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Original Research

Some conventional and non-conventional hematological and biochemical markers for characterizing some acute and chronic presentations of sickle cell patients

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Abstract

Background: Some acute and chronic presentations of sickle cell disease (SCD) are splenomegaly, acute chest syndrome (ACS), priapism and leg ulceration. Hematological and biochemical parameters are used for diagnosis and prognosis.

Method: In this cross-sectional study, carried out over a 12-month period, involving 291 patients, some conventional hematological and biochemical parameters, and two non-conventional determinations, aspartate aminotranferase: alanine aminotransferase (AST:ALT) ratio and urine color, were used to characterize the patients with some selected clinical presentations.

Results: For these subjects studied (n = 21), the mean values of hemoglobin (Hb), red blood cells (RBC), white blood cells, (WBC), reticulocytes, platelets and fetal hemoglobin (HbF) were 6.6 g/dl, 2.28×10^{12}/L; 16.3 $\times10^{9}$/L, 27.1%, 310.1×10^{9}/L and 1.84%, respectively. The mean AST:ALT ratio was 2.058 and the urine color, 2.381. These values were compared to 5 non-anemic counterparts, who had the following mean values of the parameters; Hb = 11.9 g/dl; RBC = 4.13×10^{12}/L WBC = 7.2×10^{9}/L; reticulocytes = 5.2%; platelets = 282.4×10^{9}/L; HbF = 1.48%. A Mann-Whitney non parametric two-tailed test showed several of the differences between the patients with complications and the non-anemic, were statistically significant ($p < 0.05$). The subjects with the complications were moderately anemic, having elevated levels of lactate dehydrogenase, total bilirubin, indirect bilirubin and AST:ALT ratio.

Conclusion: The four clinical presentations were characterized by anemia, accompanied by high levels of conventional markers, like reticulocytes, lactate dehydrogenase (LDH) and bilirubin, together with an elevated AST:ALT ratio, a non-conventional marker.

INTRODUCTION

Sickle cell disease is the most common genetic disorder, with the highest rate of occurrence in Africa [1]. It is a life-long hemolytic anemia caused by a single point mutation on the sixth codon of the β-globin gene on chromosome 11p 15.5 [2]. The change from a charged to a neutral, hydrophobic amino acid creates a sticky patch on the molecular surface, causing aggregation upon deoxygenation [3], seen as polymerisation of the deoxy HbS into a gel [4].

The main pathophysiology of SCD is the polymerization of the deoxygenated HbS, leading to erythrocyte rigidity, distortion, membrane damage and hemolysis [5, 6]. The cycles of erythrocyte sickling cause the cells to become fragile, allowing lysis to occur, reducing the RBC life span from the normal 120 days to 10 days, producing chronic hemolytic anemia [7].

Two sub-phenotypes have been found to be associated with hemolytic rate. One of the phenotypes is the 'viscosity-vaso-occlusion' involving red cell sickling, responsible for

complications like pain crisis, ACS and avascular necrosis [8, 9, 10]. The second phenotype is the 'hemolysis-endothelial dysfunction' a proliferative vasculopathy that involves pulmonary hypertension, priapism, leg ulcers, sudden death and stroke [9].

The viscosity-vaso-occlusion group is associated with lower hemolytic rate, marked by a higher hemoglobin level, low plasma hemoglobin, LDH, bilirubin and arginase levels [8, 9]. Patients with the hemolysis-endothelial dysfunction sub-phenotype exhibit markers of high hemolytic rate, like low hemoglobin, high plasma hemoglobin and bilirubin, culminating in low nitric oxide (NO) bioavailability [8, 9].

The foregoing biochemical and hematological parameters, which we term as conventional markers, have been used to characterize SCD patients in several studies [8, 9, 10]. In the current study, these conventional features would be used to characterize a group of SCD patients with ACS, splenomegaly, priapism (acute presentations) and leg ulcers (chronic presentation). Additionally, we are introducing two non-conventional parameters, AST:ALT ratio and urine color number scale.

We have previously proposed the AST:ALT ratio in SCD patients as a hemolytic marker [11]. Due to the hemolytic tendency, the AST:ALT ratio has been found to be higher than what is found in HbAA persons [11]. Furthermore, an SCD patient could be icteric or anicteric. Jaundice could be hemolytic, hepatic or obstructive. There is also the possibility of intravascular hemolysis leading to hemoglobinuria, and rhabdomyolysis, causing myoglobinuria. Therefore, we hypothesise that the urine of the patients would have a hue of colors. The urine colors are given a number scale or scoring, which we think can give an objective quantitative measure, allowing its comparison to other parameters.

