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Comparison of clinical and urinary features of sickle cell disease patients having elevated leucocyte levels, with their counterparts with normal counts

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ABSTRACT

Objective
The fact that leucocytes contribute to the disease process in SCD requires to be proven through various studies. In this study, it is hypothesized that in the mildly anemic SCD, the unfavorable effects due to either high or low levels of hemoglobin have been minimized, so how elevated levels of leucocytes affect the sickling pathobiology could be shown.
Patients and method
From a cross-sectional full blood count of SCD subjects, the mildly anemic group was selected, and this sub-group further stratified into two, based on WBC counts, a leucocytotic group, and the other with normal WBC count. Furthermore, the clinical and urinary features of the two groups were compared.
Results
Out of 241 mildly anemics, 150 of them; 79 males and 71 females were selected. Sixty six (66) of the selected mildly anemic showed leucocytosis. The SS male patients in crisis predominated the leucocytotic group (18/66 or 27.27%), and the mean leucocyte count of this group was 16.81 $\times 10^9$/L. Clinically, apart from the vaso-occlusive pain, other features shown were fever, cough, splenomegaly, and respiratory tract infections. Additionally, the spot urine analysis showed the patients with leucocytosis had higher incidence of hematuria, proteinuria, bacteriuria and significant pyuria.
Conclusion
Despite the fact that the mildly anemic condition offers a less stressful hemodynamic condition in terms of hemoglobin levels, patients with elevated white cells, had poorer clinical and urinary features, an indication that probably, the inflammatory response elicited in these patients, might have gone berserk due to the high levels of the white cells.

KEYWORDS

Sickle cell disease;
Hemolytic anemia;
White blood cell count;
Inflammation;
Urinary tract dysfunction.

INTRODUCTION

The primary defect in SCD is the replacement of glutamic acid by valine in the β-globin chain, rendering the HbS unstable, and polymerising deoxyHbS; the extent of polymerisation being the primary determinant of the severity of SCD[1]. Leucocytes contribute to the disease process, as leucocytosis is a risk factor for early

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SCD-related death[2]. The major laboratory risk factors for vaso-occlusive crises and acute chest syndrome are high steady state leucocyte counts and high hemoglobin levels[2, 3]. High white cell count is also associated with development of nephropathy[4]. The microvascular entrapment of red cells and leucocytes obstruct blood flow and brings about organ ischemia. Cycles of ischemia and reperfusion, cause oxidant stress, in which there is activation of vascular oxidases[5], characterized by inflammatory cytokines and leucocytosis[6].

Polymorphonuclear neutrophils release large quantities of reactive oxygen species in response to various stimuli in respiratory burst[7]. This process is critical for bacterial killing and also potentiates inflammatory reactions, sometimes inducing severe host tissue injury when activation is excessive or inappropriate[8].

High hemoglobin levels in SCD will create hyperviscosity, while low hemoglobin, caused by intravascular hemolysis, would unleash the vasulopathic potential of cell-free hemoglobin[9, 10]. Based on hemoglobin levels, persons can be categorized as non-anemic, mildly anemic, moderately anemic and severely anemic. Given the pathophysiology of SCD, it is assumed that the mildly anemic state (Hb more than 7.0 g/dl but less than 11.0g/dl) should provide the range of hemoglobin concentrations that would not be too high to present unfavorable rheological visco-elastic properties that could promote polymerization. Neither would the hemoglobin level be too low as to impair its oxygen-carrying function, or reduce the bioavailability of nitric oxide, for the unleashing of vasculopathic tendencies.

Having thus minimized the effects attributable to hemoglobin levels, in the mildly anemic state, how the levels of leucocytes affected the clinical state, as well as the urinary features of SCD patients was the objective of this study.

According to Coller[21], leucocytes are emerging as a major, yet understudied contributor to the overall pathology of ischemic vascular diseases, like SCD. Studies using a mouse model of SCD[22], showed that both the red blood cells and leucocytes interact directly with each other in the vaso-occlusive blockage in blood vessels. A flow adhesion assay by Finnegan et al[23], revealed that monocytes, neutrophils and lymphocytes can bind red blood cells, and this binding is reduced in patients on hydroxyurea.

