Iron status and hematologic parameters modifications in Congolese suffering from Sickle Cell Anemia

Profil du fer sérique et variables hématologiques chez le drépanocytaire en milieu congolais

Mikobi TM[1], Lukusa PT[2], Aloni MN[3], Mvumbi GL[1], Muyembe JJT[4], Mbuyi-Muamba JM[5]

Corresponding
Tite Minga Mikobi, e-mail: tite.mikobi@gmail.com

Résumé

L'hémolyse chronique dans la drépanocytose expose plus de 70% de drépanocytaires congolais à une poly-transfusion.

Notre objectif était d'évaluer le statut en fer sérique du drépanocytaire et son influence sur les autres paramètres hématologiques.

Il s'agit d'une étude transversale qui a porté sur 211 patients drépanocytaires homozygotes et 74 non drépanocytaires. Les drépanocytaires ont étaient repartis en trois sous-phénotypes cliniques selon la sévérité de la maladie. Les analyses suivantes ont été réalisées : l'hémogramme complet, le dosage du fer sérique.

Les variables quantitatives entre les groupes ont été comparées par le test de t de Student. Les variables de plusieurs groupes ont étaient comparées par le test d'Anova. La valeur de $p<0.05$ a été considérée comme seuil de signification.

Les drépanocytaires avaient le nombre de GB, des plaquettes, le taux des réticulocytes les plus élevés. Le VGM était semblable dans les deux groupes (80,27fl versus 80,86). La fréquence de la microcytose était plus élevée chez les drépanocytaires que dans la population non drépanocytaire (17,1 versus 5,3). Mais en fonction du phénotype clinique la fréquence de la microcytose la plus élevée a été observée dans le sous-phénotype clinique modéré (19,3%). La concentration en fer sérique était semblable dans les deux groupes de la population (17,39 µmol/l versus 16,05).

Dans la présente étude, les drépanocytaires congolais ont des taux en fer sérique semblables à la population non drépanocytaire. La microcytose est plus fréquente chez les drépanocytaires que dans la population non drépanocytaire.

Mots clé : fer sérique, variables hématologiques, drépanocytose, RD Congo

[1]Basic sciences, Faculty of Medicine, UNIKIN, DRC.
[2]Department of Paediatrics, University Hospital, UNIKIN
[3]Department of Paediatrics, University Hospital, UNIKIN
[4]INRB, Kinshasa, DRC
[5]Department of Internal Medicine, University Hospital, UNIKIN

Summary

Background: In the Democratic Republic of Congo, it is estimated that approximately 70% of sickle cell patients require blood transfusion. Despite this high rate of blood transfusion, data about iron status in sickle cell patients are unknown. We assess iron status and hematologic parameters modifications in sickle cell patients living in Kinshasa, DRC.

***Methods*:** A cross-sectional study was conducted in Kinshasa, the Democratic Republic of Congo. Study patients included 211 confirmed SCA patients and 74 subjects with normal Hb (Hb-AA) were enrolled as the control.

***Results*:** The mean white blood cell, platelets and reticulocytes levels tended to be significantly higher in subjects with Hb-SS than in non-Hb-SS subjects. The mean of MCV was similar between the two groups (($p=0.586$). In Hb-SS group, serum iron level tended to be higher than Hb-AA groups. A significantly higher proportion (17.1%) of subjects with microcytosis were subjects with Hb-SS compared to (5.3%) subjects with non-Hb-SS. Macrocytosis was a rare event and was found in 5 (2.4%) subjects with Hb-SS. The means of Hemoglobin, hematocrit and red blood cell levels were significantly higher in sickle patients with ACP ($p <0.001$). The means of reticulocytes and MCV were significantly lower in sickle patients with ACP ($p =0.009$). The means of TCMH and serum iron were similar between the three groups ($p=0.586$). The mean of serum iron level tended to be lower in Hb-SS subjects with microcytosis than in Hb-SS subjects without microcytosis. The mean of serum iron level tended to be lower in sickle cell male than in sickle cell female ($p=0.043$).

***Conclusion*:** Iron deficiency is not uncommon and may be under-reported in sickle cell patients. We recommend serum iron evaluation in patients suffering from SCA in DRC and to individualize the treatment of each patient.