Study population, materials and method

The study population comprised 291 patients, of SS and SC genotypes, recruited from the Sickle Cell Clinic of the Komfo Anokye Teaching Hospital, (KATH) Kumasi, Ghana. Cell Dynn 1800 electronic automated counter (Abbott Diagnostic Division, Abbott Park, Virginia) was used to determine the hematological indices, while biochemical parameters were determined using the Humalyzer Junior (Human Gesellschaft fùr Biochemica und Diagnostica GmbH, Wiesbaden). The kits used for the biochemical parameters were also supplied by Human.

The study participants were aged between 5-20 years. Those who were very sick and weak who had been given some emergency drug therapy were excluded. The patients were examined by the clinicians and those with splenomegaly, ACS, priapism and leg ulceration, identified. Splenomegaly was diagnosed through palpation. ACS diagnosis was done clinically and radiologically; chest pain, cough, fever and appearance of new infiltrate on chest x-ray radiography. Priapism cases were identified in males who had reported prolonged penile erection without sexual arousal. Leg ulcer cases were those who had reported of sores persisting for over three months. The ages of the patients and number of visits they had made in the preceding 12 months were extracted from their medical records.

From the blood sample taken from the antecubital vein, biochemical and hematological parameters were measured. Hemoglobin, RBC, WBC and platelet counts were determined using the Cell Dynn autoanalyzer. However, a manual method (involving supravital staining with brilliant cresyl blue), was used for determining the reticulocyte count, recorded as the percentage of red cells counted in a field, per thousand red cells, by light microscopy. HbF measurement was by the alkali denaturation method [12]. The biochemical indices determined were enzymatic (LDH, ALT, AST) and non-enzymatic (total, direct and indirect bilirubin). A spot urine sample was also collected for visual observation. The hues of urine color seen were given number scores, from 1 to 4; 1 for colorless, 2 for straw-colored or amber, 3 for deep amber and 4, for reddish brown or coke-like.

From the hemoglobin measurements, SS patients who were not anemic (Hb $\geq$ 11.0 g/dl), were also identified, and the characteristics of this group recorded, to serve as a control group.

Statistical analysis

The values of the various measured parameters were subjected to statistical analyses using Statgraphics 2006, StatPoint Inc., USA, statistical package. The mean values and standard deviations were calculated. Statistical significance of differences between means were found using students' t-test and two-tailed Spearman nonparametric correlation analysis for association between some of the parameters was also done.

Ethical Approval and informed consent

The Committee for Human Research, Publication and Ethics of the School of Medical Sciences, KNUST and KATH, granted ethical approval to this study. Informed consent was provided by either patients who were 18 years and above or by the parents/caregivers of minors.

RESULTS

According to Table 1 there were 7 cases each, for splenomegaly and ACS, 4 cases of ulcer and 3 of priapism. Only 2 SC subjects had the conditions; 1 each of ACS and leg ulcer. The other conditions were found in the SS genotype. The overall population of SS and SC in this study is 291, so the prevalence of both ACS and splenomegaly is 7/291 or 2.4%. If the two genotypes are segregated, the prevalence rates of ACS for SS and SC are 3.2% (6/187) and 0.96% (1/104), respectively. The prevalence of leg ulcer is 1.4% and priapism, a male problem, 3/95 or about 3.2%.

The condition with the highest mean level of hemoglobin (7.4 g/dl) was leg ulcer, but the others had similar levels of hemoglobin. The lowest mean hemoglobin of 6.3 g/dl in the splenomegalics is noteworthy. Indeed, one of the splenomegalics had the lowest hemoglobin of 4.4 g/dl (result not shown). The differences in mean levels of hemoglobin between the various pairs of complications are not statistically significant. For the hematological parameters, the patients with leg ulcers had the highest mean levels of hemoglobin, platelets and reticulocytes, but these did not significantly differ from the other conditions.

The differences in mean levels of hemoglobin and red cells

between the non-anemic subjects (n = 5) and their counterparts with complications (n = 21), is so obvious, requiring no statistical analysis. However, for the other hematological parameters where the differences were not that obvious, the Mann Whitney non-parametric analysis showed that the WBC and reticulocyte counts are significantly elevated in the patients with complications. On the other hand, the HbF and platelets showed no differences between the two groups.

