state and VOC were also compared with control using the student't' test.

Results

Two hundred and forty-six patients with a diagnosis of SCA who met the inclusion criteria were recruited comprising of 116 males (47.2%) and 130 females (52.8%). Two hundred patients (81.3%) were seen in steady state and forty-six patients (18.7%) were seen during VOC. The mean age was 23.69 years (S.D±10.94) with a range of 5 years to 70 years.

The haematological values obtained in steady state and VOC is shown in table1. There was a statistically significant difference in the neutrophil count ($P<0.01$), mean cell volume ($P<0.01$), mean cell haemoglobin concentration ($P<0.05$), red cell distribution width ($P<0.05$) and haemoglobin A_2 ($P<0.01$). The haematological variables in the steady state and in control are shown in table 2. The haematological values in VOC and in control are shown in table 3. There was a statistical difference in all the variables tested except the mean cell volume and the mean cell haemoglobin concentration.

Table 1: Haematological values in sickle cell anemia patients in steady state and vaso-occlussive crisis

Variable	HbSS (n=200) Steady statee Mean ± S.D	HbSS (n=46) Vaso-occlusive crisis Mean ± S.D	P
Haemoglobin (g/dl)	7.54 ± 2.26	7.77±2.25	>0.05
Haemoglobin (l/l)	0.23 ± 0.06	0.24±0.06	>0.05
White Blood Cell Count (x 10^9/l)	12.72± 7.98	13.67±7.57	>0.05
Neutrophils (x 10^9/l)	5.2 ± 1.6	6.4 ± 2.0	<0.01
Lymphocytes (x 10^9/l)	6.5 ± 1.6	6.3 ± 2.0	>0.05
Monocytes (x 10^9/l)	1.0 ± 0.78	1.0 ± 0.04	>0.05
Platelet count (x 10^9/l)	342.62±143.03	352.89±144.78	>0.05
Mean Cell Volume (fl)	79.38±22.41	85.50±8.14	<0.01
Mean Cell Haemoglobin (pg)	28.31±3.58	28.79±2.78	>0.05
Mean Cell Haemoglobin Concentration (g/dl)	32.56±2.27	33.76±3.44	<0.05
Red Cell Distribution Width (%)	23.76±6.49	21.62± 5.11	<0.05
Haemoglobin F (%)	2.17±1.81	2.05 ± 1.19	>0.05
Haemoglobin A_2 (%)	4.52±1.16	3.82 ± 1.27	<0.01

Table 2: Haematological values in sickle cell anaemia patients in the steady state and in control

Variable	HbSS (n=200) Steady state Mean ± S.D	HbAA (n=84) Control Mean ± S.D	P
Haemoglobin (g/dl)	7.54 ± 2.26	12.93±2.22	<0.01
Haemoglobin (l/l)	0.23 ± 0.06	0.37±0.06	<0.01
White Blood Cell Count (x 10^9/l)	12.72± 7.98	5.71±0.97	<0.01
Neutrophils (x 10^9/l)	5.2 ± 1.6	2.3 ± 0.51	<0.01
Lymphocytes (x 10^9/l)	6.5 ± 1.6	2.9 ± 0.69	<0.01
Monocytes (x 10^9/l)	1.0 ± 0.78	0.4 ± 0.17	<0.01
Platelet count (x 10^9/l)	342.62±143.03	304.24±61.47	<0.01
Mean Cell Volume (fl)	79.38±22.41	8 4.46±5.26	<0.01
Mean Cell Haemoglobin (pg)	28.31±3.58	30.16±2.99	<0.01
Mean Cell Haemoglobin Concentration (g/dl)	32.56±2.27	2.62± 1.04	<0.01
Red Cell Distribution Width (%)	23.76±6.49	2.62 ± 1.04	<0.01
Haemoglobin F (%)	2.17±1.81	1.28 ± 1.04	<0.01
Haemoglobin A_2 (%)	4.52±1.16	2.13±0.98	<0.01

Table 3: Haematological values in sickle cell anaemia patients in vaso-occlusive crisis and in control