This study, a cross-sectional, one looks at how two categorical levels of white blood cells affect the clinical and urinary markers of SCD patients with 'apparently uniform and optimal range of hemoglobin levels.'

METHOD

Study design

This was a cross-sectional study.

Subjects and setting

The subjects were sickle cell patients reporting to the Sickle Cell Clinic (SCC) at the Komfo Anokye Teaching Hospital (KATH), Kumasi.

Sampling and analysis

The attending clinicians, after the physical and clinical examination of the subjects identified those in crises and steady state, and referred them to an interviewer and phlebotomist. Blood sample from each patient was then taken from the antecubital vein into a sample tube. The collected blood samples were kept in an ice chest and taken to the Hematology Department of the KATH for the determination of full blood count, including hemoglobin, total white blood cell count, and the differential counts for the levels of neutrophils and monocytes.

Spot urine samples were collected between 2-5 pm from the patients, into clean plastic bottles, and kept on ice in an ice chest, and the analysis was done within three hours after the collection.

A visual observation of the urine was made for the colour and cloudiness, followed by microscopic examination of spun urine samples. Then a dipstick test was carried out on the unspun urine specimen, using Cybow™ reagent strips for urinalysis (manufactured by DFI Co Ltd., 542-1, Daman-Ri, Republic of Korea).

Cell Dynn 1800 electronic automated counter (Abbott Diagnostic Division, USA) was used to determine the full blood count, and based on the WHO classification of anemia, the mildly anemic patients were identified. This sub-group was further stratified into two, based on their leucocyte counts; levels above and below 10×10^9/L. The neutrophil and monocyte counts were also noted. The clinical and urinary features of the leukocytotic were then compared with the non-leuco-

cytotic counterparts.

Ethical approval and informed consent

Ethical approval for this study was granted by the Committee for Research into Human Subjects of the School of Medical Science and KATH, Kumasi. A written informed consent was also obtained from either the parents/caretakers or the subjects themselves.

Statistical analysis

Data on quantitative variables were statistically analysed using Statsgraphics 2006 (StatPoint Inc, USA), to obtain mean values and standard deviations.

RESULTS

Of the 330 SCD patients, 214 were mildly anemic, but after excluding patients with confounding factors of malaria parasitemia, HBsAg, thrombocytopenia (platelet count $<150\times10^9$/L) and thrombocytosis (platelet count $>600\times10^9$/L), 150 of the subjects (71 males and 79 females) were selected. Of this number, the 19 whose genotype had not been determined were also excluded. Thus, the mildly anemic under consideration were made up of 80 SS and 51 SC subjects. Based on the leucocyte cut-off level of = 10×10^9/L, TABLE 1 shows the grouping of the patients between the two categorical levels of leucocytes, according to sex, genotype and clinical states (steady or crisis).

The age range for the subjects was between 5 and 20 years. The mean age of the 3 SC females in the steady state, who were leucocytotic, was 7.5 years, and of the SC leucocytotic females in crisis, 9.75 years. The groups with sample sizes of up to four (n = 4) is too small and so are ignored in the statistical analysis for significance. All the other groups (with n > 4) had mean ages between 11.75 and 15.22 years, with no statistically significant differences. Thus age was not a confounding factor.

The number of patients with leucocytosis was 66, constituting 50.4% of the sub-group of mildly anemics under investigation. There were 35 males (53.0%) with leucocytosis, and the females were 31. The patients showed more leucocytosis in the crisis state, 42 of them (63.6%), compared to the 24, who had elevated leucocytes, even in the steady state.