Of the six biochemical parameters, while the mean levels of LDH, total bilirubin and indirect bilirubin were elevated in the patients with complications, AST, ALT and direct bilirubin, showed non-significant differences.

Comparing the mean values between pairs of clinical presentations, it is only the WBC and LDH that show significant differences. For example, the splenomegalic and the patients with ACS show higher white cell counts than the patients with priapism, while the splenomegalic also has higher LDH than those with priapism.

Since some background information on the patients would put the clinical picture of the 21 patients in the proper context, we also provide a summary of the clinical states of the patients twelve months prior to their being drafted into the study. The information was extracted from their medical records.

In the previous visits, the patients had reported to the clinic with other clinical presentations. For example, in the case of the patient with leg ulcer who made the highest number of visits of 15 times, some of the clinical notes on her records included, vomiting, diarrhea, fever, abdominal pain, mild vasoocclusive crisis, steady state, jaundice, dysuria. Some drugs that had been prescribed for her were wokadin ointment, glucosamine, zincovit, ciprofloxacin, folic acid, daraprim, diclofenac, ibuprofen flucoxacillin, germidine solution, dalacin, plasmotrium. All the other patients, in their visits, had similar presentations, with the two predominant being malaria and vaso-occlusive crises. Their drug prescriptions had been similar, made up of antimalarials like camoquin, artesunate, pain killers and antibiotics. It was an eight-year old girl with splenic sequestration, with hemoglobin of 4.4 g/dl, who was admitted in the Pediatric Emergency Unit for blood transfusion.

Table 1. Mean ± SD hematological and biochemical parameters for some clinical presentations

Parameter	S'megaly n = 7	ACS n = 7	Leg ulcer n = 4	Priapism n = 3	Total n=21	Non-anemic n = 5
Hb (g/dl)	6.3 ±0.55	6.7 ±0.59	7.4 ±0.71	6.6 ±0.45	6.7 ±0.29	11.9±0.12
HbF (%)	1.6 ±0.31	2.5 ±1.02	0.7 ±0.31	2.2 ±0.23	1.8 ±0.40	1.5 ±0.25
RBC	2.18 ±0.27	2.31 ±0.84	2.39 ±0.04	2.49 0.16	2.33 ±0.14	4.13 ±0.35
WBC	19.3±4.40g	16.3 ±(2.09)f	14.8 ±2.86	7.5±1.19f,g	15.8±1.78d	7.2 ±0.92d
Platelets	224.3±35.5	299.0±55.1	500.8±174.3	287.3±24.0	310.3±41.35	282.4±29.8
Retics (%)	18.0 ±6.80	23.5 ±5.76	27.3 ±4.52	14.3 ±3.28	26.2 ±3.31e	5.2 ±0.49e
ALT (U/L)	27.4 ±2.81	24.5±3.84	36.8 ±6.65	23.3 ±3.71	24.5 ±3.84	22.4 ±5.16
AST (U/L)	54.6 ±6.70	56.5±14.85	60.0 ±13.98	36.3 ±3.18	53.8 ±6.02	47.8 ±13.60
LDH (U/L)	1470±274h	1166 ±203	1036 ±100	828 ±49h	1193 ±120a	603 ±95a
TB	52.1±23.03	18.0 ±2.68	35.6 ±13.52	38.9±11.82	34.9 ±8.07b	12.3 ±2.06b
DB	21.4 ±9.90	8.6 ±2.03	15.2 ±5.12	19.8 ±8.49	15.4 ±3.54	4.8 ±1.21
IB	30.7±13.46	9.5 ±0.73	20.4 ±9.19	19.1 ±5.03	19.5 ±4.76c	7.5 ±1.22c

Values with identical letters show statistically significant difference.
a; p = 0.0052, b; p = 0.0374; c; p = 0.0293, d; p = 0.0063, e; p = 0.0007
f; p = 0.0187, g; p = 0.0333, h; p = 0.0333
Units RBC: $\times 10^{12}$/L, WBC $\times 10^{9}$/L, $\times 10^{9}$/L, TB, DB, IB; µmol/L
SD: standard deviation, TB: total bilirubin, IB: indirect bilirubin, DB: direct bilirubin.

Table 2. Mean ± SD age, visits, urine color and AST:ALT ratio of patients with various presentations compared with non-anemic counterparts.