Variable	HbSS (n=46) Vaso-occlusive crisis Mean ± S.D	HbAA (n=84) Control Mean ± S.D	P
Haemoglobin (g/dl)	7.77±2.25	12.93±2.22	<0.01
Haemoglobin (l/l)	0.24±0.06	0.37±0.06	<0.01
White Blood Cell Count (x 10^9/l)	13.67±7.57	5.71±0.97	<0.01
Neutrophils (x 10^9/l)	6.4 ± 2.0	2.3 ± 0.51	<0.01
Lymphocytes (x 10^9/l)	6.3 ± 2.0	2.9 ± 0.69	<0.01
Monocytes (x 10^9/l)	1.0 ± 0.04	0.4 ± 0.17	<0.01
Platelet count (x 10^9/l)	352.89±144.78	304.24±61.47	<0.05
Mean Cell Volume (fl)	85.50±8.14	8 4.46±5.26	>0.05
Mean Cell Haemoglobin (pg)	28.79±2.78	30.16±2.99	<0.01
Mean Cell Haemoglobin Concentration (g/dl)	33.76±3.44	2.62± 1.04	>0.05
Red Cell Distribution Width (%)	21.62± 5.11	2.62 ± 1.04	<0.01
Haemoglobin F (%)	2.05 ± 1.19	1.28 ± 1.04	<0.01
Haemoglobin A_2 (%)	3.82 ± 1.27	2.13±0.98	<0.01

Discussion

It is now believed that with purposeful alteration of certain red cell parameters, it is possible to improve the haematological and biological characteristics of SCA patients and consequently improve the clinical course of the disease. These parameters include increasing intra-corpuscular HbF concentration as well as cellular hydration, increasing HbA_2, MCV below 72fl and MCHC below 32g/dl.[10]

The mean age was 23.69 years (S.D±10.94). The peak age incidence in this study was in the third decade. This is high because the study was carried out mainly in adult clinics. Life expectancy of SCA patients is said to be short.[11,12] The 1989 cooperative study of SCD (CSSCD) revealed that approximately 85% of children and adolescents with SCA and 95% with SC survived to 20 years of age in contrast to the estimated 14.3 years in 1973.[11] There were more females 130 (52.8%) than males 116 (47.2%) in this study. A review of the age and sex distribution in this study showed that only females were seen above the age of 50 years. This is in agreement with the findings that females may have longer life expectancy, which reported 42-53 years for men and 48-58 years for women.[12]

The mean haemoglobin and Hct values in steady state, VOC and controls are in agreement with previous studies.[13] The SCA patients are continually haemolysing their red cells with a short survival rate of the erythrocytes between 12-14 days.[13] Hence, the haemoglobin and Hct values are usually lower than normal healthy individuals. This is shown in this study where the haemoglobin and Hct values in steady state and VOC were significantly less than in control (P<0.01). The paradox was found in the results for the controls where a mean haemoglobin value of 12.9g/dl and Hct value of 0.37l/l were recorded as compared to Ezeilo's report of 15.6g/dl and 0.49l/l.[14] This could be as a result of the downturn in our economy. There was however no statistically significant difference in haemoglobin and Hct values in steady state and VOC. This is in agreement with another study carried out in Benin City.[13]

The mean total white blood cell count (WBCC) recorded in this study for steady state, VOC and control are of similar values to those reported by other authors.[9,15] As expected, the total WBCC and differential counts in steady state and VOC were significantly higher than in controls (P<0.01).This is expected because of the basic mechanisms which cause an increase concentration of neutrophils in

venous blood of SCA patients which include demargination of intravascular neutrophils, accelerated release from the bone marrow and reduction in the rate at which neutrophils leave the blood.[15]

Patients with SCA are known to have significantly higher mean total WBCC and differentials than people with AA genotype thereby blunting out the utility of this measurement in assessment of infection.[16] Akinola et al[17] suggested the generation of a covert inflammatory response leading to the release of cytokine mediators, one of whose main function is increased neutrophils production by the bone marrow. There was no significant difference in mean total WBCC between steady state and VOC (P>0.05). The neutrophil count was however significantly higher in VOC than in the steady state (P<0.01). This is in agreement with other studies, which show an increase in polymorphonuclear neutrophils in VOC over steady state values. This may also be due to increased demargination of intravascular neutrophils.[15] The resulting raised total WBCC in steady state and during VOC when compared with controls was statistically significant (P<0.01). This has continued to affect the ability of clinicians to predict the presence of bacteria infections from leucocyte counts in sickle cell anaemia patients.[16, 18]