Key words: Sickle cell anemia; clinical phenotype; gender; serum iron; microcytosis; Kinshasa; Democratic Republic of Congo

Introduction

Sickle cell disease (SCD) is common inherited hemoglobin and an autosomal recessive genetic condition due to a single mutation in the beta-globin gene that substitutes valine for glutamic acid in position 6 of the beta-globin subunit resulting in an abnormal haemoglobin HbS molecule (1). The pathogenesis of SCD is centered on the sequence of events that occur between polymerization of deoxyhemoglobin (Hb S) and causes distortion of red blood cells, leading to vaso-occlusive events, chronic hemolysis and organ dysfunction (1).

Sickle cell anemia is the most common genetic disease worldwide. Africa is the continent with the greatest burden of disease (2). The highest frequencies of sickle cell anemia in the world occur in sub-Saharan Africa where 3.0 to 4.0% of the populations are affected (3, 4).

In the Democratic Republic of Congo (DRC), the prevalence of sickle cell trait is 25.0% in adults and the prevalence rate ranges from 0.97% to 1.4% of newborns with homozygous sickle cell anemia (5, 6). The country is the second, about 40,000 newborns per year are born with sickle cell anemia (4).

The main and commonest manifestation of SCA is acute anemic crisis with high risk of blood transfusion. Normal transfusion in some sickle patients increases the risk to develop secondary iron overload (7-9). In other part, previous reports showed that low hemoglobin rate was associated with high risk of mortality (10, 11).

Sub-Saharan Africa contributes significantly to the high global rate of transfusion reported in sickle cell patients (1, 2). This alarming rate is attributed to either tropical infectious diseases or non-infectious complications including acute anemic crisis (12-14).

In DRC, the main haplotype of SCD is the Central African Republic (CAR) –globin gene, the most severe form of the disease (15). In this country, it is estimated that approximately 70% of sickle cell patients require blood transfusion and the average blood transfusion requirement was 0.4 units per patient-year (12, 14).

Hematologic effects of iron overload were increased MCHC, reticulocyte count, RDW, and dense cells. The increase of this hematologic parameters leads to vasoocclusive events and organ damages (16, 17). However, population with sickle cell anemia living in Sub-Saharan Africa are confronted to malnutrition, malabsorption, growth spurt, infections, geohelminth infections and iron deficiency as reported in previous studies (11, 18-20).

Despite this high prevalence of the disease and the rate of blood transfusion in our midst, information about iron status in population suffering from SCA in DRC are unknown. Probably this data is under-reported in sickle cell Congolese patients, poverty and the paucity of pediatricians and hematologists in this country should contribute to this fact. To our knowledge, in Central Africa region, none available epidemiological data have been reported. In this context, dietary iron requirements are unclear in patients suffering from sickle cell anemia in this region. We hypothesized than iron status influences the severity of sickle cell anemia, especially in Congolese patients living in Kinshasa, the Democratic Republic of Congo.

Our ultimate goals are to develop the basis for designing and implementing effective preventive interventions for post-transfusional complications in sickle cell patients. In this first report, we assess iron status in sickle cell patients living in Kinshasa, DRC.

Methods

Study design and participants

This was a cross-sectional and multicenter study conducted over a 3-month period. The study was conducted in Sickle cell centre of Yolo. This hospital provides most of the non-private paediatric beds in Kinshasa for sickle

cell patients. The number of consultations per year is approximately 6,500.

Patients were selected in the outpatient clinic of Sickle Cell Centre of Yolo. Study patients included 211 confirmed SCA patients consecutively recruited while 74 with normal Hb (Hb-AA) were enrolled as the control in the study. The characteristics of the studied patients are summarized in table-1.

We excluded subject with (i) history of sickle cell crisis during the period of study; (ii) initiated antibiotics treatment prior to seeking medical care; (iii) previous blood transfusion in the 3 months prior to the study; (iv) initiated hydroxyurea therapy (v) patient on red cell exchange transfusion.

Laboratory analysis

Five ml of venous blood sample was drawn from each study participant into an EDTA tube, used to determine hematologic parameters. Hematologic parameters analysis was performed at Institut National de Recherche Biomédicale (INRB) of Kinshasa using an automate Sysmex XS – 1000 *i* (Lincolnshire, USA).

The blood sample from each participant was collected into an EDTA tube, and used to determine haemoglobin and protein electrophoreses. Sickle cell screening was performed using semi-automated agarose gel electrophoresis technique with the Hydrasis II apparatus (SEBIA, France). Sickle cell anemia was diagnosed in presence of production of mostly Hb S with no Hb -A.