	Age	Visits	Urine color	AST:ALT ratio
S'megalic	11.14 ±2.73	4.71 ±0.84	2.857 ±0.26a	2.007 ±0.17
ACS	11.00 ±0.98	5.00 ±0.80	2.130 ±0.13a	2.27 ±0.31
Priapism	13.67 ±2.19	4.67 ±1.86	2.000 ±0.00	1.60 ±0.14
Leg ulcer	17.25 ±1.11	5.50 ±3.30	2.250 ±0.25	1.87 ±0.52
Non-anemic	12.40 ±2.29	3.20 ±1.56	2.000 ±0.00	2.072 ±0.12
Total	12.67 ±3.61	5.33 ±3.34	2.381 ±0.59	2.058 ±0.73

a: Statistically significant; p = 0.0417

From Table 2, apart from the patients with leg ulcers who have significantly higher mean age, the patients with the other conditions and non-anemics have similar ages; they are younger. Whether for the subjects with the complications or the non-anemics, there are no differences in the visits made to the clinic. The patients with priapism, like the non-anemics, are lowest in the urine colour score, while the splenomegalic is the highest. There is statistically significant difference in the urine color between the splenomegalic and the subjects with ACS, but the difference between the splenomegalic and leg ulcer is not significant. For the AST:ALT ratio, the value obtained for ACS was the highest, while the level for priapism, the lowest. The lowest p-value of 0.2619 was seen in the difference between splenomegalics and subjects with priapism.

Table 3. Correlation coefficient (r) and p-values of AST:ALT ratio vs Hb and urine color for the various conditions

Presentation	Hb		Urine color	
	r	p	r	p
Splenomegaly	-0.3571	0.4444	-0.3187	0.4976
ACS	-0.8108	0.0341*	0.4082	0.3536
Leg ulcer	-0.6000	0.4167	-0.7746	0.3333
Priapism	1.0000	0.3333	-	-
Non-anemic	-0.5643	0.3500	-	-
Total	-0.5172	0.0163*	-0.0849	0.3500

* Statistically significant; p< 0.05

From Table 3, generally, there is negative correlation between the AST:ALT ratio and hemoglobin levels and urine color. For ACS and the entire group of subjects with the 4 presentations (total), the association was statistically significant. Because the subjects with priapism and the non-anemic had no urine color variation (all urine samples were straw-colored or 2.0), no correlation analysis could be done.

DISCUSSION

The characteristics of these four clinical presentations; ACS, splenomegaly, priapism and leg ulcers are shown in Tables 1 and 2. It has to be conceded that due to the inclusion criteria, particularly, the age range used and the exclusion of the severely ill, the prevalence rates in this study could be an under-estimation of the actual rates. Another bias factor is the study being hospital-based

The background information on the clinical conditions of the 21 patients with the complication is a reflection of the common conditions clinicians and other healthcare providers encounter in a clinic dedicated to sickle cell management. Also provided are some of the common therapies prescribed for patients in a West African setting. Unlike developed countries where the use of hydroxyurea is rife, clinicians in the Kumasi Sickle Cell Clinic and other similar clinics in the country have not adopted hydroxyurea as part of their treatment regimen for sickle cell disease patients. There was one case of blood transfusion, in a patient who had acute splenic sequestration with a hemoglobin level of 4.4 g/dl. This was a top-up mode of blood transfusion. Exchange transfusion is to be used in our clinic.

That the SS genotype, compared to the SC, presents the more severe clinical expressivity, is shown by the overwhelmingly larger number of SS patients, 19, who had the presentations, as against 2 of the SC, or 90.5% against 9.5%. Again, while the SS showed all the four conditions, the SC showed two of them; leg ulcer and ACS.

The highest prevalence of 3.2% was recorded for both ACS in SS patients and priapism in SS males, but leg ulcers showed the lowest prevalence of 1.4%. In a study in Accra, Ghana, by Konotey-Ahulu [13], the incidence of priapism in SS patients (n = 387) was 2.5%, and SC (n = 385) was 1.3%. The youngest patients to be affected were 7 and 8 years. Incidence of splenomegaly in a random sample of a West African population varied from 0% at birth to 70% in the 2-5 age range and declining to about 10% at 20 years and above [14]. While 10.6% of SS patients had cutaneous ulcers, 2.2 % of SC had this dermatopathy [14]. The age group of peak incidence was 15-19 years in Accra, compared to 10-14 years, in the West Indies. In the United States, 2·5% of patients with all common genotypes of sickle cell disease had leg ulcers [15]. It is estimated that 8-10% of homozygous patients will develop leg ulceration between 10 and 50 years of age, but higher rates of more than 50% has been reported [15]. The highest mean age recorded for the patients with leg ulcer in this present study, 17.25 years (Table 2), is similar to the study of Akinyanju and Akinsete [16] in Nigeria, in which the ulcers were found in patients above twelve years, and also to the findings of Konotey-Ahulu, in the Accra study [13].