The mean platelet count for steady state value is similar to the 320-327x10^9/l reported in other studies.[13, 18] There was no significant difference between values in steady state and VOC. However, the steady state platelet count was significantly higher than control (P<0.01). The platelet count in VOC was also significantly higher than control (P<0.05). This result is in agreement with previous studies, which show that platelet count is higher in SCA than in healthy control.[13, 18] This may be due to loss of splenic platelet pool function in adult sickle cell patients consequent upon autosplectomy.[19]

The mean cell volume (MCV) and the mean cell haemoglobin concentration (MCHC) were significantly higher in VOC than in steady state (P<0.01 and P<0.05 respectively) while the red cell distribution width (RDW) was significantly higher in the steady state than in VOC (P<0.05). This may be because SCA being a chronic haemolytic state stimulates haemopoiesis and haemopoietic activity is much higher during VOC than in the steady state. The cohort of fresh red blood cells being produced will have higher haematological indices .A higher MCHC may result from release of endogenous iron from broken down red cell haemoglobin synthesis. Also a higher MCHC may predispose to polymerization and hence VOC. The RDW, which is a measure of erythrocyte anisocytosis, was significantly higher in steady state and VOC than in control (P<0.01). This is in agreement with previous studies[20] that show that SCA is associated with marked anisocytosis. This may be because with more rapid erythropoiesis, cells at different stages of maturation with different sizes are present at the same time.

The mean HbF level found in this study in steady state, VOC and control are 2.17%±1.81, 2.05%± 1.19 and 1.28%± 1.04 respectively. Although the HbF level in

steady state is higher than during VOC, the result is not statistically significant. This is in agreement with an earlier study which reported no significant change in the HbF level during VOC and steady state period.[21] A similar low mean HbF value of 3.60%±1.96 in SCA patients was reported by Olatunji et al[22] and also by Falusi et al.[23] In contrast to these, other authors have reported much higher mean HbF value of 6.7-9.5%.[21,24] This may be because many of these studies have a higher proportion of paediatric population than in this study, a coinheritance trait with thalassaemia gene or a strong genetic component controlling the number of HbF containing cells (F cells) and the clinical status of the patient. Morrison et al[25] has suggested that HbF level may fall following painful crisis. The HbF level has been shown to decline with age from about 80.4% at birth to 9.2% at 24 months.[26] HbF values in steady state and VOC were significantly higher than in control (P<0.01), which is in agreement with other studies.[21]

Recent therapeutic approaches to SCD focus on attempt to reduce intracellular HbS polymerization by altering the haemoglobin specie.[27] Pharmacological elevation of HbF has become the central focus of much laboratory and clinical research in recent years. Agents such as hydroxyurea (with or without recombinant human erythropoietin and butyrate compounds) elevate HbF and reduce HbS in a majority of sickle erythrocytes thus decreasing intracellular polymerization.[27]

The mean haemoglobin A_2 (HbA_2) level in this study for steady state, VOC and control were 4.52%±1.61, 3.82%±1.27 and 2.13%±0.98 respectively. The mean HbA_2 for steady state is in agreement with the study by Hall et al[28] who reported a mean HbA_2 level of 5% with a range of 3.6-7.0% It also agrees with the 3.48%±0.50 for males and 3.38%±0.62 for females reported by Serjeant et al.[29] The mean HbA_2 level in steady state and in VOC was significantly higher than in control (P<0.01). This is also in agreement with previous studies.[30] HbA_2 value in steady state was significantly higher than in VOC, t-cal=3.43; P<0.01.It is taught that HbA_2 may have a protective effect against complications of SCA by reducing minimum gelling concentration of haemoglobin S but only at considerably greater concentrations of HbA_2.[31]

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