TABLE 1 : Mildly anemic SS and SC patients showing normal and elevated levels of leucocytes

	SS Males crisis		SS males steady state	
	WBCs $<10\times10^9$/L	WBCs $>10\times10^9$/L	WBCs $<10\times10^9$/L	WBCs $>10\times10^9$/L
n	9	18	4	9
Min	5.0	10.2	5.3	10.4
Max	9.9	35.7	9.9	18.7
Mean	8.189	16.81	7.4	13.97
	SS males crisis		**SS males steady state**	
	WBCs $<10\times10^9$/L	**WBCs $>10\times10^9$/L**	**WBCs $<10\times10^9$/L**	**WBCs $>10\times10^9$/L**
n	5	14	11	10
Min	6.6	11.0	4.6	10.2
Max	9.8	30.2	9.6	27.6
Mean	8.48	18.72	7.44	16.20
	SS Males crisis		**SS males steady state**	
	WBCs $<10\times10^9$/L	**WBCs $>10\times10^9$/L**	**WBCs $<10\times10^9$/L**	**WBCs $>10\times10^9$/L**
n	8	6	11	2
Min	4.8	10.2	3.1	10.1
Max	9.4	13.8	9.2	10.5
Mean	7.38	11.68	6.15	10.30
	SS males crisis		**SS males steady state**	
	WBCs $<10\times10^9$/L	**WBCs $>10\times10^9$/L**	**WBCs $<10\times10^9$/L**	**WBCs $>10\times10^9$/L**
n	9	4	8	3
Min	3.0	12.50	5.1	10.0
Max	9.6	16.50	9.7	18.6
Mean	6.94	14.15	7.54	14.87

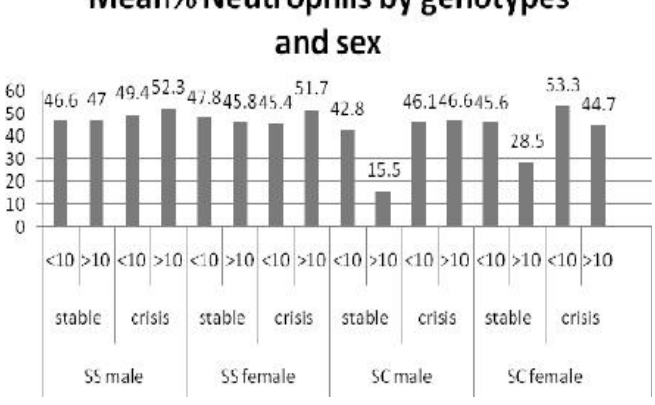

Figure 1 : Mean percentage neutrophils by genotypes and sex. <10 stands for WBC counts less than 10×10^9/L, while >10 denotes more than 10×10^9/L.

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Of the 66 leucocytotic, 51 (77.3%) were SS and 15 (22.7%), SC. The male SS group in crisis formed the majority (18/66) of the patients with elevated white blood cells, but the greatest elevation of the white blood cells occurred in the female SS patients in crisis, who had a mean level of 18.72×10^9/L. On the contrary, the SC male in steady state had the lowest mean leucocyte count of 6.15×10^9/L.

From the pattern of distribution of the neutrophils (Figure 1), the three highest neutrophil counts (levels above 50%) were found in the crisis state of SS males and females, and SC females. In the SS, these highest neutrophil counts were found in the leucocytotic group, whereas in the SC, the highest was in the non-leucocytotic group. The SC males stable (n = 2) and females (n = 3) recorded lowest values of neutrophils (15.5 and 28.5%). Because of the small sample sizes, these are ignored.

The majority (89/131 or about 68%) of the subjects had no monocytes in their peripheral count, and for those who had these cells, the levels ranged between 1-7%. The proportion of the subjects with monocytes were as follows; SS males stable (2/13), SS males crisis (8/27), SS females stable (6/21), SS females crisis (11/19), SC males stable (4/12), SC males crisis (3/14), SC females stable (4/12) and SC females crisis (4/13). In the SS, for both males and females, the proportion with monocytes doubled from steady to crisis (15.4 to 29.6% and 28.6 to 57.9% respectively). On the other hand, in the SC, the proportion with monocytes decreased from steady to crisis in males, but in females, there was no difference between the steady and crisis states.

The urinary parameters identified as markers of some disorder, either in the kidney, or systemic circulation were as follows, according to their decreasing order of occurrence; pus cells, cloudiness, yeast, blood, protein, red blood cells and epithelial cells. The others were nitrite, urobilinogen, bacteria, ketones, bilirubin and coke-like color of urine.