The trend shown in the hematological and biochemical parameters in Table 1 is a reflection of the vaso-occlusive and hemolytic tendencies, characteristic of the sickling hemoglobinopathy. According to Kato *et al*. [9], both vaso-occlusion and hemolysis are major determinants of sickle cell-related organ damage.

Attention is first drawn to the five non-anemic SS patients, (forming about 2.7% of the study population with this genotype) having a mean hemoglobin of 11.9 g/dl. A greater number of the SC was non-anemic, but this group has been ignored, as the sub-set of 21 patients under investigation are predominantly SS. Despite the close association of SCD with anemia, it is still possible to come across some persons with this condition who are non-anemic. This is another indication of the heterogeneous phenotypic features exhibited by this disorder.

Ahmed *et al* [17], studying 18 SCD patients with priapism, found the patients had significantly higher hematocrit than those without priapism. The mean reticulocyte count of those with priapism was lower than that of the patients without (8% vs 12%). However, there was no difference in the HbF levels, 7% and 6%. The conclusion drawn from that study was that SCD patients with priapism had lower rate of hemolysis, resulting in higher hematocrit and greater viscosity, which increased the risk of sickling in the corpora cavernosa. Similar observation had been made in a Nigerian study by Adedeji *et al*. [18] involving 23 SCD male patients, in which patients with priapism had a mean hemoglobin level of 8.63 g/dl, while those without priapism had a mean hemoglobin of 6.67 g/dl and hematocrit of 20.43%. Therefore, relatively

high hemoglobin and hematocrit had been suggested as a risk factor for the development of priapism.

Our present study rather supports priapism arising from increased breakdown of red cells.

In contrast to the non-anemic, the mean hemoglobin level of all the patients with the complications, show they are moderately anemic, while showing elevated levels of reticulocytes. Thus, the hemolytic disposition, had resulted in the complications of the 21 subjects, marked by the mean hemoglobin range of 6.3-7.4 g/dl and corresponding mean red cell count of $2.18\text{-}2.49\times10^{12}$/L (Table 1).

Compared to the non-anemic red cell count of 4.13×10^{12}/L, there has been about 1.7 times decrease in the red cell count in the patients with the complications, due to hemolysis, during which there is growing evidence that hemoglobin becomes decompartmentalized [8, 19, 10]. The cell-free hemoglobin so formed mops up nitric oxide, the physiological endothelial vasodilator. The decreased nitric oxide bioavailability, due to the ectopic localization of hemoglobin [20], results in endothelial dysfunction contributing to chronic vasoconstriction, together with hemostatic activation and vascular smooth muscle proliferation: these are features associated with pulmonary hypertension, priapism, and cutaneous leg ulceration in SCD and other disorders in which intravascular hemolysis is so severe as to overwhelm the hemoglobin-scavenging mechanism [8, 21].

Comparing the overall population of the subjects having the complications (n = 21), with the non-anemic (n = 5), significant differences were seen in LDH, reticulocytes, total bilirubin and indirect bilirubin, known markers of hemolysis. Nolan *et al*. [22], reviewing data for the Cooperative Study on SCD, indicated that the patients with priapism had significantly lower hemoglobin levels, higher reticulocyte counts, along with higher serum total LDH, total bilirubin and AST. The patients with priapism also had significantly higher WBC and platelet counts than those without priapism. In another CSSCD study by Nolan *et al*. [23] on leg ulceration, it was found that the patients with ulcer had minimal but statistically significant reduction in hemoglobin levels and elevation of serum total LDH, total bilirubin and AST, while the reticulocyte counts of the patients with ulcer and those without, were identical.

It has to be noted from this current study that in addition to total bilirubin, indirect or unconjugated bilirubin showed significant difference. The rise in the indirect bilirubin is a further confirmation of the hemolytic tendency, an observation, not reported in the other studies. The other parameter that showed significant difference was the WBC count. The higher leucocyte count in leg ulcer cases, when compared with controls, could reflect an increased hematopoiesis associated with hemolysis, although leucocytosis might also be a marker of more severe vasculopathy [23].