Serum iron assay was performed at 562ηm with a Thermo Genesys 10S Bio spectrophotometer (USA). Serum iron concentration was calculated in µg/dl, then converted in µmol/L with the conversion factor given by the manufacturer: µg/dl x 0.179= µmol/L. The reference values were: Men (11.6-31.3 µmol/L), Women (7.16-26.85).

Hematological and biochemical analyzes were performed in the laboratory of the Institut de Recherche Biomédicale (INRB) at Kinshasa, the DRC.

Case definitions

We conceive a clinical phenotype score built up by recording the individual scores related to the most relevant medical history parameters (Table-2).

The following definitions were applied: asymptomatic clinical phenotype (ACP) (score $\leq$ 5), moderate clinical phenotype (MCP) (score between 6 and 15), and severe clinical phenotype (SCP) (score $\geq$ 16).

Microcytic anemia was defined as a mean red cell corpuscular volume < 75 Fl; Macrocytic anemia was defined as a mean red cell corpuscular volume < 105 Fl

Ethical consideration

Ethical approval for the study was granted by the Ethical Committee of the Public Health School of the University of Kinshasa, Kinshasa, DRC (ESP/CE/027B/2011), in compliance with the principles of the Helsinki Declaration II. The aim and the procedures of the study were explained to the participants. Written informed consent was obtained from the patients before the samples and patient data were collected. The participants were informed that they could withdraw anytime without further obligation.

Data management

All data obtained from the study were manually entered into a microcomputer using the Excel Microsoft 2003. After data cleaning (control for quality and coherence), they were exported on SPSS 17.0 for further analysis. Data are represented as means ±SD when the distribution was normal and median with range when the distribution was not normal. Frequency of various clinical and laboratory findings are expressed as proportions (%). The analysis of Student's t-test was used for comparisons of means. The ANOVA, Chi² or Fischer's exact (for the cell with expected frequency less than 5 in two by two table more

than 20%) tests were used to compare differences among categorical variables. A p value <0.05 was considered significant.

Results

The mean hemoglobin, hematocrit and red blood cell levels tended to be lower in subjects with Hb-SS than in non-Hb-SS subjects. The difference between the two groups was statistically significant (Table 1).

The mean white blood cell, Platelets and reticulocytes levels tended to be higher in subjects with Hb-SS than in non-Hb-SS subjects. This tendency was significantly decreased compared to corresponding values in control group (Table 2).

The mean of MCV was similar between the two groups (p=0.586).

A significantly higher proportion (17.1%) of subjects with microcytosis were subjects with Hb-SS compared to those with non-Hb-SS (5.3%).

Macrocytosis was a rare event and was found in 5 (2.4%) subjects with Hb-SS. No case of macrocytosis was detected in non-Hb-SS group (table 3).

In Hb-SS group, serum iron level tended to be higher than in Hb-AA one. However, there was no statistically significant difference between the two groups (Table 4).

The means of Hemoglobin, hematocrit and red blood cell levels were significantly higher in sickle patients with ACP (p <0.001). Details of comparative value are given in Table 5.

The means of reticulocytes and MCV were significantly lower in sickle patients with ACP (p =0.009).

The means of TCMH and serum iron were similar between the three groups (p=0.586).

Microcytosis was found in 18.6% of sickle cell patients with ACP phenotype, in 19.3% of patients with MCP phenotype and 11.3% of children with SCP phenotype. Macro-cytosis was a rare event and was found in 2.3% of sickle cell patients with ACP phenotype, in 0.9% of patients with MCP phenotype and 5.7% of subjects with SCP phenotype.

The means of hemoglobin, hematocrit, MCV and TCMH were similar between males and females with Hb-SS. However, in the non-Hb SS group, hemoglobin, hematocrit, MCV and TCMH tended to be significantly higher in males than in female subjects.

The means of hemoglobin levels were similar in males with ACP, MCP in comparison with female with the same phenotypes, respectively. However, this value tended to be significantly higher in males with SCP than in females with the same phenotype.

The means of serum iron levels tended to be higher in males with ACP, MCP and SCP in comparison with female with the same phenotypes, respectively.

In the Hb-SS group, the MCV and TCMH tended to be lower in Hb-SS subjects with microcytosis than in Hb-SS subjects without microcytosis. The difference between the two groups was statistically significant.

The mean of serum iron level tended to be lower in Hb-SS subjects with microcytosis than in Hb-SS subjects without microcytosis. However, there was no statistically significant difference between the two groups.

The MCV tended to be significantly higher in Hb-SS subjects with macrocytosis than in Hb-SS subjects without macrocytosis.