From TABLE 2, a total of 23 (35.4%) of the patients who had their leucocyte counts within the normal range had some form of abnormality in the urine. On the other hand, for those with leucocytosis, the number with abnormal urinary features was 33, forming 50% of this sub-group.

TABLE 2 : The relation between level of leucocytes and the proportion of subjects in the different genotypes who showed some abnormality in the urinary features.

	Leucocytes<10×10^9/L	Leucocytes >10×10^9/L
SS male steady state	1/4 (25.0)	3/9 (33.3)
SS male crisis	2/9 (22.2)	6/18 (33.3)
SS female steady state	5/11 (45.5)	7/10 (70.0)
SS female crisis	2/5 (40.0)	10/14 (71.4)
SC male steady state	2/11 (18.2)	½ (50.0)
SC male crisis	1/8 (12.5)	0/6 (0)
SC female steady state	4/8 (50.0)	3/3 (100.0)
SC female crisis	6/9 (66.7)	¾ (75)
Overall	23/65 (35.4)	33/66 (50.0)

Percentages in parentheses

For the SS males in steady state, only one patient out of four with normal leucocyte count had an abnormal urine feature, compared to the 3 out of nine leucocytotic who showed cloudiness in the urine.

In the crisis state, 2 out of the 9 patients with normal WBC count showed urine cloudiness or pus cells or both, whereas for the leucocytotic group, 6 out of 18, showed some markers of kidney lesion, including blood, protein, in addition to cloudiness.

In the case of the SS females in the steady state, 5 of the 11 within the normal range of WBCs showed some markers of abnormality, compared to the 7 out of 10 leucocytotics. Apart from the cloudiness, proteins and pus cells, the females also showed yeasts, nitrite, bacteria and urobilinogen. The intensity of the pus cells was also higher, recording levels as high as 100-150 pus cells.

The urinary picture of the SS females in crisis was worse than those in the steady state. Two out five subjects with normal WBC count, showed protein, RBCs and blood. The vaso-occlusive crisis of a third patient was so serious that she was referred to the Paediatric Emergency Unit. Of the 14 who had elevated leucocytes, 10 of them showed varied markers from cloudiness, yeasts, pus cells, proteins, nitrite and red blood cells.

The SC males presented the best picture of the urinary features. In the steady state, 11 of them had nor-

mal WBC counts, and of this number, 2 showed urinary cloudiness and pus cells. Only two had leucocytosis, with one of them having a slight pyruria. For the 5 leucocytotic male SC patients in crisis, no marker of systemic or kidney dysfunction was shown, and for the 8 within normal-range of WBC count, only one showed ketonuria.

Vaso-occlusive pain or crisis was the most common diagnosis, irrespective of the leucocyte count (TABLE 1). However, the patients with elevated white cell counts were more frequently associated with other complications such as respiratory tract infections, splenomegaly, osteomyelitis and leg ulceration.

DISCUSSION

The higher proportion of leucocytosis in the crisis state, 63.6% attests to the greater degree of inflammation in this state, than in the steady state. Of the two genotypes, the SS showed relatively higher degree of leucocytosis than the SC: the SS males in crisis predominated the leucocytotic (77.3%), and the highest mean WBC count was seen in SS females in crisis (TABLE 1).

It had been observed by Platt et al.[2], likewise Benkorrou et al.[3], that the major laboratory factors for vaso-occlusive crises and acute chest syndrome, two acute events of SCD, are high steady state leukocyte counts and high hemoglobin levels. White cell counts are significantly elevated and are highly correlated with stroke in children with sickle cell anemia[2]. High white cell count is also associated with development of nephropathy[4]. The base-line leucocyte count has also been shown to be a strong predictor of which infants with sickle cell anemia will develop clinically severe disease[11]. In another study by Litos *et al.*[12], pregnant women who developed SCD-related complications had significantly higher total WBC count, compared to asymptomatic subjects. Clinical studies have suggested a link between elevation of WBC counts and poor outcome of SCD[6].