From Table 2, all the AST:ALT ratios are above unity, with the lowest of 1.60 in priapism, and the highest of 2.27 in ACS. This ratio has been proposed as a hemolytic marker in SCD subjects [11], assuming there are no hepatic and cardiac dysfunctions. The high values of the ratio for all the conditions are just a reflection of the underlying hemolytic propensity. What at a first glance, may appear unexpected, is the high value of the ratio for the non-anemic. However, when this ratio of the non-anemic SS, is compared to the ratio of between 1.070 and 1.112 obtained for normal HbAA persons who were non-anemic, in an earlier study [11], it would be appreciated that the hemolytic tendency in the SCD would still be at play, despite the normal hemoglobin level. Thus, what looked like a normal level of hemoglobin was a 'façade' of normalcy. Once hemolysis was taking place due to the hemoglobinopathy, the increase of AST would be higher relative to ALT, while in the normal non-anemic, the relative changes in the two transaminases, were almost equal, hence the ratio being close to unity. It has to be further pointed out that the high AST: ALT ratios could be obtained either in the low-range or high-range values of the transaminases. For the non-anemic SCD, the high ratio could usually come from low-range values.

The urine color number score is another innovation in this study, whereby the different urine colors have been assigned numbers, from 1-4, from colorless, straw, amber to brownish. The non-anemic and the subjects with priapism were lowest on the scale, 2.000, while the splenomegalic, were highest 2.857 (Table 2). The urine color difference between the splenomegalics and the ACS subjects is statistically significant. In this case, it could be said that the extent or degree of hemolysis was different, being greater in splenomegaly than in ACS. The general negative correlation (Table 3) between the AST:ALT ratio and hemoglobin shows the inverse relationship between the two. Due to the very small sample sizes, we find it quite inappropriate to make any further deductions. Table 3 shows the correlation analysis between urine color and either the ratio or hemoglobin.

A deeper urine color could be traced to hyperbilirubinemia arising particularly, from hemolysis or from hepatic crisis, or from intravascular hemolysis. Unconjugated hyperbilirubinemia and jaundice are common findings in SCD because of the chronic hemolysis that characterizes the disease [24, 25]. Hepatic derangement may also cause hyperbilirubinemia, following intra-sinusoidal sickling, intra-canalicular cholestasis and Kupffer cell hyperplasia [26]. Myoglobinuria, resulting from rhabdomyolysis as a result of excessive muscular activity, could also lead to brownish urine color, but this is ruled out in our study, as none of the subjects had engaged in any recognizable vigorous activity, and none had reported of muscular pain.

The diagnosis of splenomegaly is a clinical one, based on sudden and massive enlargement of the spleen, which is associated with a rapid fall in hemoglobin due to the extensive pooling of blood in the spleen [27]. Major crisis is identified by a fall in hemoglobin level to less than 6 g/dl, while hemoglobin of more than 6 g/dl indicates a minor episode. A study of Aquino *et al*. [28], of splenomegalic SCD patients showed two young children (aged 4 and 6 years) had a hemoglobin value less than 2 gm/dl. Apart from the one case of splenomegalic who showed severe anemia, the remaining six were moderately anemic. Okuonghae *et al*. [29] had observed that acute sequestration is a continuum, from the very mild to the most severe. It is therefore, possible that minor forms of sequestration crisis may have occurred in the spleen, in the patients under study, just as has been reported

in the study of Okuonghae et al. [29].

On the whole, this study, using non-anemic SCD subjects as controls has proven 21 patients with ACS, splenomegaly, leg ulcer and priapism to be moderately anemic, associated with lowered red cell and hemoglobin levels, while showing elevated levels of reticulocytes, LDH, total bilirubin and indirect bilirubin. Apart from the changes seen in the foregoing conventional markers, we have also reported for the first time, on the trend of two non-conventional parameters, AST:ALT ratio and urine color.

One major shortcoming of the study is the cross-sectional nature. The other is the small sample size of the overall population, and the consequent smaller sample sizes of the complications: the statistical power of the study is thus low.

The strengths in the study are several. First, the case of studying four different clinical conditions at the same time. Many other studies involved only single complications. Second, our ability to measure many hematological and biochemical parameters for selected complications. Third, our report on the application of AST:ALT ratio and urine color to some clinical conditions in SCD is a novelty.

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