The mean of serum iron level tended to be lower in sickle cell male than in sickle cell female. The difference between the two groups was statistically significant (p=0.043). In the non-Hb SS group, the mean of serum iron level tended to be significantly higher in males than in females (p= 0.039).

Discussion

The present study is the first attempt to describe the iron status in the sickle cell population in Central Africa. Despite the high prevalence of SCA and the high rate of blood transfusion, serum iron status screening is not recommended in health care policy in DRC. This situation is mainly due to resource deficiencies that range from inadequate healthcare budgets to scarce laboratory facilities (21).

In addition, the iron status remains a matter of controversy (22). Few previous papers show that iron deficiency leads to a reduction of the MCHC induced and may ameliorate sickling (23-25).

This situation of controversy is also due to availability of an adequate iron source potentially from increased red cell turnover and from the increased risk of blood transfusions, particularly in low resource-settings and endemic malaria area such as the DRC **(**12, 13, 26**).**

In Hb-SS group, Hb levels tended to be significantly lower in comparison with Hb-AA group. These results are in consonance with previous studies (27, 28). Among Hb-SS group, the present study shows that sickle cell patient with ACP have a high mean steady-state hemoglobin concentration compared to those with MCP and SCP ($p < 0.001$). A high mean steady-state hemoglobin concentration has previously been shown to correlate with a decreased risk of severity of the disease (10, 29, 30).

In the Hb-AA group, Hb was markedly decreased in female group compared to male group. This observation appears in line with such evidence that increase of Hb is predominantly in male. The major factors influencing Hb level in human are testosterone level and the presence of menstruation (31-33).

In females' subjects, Hb levels tended to be lower in comparison with males. However, there was no significant difference. These results suggest that specific patho-physiological models should be defined in sickle cell patients living in our midst.

Our study showed that the sickle cell subjects had a serum iron levels in the normal range and comparable to that of non-SCD.

In the Hb-AA group, iron levels tended to be significantly lower in female in comparison with male. These results are in consonance with previous studies (32, 34). However, in the Hb-SS group, iron levels tended to be significantly higher in female in comparison with male**.**

Sickle cell disease is a chronic inflammatory disease and in this condition a low serum iron may not represent iron deficiency. The concentrations of iron in the serum are significantly affected, and decrease rapidly as part of the acute phase response after the onset of the inflammation irrespective of the status of the iron stores in the body (35).

Evaluation of serum iron requires the association of biochemical tests. Iron deficiency causes a microcytic anemia (MCV< 75 fl) and macrocytic anemia (MVC >105 fl) is caused by folates deficiencies.

In our cohort, mean of MCV between Hb-SS group and Hb-AA was comparable. Chronic hemolysis and polytransfusion may suggest that iron deficiency is low in sickle cell patients living in our environment (36). However, most cases of microcytic anemia were reported in Hb-SS group (17.1%) compared to 6.1% in Hb-AA group (table-1). In tropical environment, microcytic anemia may be associated with inadequate food intake and spoliation of iron due to infectious and parasitic diseases (37, 38). In our cohort, serum iron, MCV and reticulocytes were markedly decreased in Hb-SS group with microcytic anemia compared to Hb-SS group without microcytic anemia. However, the mean Hb and Ht levels were comparable between the Hb-SS group with microcytic

anemia and Hb-SS group without microcytic anemia. These observations can be explained by the fact that iron deficiency resulted in increased production of red blood cells (39).

Regarding the clinical phenotype in our cohort, the distribution of microcytic anemia was comparable between the three groups. These observations can be explained by the fact that nutritional status may influence the severe nature of the disease but probably the severity of the disease is not necessarily related to the nutritional status.

Macrocytic anemia (> 105 fl) was present in only 2.4% of patients suffering from SCA. Hb level, red blood cell count and reticulocytes were significantly decreased in Hb-SS group with macrocytic anemia compared to Hb-SS group with microcytic anemia. In contrast, serum iron and reticulocytosis were significantly increased in Hb-SS group with macrocytic anemia compared to Hb-SS group with microcytic anemia. In our context, the macrocytic anemia may be associated with hypermetabolism in sickle cell anemia and due to nutritional deficiency (18, 40, 41).

Conclusion

Iron deficiency is not uncommon and may be under-reported in sickle cell patients living under tropical environment. In this study, it appears that one in 5 patients with SCA had iron deficiency and only 2.4% had folate deficiency. Men are more affected than women. There is no difference between the different clinical phenotypes. We recommend serum iron evaluation in the patients suffering from SCA in DRC and to individualize the treatment of each patient.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

Acknowledgements

The authors thank all patients who participated in this study. We thank all our colleagues involved in the collection of samples, all the nurses of CMMASS and technicians of INRB for the support given for the present study.