In the presence of the elevated WBCs, there could be the entrapment of these cells, red cells and other blood factors, to obstruct blood flow, causing ischemia: and cycles of ischemia-reperfusion can lead to oxidant stress, activating vascular oxidases[5], and inflammatory stress, marked by inflammatory cytokines and leucocytosis[6, 13]. Rother et al.[14] had also observed that the release of hemoglobin by lysed erythrocytes into the blood plasma, unveils its vasculotoxic potential by directly impairing endothelial function and generating inflammatory and oxidative stress.

The crisis states were associated with the greater elevations of neutrophils (Figure 1), and to a less extent monocytes. White blood cells, particularly, neutrophils and monocytes are regarded as markers of inflammation[20]. A large number of neutrophils are rapidly recruited to the site(s) of infection where they function to destroy invading pathogens, using an array of oxygen-depedent and oxygen-independent microbicidal weapons to destroy infectious agents[15]. Oxygen-dependent mechanisms involve the production of reactive oxygen species, which can be microbicidal[7], and oxygen-independent mechanisms include most other neutrophil functions, like chemotaxis, phagocytosis, degranulation and release of bactericidal peptides[15]. Brittain et al[24] had shown that interactions of monocytes with reticulocytes can potentially activate the monocytes for the release of inflammatory factors from monocytes, apart from inducing the adhesivity of same to the endothelium.

This study further looked at the link between the extent of increase of WBC count to the clinical presentations and the frequency of occurrence of markers indicative of renal damage, using a spot urine analysis. As shown in TABLE 1, vaso-occlusive crisis was common to both the leucocytotic and the non-leucocytotic. However the leucocytotic had more crises and complications; and greater severity of their conditions, evidenced by presence of such complications as respiratoy tract infections, splenomegaly, osteomyelitis and leg ulceration, and the admission of patients in the Paediatric Emergency Unit of the hospital.

Whereas 35.4% of patients with normal levels of WBC showed some markers of kidney lesion, 50.0% of the leucocytotic showed such lesions (TABLE 2). Thus, there was greater likelihood of encountering a kidney disorder in those with leucocytosis. These disoders were also most common in female SS in crisis, while the male SC showed the least. As to whether the kidney disorder was as a result of the leucocytosis or a urinary tract infection could not be determined,

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except to say that the two possibilities exist.

SCD may present with a variety of renal dysfunction as a result of several structural and functional abnormalities that occur along the entire length of the nephron[16]. According to Allon[17], SCD causes defects in the tubulomedullary function, leading to proteinuria, progressive renal insufficiency and end stage renal disease.

Urinary tract infection (UTI) is frequently caused by organisms which are normal commensals in the distal urethra and adjacent sites[18]. The most common route of infection is by ascension. Because the female urethra is shorter than that of male, there tends to be higher UTI prevalence in females than males. This sexual dimorphism in UTI prevalence has also been attributed to a relative deficiency of secretory IgA antibody response from the mucosal surface in the urogenital tract of females, compared to males[19].

The possible roles of neutrophils and monocytes have been shown, though circumstantially. The observation that the neutrophils formed more than 50% of the WBC in the crisis state of both males and females of SS genotype, and that these high percentages were found in the leucocytotic, is supportive of the deleterious role they could play. For the monocytes, this study has shown that their numbers doubled from the steady to crisis states of male and female SS subjects. Monocytes activate the endothelium by secreting the inflammatory cytokines, tumour necrosis factor-α and interleukin-1β and such activated endothelial cells increase their expression of ligands for adhesion molecules on leuocytes and erythrocytes. In this way, monocytes facilitate adherence of blood cells to vessel walls, and promote vaso-occlusion[25, 26].

LIMITATIONS OF STUDY

The cross-sectional design of the study was a limitation, as the reproducibility of the observations was not proven. Even though attempts were made to eliminate confounding factors like thrombocytosis, thrombocytopenia, malaria parasitemia and hepatitis B antigenemia, there could be several other factors that could have affected the results, for example, the effect due to adhesivity from reticulocytes. The fact that spot urine samples were used for the urinalysis is another limitation.

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