This research project was supported by the World Bank through its scholarship program Robert S McNamara.

References

1. Sun K, Xia Y. New insights into sickle cell disease: a disease of hypoxia. *Curr Opin Hematol*. 2013 May; **20**(3):215-21.
2. Piel FB, Patil AP, Howes RE, Nyangiri OA, Gething PW, Dewi M, Temperley WH, Williams TN, Weatherall DJ, Hay SI. Global epidemiology of sickle haemoglobin in neonates: a contemporary geostatistical model-based map and population estimates. *Lancet*. 2013 Jan 12; **381**(9861):142-51.
3. Weatherall D, Akinyanju O, Fucharoen S, Olivieri N, Musgrove P. Inherited disorders of hemoglobin. Disease control priorities in developing countries. 2nd edn. Oxford University Press; New York: 2006. pp. 663–680.
4. Piel FB, Hay SI, Gupta S, *et al*. Global burden of sickle cell anaemia in children under five, 2010-2050: modelling based on demographics, excess mortality, and interventions. *PLoS Med*. 2013; **10**(7): e1001484. doi: 10.1371/ journal.pmed. 1001484.
5. Agasa B, Bosunga K, Opara A, Tshilumba K, Dupont E, Vertongen F, Cotton F, Gulbis B. Prevalence of sickle cell disease in a northeastern region of the Democratic Republic of Congo: what impact on transfusion policy? *Transfus Med*, 2010 Feb; **20**(1):62-5.
6. Tshilolo L, Aissi LM, Lukusa D, *et al*. Neonatal screening for sickle cell anaemia in the Democratic Republic of the Congo: Experience from a pioneer project on 31 204 newborns. *J Clin Pathol*, 2009; **62**(1):35-38.
7. Stettler N, Zemel BS, Kawchak DA, Ohene-Frempong K, Stallings VA. Iron status of children with sickle cell disease. *JPEN J Parenter Enteral Nutr*, 2001 Jan-Feb; **25**(1):36-8.
8. Marsella M, Borgna-Pignatti C. Transfusional Iron Overload and Iron Chelation Therapy in Thalassemia Major and Sickle Cell Disease. *Hematol Oncol Clin North Am*, 2014 Aug; **28**(4):703-727.
9. Coates TD. Physiology and pathophysiology of iron in hemoglobin-associated diseases. *Free Radic Biol Med*, 2014 Jul; **72**: 23-40.

10. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, *et al*. Mortality in sickle cell disease. Life expectancy and risk factors for early death. *N Engl J Med*, 1994; **330**(23): 1639–44.
11. Makani J, Cox SE, Soka D, Komba AN, Oruo J, Mwamtemi H, Magesa P, Rwezaula S, Meda E, Mgaya J, Lowe B, Muturi D, Roberts DJ, Williams TN, Pallangyo K, Kitundu J, Fegan G, Kirkham FJ, Marsh K, Newton CR. Mortality in sickle cell anemia in Africa: a prospective cohort study in Tanzania. *PLoS One*, 2011 Feb 16; **6**(2):e14699.
12. Tshilolo LM, Mukendi RK, Wembonyama SO. Blood transfusion rate in Congolese patients with sickle cell anemia. *Indian J Pediatr*, 2007 Aug; **74**(8):735-8.
13. Aloni MN, Tshimanga BK, Ekulu PM, Ehungu JL, Ngiyulu RM. Malaria, clinical features and acute crisis in children suffering from sickle cell disease in resource-limited settings: a retrospective description of 90 cases. *Pathog Glob Health*, 2013 Jun; **107**(4):198-201.
14. Aloni MN, Nkee L. Challenge of managing sickle cell disease in a pediatric population living in Kinshasa, democratic republic of Congo: a sickle cell center experience. *Hemoglobin*, 2014; **38**(3):196-200.
15. Tshilolo L, Summa V, Gregorj C, Kinsiama C, Bazeboso JA, Avvisati G, Labie D. Foetal haemoglobin, erythrocytes containing foetal haemoglobin, and hematological features in congolese patients with sickle cell anaemia. *Anemia*, 2012; **2012**:105349.
16. Cox SE, L'Esperance V, Makani J, Soka D, Prentice AM, Hill CM, Kirkham FJ. Sickle cell anemia: iron availability and nocturnal oximetry. *J Clin Sleep Med*, 2012 Oct 15; **8**(5):541-5. doi: 10.5664/jcsm.2152.
17. Ballas SK. Effect of alpha-globin genotype on the pathophysiology of sickle cell disease. *Pediatr Pathol Mol Med*. 2001 Mar-Apr; **20**(2):107-21.
18. Hyacinth HI, Adekeye OA, Yilgwan CS. Malnutrition in Sickle Cell Anemia: Implications for Infection, Growth, and Maturation. *J Soc Behav Health Sci*. 2013 Jan 1; **7**(1). doi: 10.5590/JSBHS.
19. Osei-Yeboah C, Rodrigues O, Enweronu-Laryea C. Nutritional status of children with sickle cell disease at Korle Bu Teaching Hospital, Accra, Ghana. *West Afr J Med*. 2011 Jul-Aug; **30**(4):262-7.
20. Cox SE, Makani J, Fulford AJ, Komba AN, Soka D, Williams TN, Newton CR, Marsh K, Prentice AM. Nutritional status, hospitalization and mortality among patients with sickle cell anemia in Tanzania. *Haematologica*, 2011 Jul; **96**(7):948-53.
21. Wembonyama S, Mpaka S, Tshilolo L. (Medicine and health in the Democratic Republic of Congo: from Independence to the Third Republic). *Med Trop*, (Mars) 2007 Oct; **67**(5):447-57.
22. Mohanty D, Mukherjee MB, Colah RB, Wadia M, Ghosh K, Chottray GP, Jain D, Italia Y, Ashokan K, Kaul R, Shukla DK, Muthuswamy V. Iron deficiency anaemia in sickle cell disorders in India. *Indian J Med Res*, 2008 Apr; **127**(4):366-9.
23. Rao KR, Patel AR, Honig GR, Vida LN, McGinnis PR. Iron deficiency and sickle cell anemia. *Arch Intern Med*, 1983 May; **143**(5):1030-2.
24. Koduri PR. Iron in sickle cell disease: a review why less is better. *Am J Hematol*, 2003 May; **73**(1):59-63.
25. Castro O, Poillon WN, Finke H, Massac E. Improvement of sickle cell anemia by iron-limited erythropoiesis. *Am J Hematol*, 1994 Oct; **47**(2):74-81.
26. Batina Agasa S, Dupont E, Kayembe T, Molima P, Malengela R, Kabemba S, Andrien M, Lambermont M, Cotton F, Vertongen F, Gulbis B. Multiple transfusions for sickle cell disease in the Democratic Republic of Congo: the importance of the hepatitis C virus. *Transfus Clin Biol*, 2010 Oct; **17**(4):254-9.
27. Taylor JG, Nolan VG, Mendelsohn L, Kato GJ, Gladwin MT, Steinberg MH. Chronic hyper-hemolysis in sickle cell anemia: association of vascular complications and mortality with less frequent vasoocclusive pain. *PLoS One*, 2008; **3**: e2095.
28. Connes P, Lamarre Y, Hardy-Dessources MD, Lemonne N, Waltz X, Mougenel D, Mukisi-Mukaza M, Marie-Laure Lalanne-Mistrih ML, Tarer V, Tressières B, Maryse EJ, Romana M. Decreased Hematocrit-To-Viscosity Ratio and Increased Lactate Dehydrogenase Level in Patients with Sickle Cell Anemia and Recurrent Leg Ulcers. *PLOS ONE*, 2013, (11) e79680.
29. Powars D, Weiss JN, Chan LS, Schroeder WA. Is there a threshold level of fetal hemoglobin that ameliorates morbidity in sickle cell anemia? *Blood*, 1984; **63**(4):921-926.
30. Steinberg MH, Forget BG, Higgs DR, Weatherall DJ, editors. Disorders of Hemoglobin: Genetics, Pathophysiology, Clinical Management (2nd ed). Cambridge, United Kingdom: Cambridge University Press; 2009.
31. Bachman E, Travision TG, Basaria S, Davda MN, Guo W, Li M, Connor Westfall J, Bae H, Gordeuk V, Bhasin S. Testosterone induces erythrocytosis via incerased erythropoietin and suppressed hepcidin : evidence for a new erythropoietin/hemoglobin set points : *J Gerontol A Biol Sci Med Sci*, 2014 Jun; **69**(6): 725-35,

32. Wen Gu, Eric Bachma, Michelle Li, Cindy N. Roy, Jerzy Blusztajn, Siu Wong, Stephen Y. Chan, Carlo Serra, Ravi Jasuja, Thomas G. Travison, Martina U. Muckenthaler, Elizabeta Nemeth, and Shalender Bhasin: Testosterone Administration Inhibits Hepcidin Transcription and is Associated with Increased Iron Incorporation into Red Blood Cells, *Aging Cell*, 2013 April; **12**(2): 280-291.
33. Blanco-Rojo R, Toxqui L , López-Parra AM, Baeza-Richer C, Pérez-Granados AM , Arroyo-Pardo E and Vaquero MP. Influence of Diet, Menstruation and Genetic Factors on Iron Status: A Cross-Sectional Study in Spanish Women of Childbearing Age: *Int. J. Mol. Sci*, 2014; **15**: 4077-4087; doi: 10.3390/ijms 15034077.
34. Bachman E, Travision TG, Basaria S, Davda MN, Guo W, Li M, Connor Westfall J, Bae H, Gordeuk V, Bhasin S. Testosterone induces erythrocytosis via incerased erythropoietin and suppressed hepcidin : evidence for a new erythropoietin/ hemoglobin set points : *J Gerontol A Biol Sci Med Sci*, 2014 Jun; **69**(6): 725-35.
35. Nemeth E, Ganz T. Anemia of Inflammation. *Hematol Oncol Clin North Am*, 2014 Aug; **28**(4): 671-681.
36. Olufemi AS, Omolara AK, Diaku-Akinwumi IN and Njokanma OF. Iron Deficiency Anaemia among Pre-School Children with Sickle Cell Anaemia: Still a Rare Diagnosis? *Mediterr J Hematol Infect Dis*, 2013 ; **5**(1): e2013069.
37. Mukaya JE, Ddungu H, Ssali F, O'Shea T, Crowther MA. Prevalence and morphological types of anaemia and hookworm infestation in the medical emergency ward, Mulago Hospital, Uganda. *S Afr Med J*, 2009 Dec 7; **99**(12): 881-6.
38. Kalenga MK, Nyembo MK, Nshimba M, Foidart JM. (Anemia associated with malaria and intestinal helminthiasis in Lubumbashi). *Santé Publique*, 2003 Dec; **15**(4): 413-21.
39. Bartal M, Mazor D, Dvilansky A, Meyerstein N. Iron deficiency anemia: recovery from in vitro oxidative stress. *Acta Haematol*, 1993; **90**(2):94-8.
40. Thachil J. The possible role of reticulocytes in sickle cell disease associated thromboembolism. *Hemato-logy*, 2008 Feb; **13**(1):68-70.
41. Browne PV, Hebbel RP. CD36-positive stress reticulocytosis in sickle cell anemia. *J Lab Clin Med*, 1996 Apr; **127**(4):340-7.

Table 1: Relationship between haematological variables in study population

Variables	Group 1 (Hb-SS) n = 211	Group 2 (Hb-AA) n = 74	*P*
Age (years)	21.20±10.72	29.75±15.43	0.001
Hb (g/dl)	7.66±1.65	12.54±1.81	0.001
Htc (%)	23.54±4.98	39.28±4.77	0.001
RBCs ($\times 10^6$/µl)	3.02±0.80	4.87±0.56	0.001
WBC ($\times 10^3$/µl)	12.64±6.18	5.16±1.24	0.001
Reticulocytes (%)	15.53±10.53	0.84±2.33	0.001
Platelets ($\times 10^3$/ µl)	295.68±175.31	207.19±65.74	0.001
MCV (fl)	80.27±11.13	80.86±6.62	0.586
TCMH (g/dl)	32.53±1.60	31.84±1.25	0.001
Microcytosis (%)	36 (17.1%)	4(5.3%)	0.011*
Macrocytosis (%)	5 (2.4%)	0 (0)	0.33**
Serum iron (µmol/l)	17.39 ± 10.62	16.05 ± 6.42	0.2

* : *Chi-square test ; ** : Fisher test*

Table 2: Relationship between hematological variables of sickle cell disease by clinical phenotype

Variables	GROUP 1 (Hb-SS) n = 211			
	ACP (n = 43)	MCP (n = 115)	SCP (n = 53)	*P (anova)*
Age (years)	25.91±10.0	20.47±11.20	18.94±9.14	0.003
Hb (g/dl)	9.12±1.71	7.50±1,27	6.81±1.56	0.001
HbF (%)	15.72±7.37	2.88±4.55	0.00	0.001
Htc (%)	28.09±5.40	23.02±3.75	20.96±4.61	0.001
RBCs ($\times 10^6$/µl)	3.65±0.85	2.99±0.65	2.56±0.74	0.001
WBC ($\times 10^3$/µl)	8.91±4.05	12.97±6.33	14.98±5.98	0.001

Reticulocytes (%)	11.33±9.14	16.16±10.30	17.57±11.31	0.009
Platelets (×10^3/ µl)	249.07±104.35	288.41±157.35	349.11±236.86	0.016
MCV (fl)	78.66±11.63	79.01±10.39	84.31±11.49	0.009
TCMH (g/dl)	32.56±1.24	32.55±1.46	32.48±2.11	0.958
Microcytosis (%)	8(18.6%)	22(19.3%)	6(11.3%)	0.427*
Macrocytosis (%)	1(2.3%)	1(0.9%)	3(5.7%)	
Serum iron (µmol/l)	16.46 ± 8.15	18.51 ± 12.16	15.75 ± 8.52	0.24

* : *Chi-square test*

Table 3: Relationship between serum iron and hematologic variables by gender

Variables	Group 1 (Hb-SS) n = 211			Group 2 (Hb-AA) n = 74		
	Males (n = 96)	Females (n =115)	p	Males (n = 20)	Females (n = 54)	p
Hb (g/dl)	7.77 ± 1.75	7.56 ± 1.56	0.349	14.05 ± 1.44	11.99 ± 1.61	0.001
Hct (%)	23.98 ±5.22	23.16 ± 4.77	0.237	43.29 ± 3.99	37.82 ± 4.17	0.001
MCV (fl)	78.60 ± 10.37	81.68 ± 11.60	0.046	83.73 ± 6.49	79.82 ± 6.41	0.023
TCMH (g/dl)	32.38 ± 1.69	32.67 ± 1.51	0.191	32.43 ± 0.80	31.63 ± 1.33	0.014
Platelets (×10^3/ µl)	279.43 ± 131.07	309.36 ± 204.91	0.202	184.55 ± 62.92	215.42 ± 65.35	0.072
Serum iron (µmol/l)	15.78 ± 9.56	18.75 ± 11.31	0.043	18.58 ± 6.15	15.13 ± 6.32	0.039

Table 4: Relationship between haematological variables by sex in the three clinical phenotypes

Variables	ACP (n = 43)			MCP (n = 115)			SCP (n = 53)		
	M (n=11)	F (n=32)	p	M (n=55)	F (n=60)	p	M (n=31)	F (n=22)	p
Hb (g/dl)	9.93 ± 1.49	8.84 ± 1.72	0.069	7.63 ± 1.62	7.38 ± 0.85	0.32	7.26 ± 1.52	6.18 ± 1.43	0.012
Hct (%)	30.97 ± 4.71	27.10 ± 5.33	0.039	23.38 ± 4.68	22.70 ± 2.65	0.351	22.56 ± 4.42	18.71 ± 3.95	0.002
MCV (fl)	77.48 ±10.28	79.06 ±12.18	0.702	77.36 ±10.25	80.49 ±10.37	0.108	81.17 ±10.47	88.72 ± 11.66	0.017
TCMH (g/dl)	32.32 ± 1.03	32.65 ± 1.31	0.455	32.55 ± 1.66	32.55 ± 1.26	0.99	32.09 ± 1.92	33.02 ± 2.27	0.117
Platelets (×10^3/ µl)	225.36 ± 99.16	257,22 ±106.36	0.389	279.98 ±114.38	296.00 ± 188.55	0.581	297.65 ±163.10	421.64 ±302.48	0.06
Serum iron (µmol/l)	14.20 ± 5.33	17.24 ± 8.86	0.292	16.76 ± 11.19	20.09 ± 12.86	0.145	14.64 ± 7.41	17.31 ± 9.34	0.265

M : Males, F : Females

Table 5: Relationship between haematological variables in the sickle population with microcytosis and in the sickle cell population with macrocytosis in study group

Variables	(Hb-SS) (n = 211)					
	Microcytosis + n = 36	Microcytosis - n = 175	P*	Macrocytosis + n = 5	Macrocytosis – n = 206	p**
Hb (g/dl)	7.56 ± 1.38	7,68 ± 1.70	0.684	6.0 ± 0.93	7.69 ± 1.64	0.009
Hct (%)	23.91 ± 4.03	23.46 ± 5.17	0.626	18.44 ± 1.50	23.66 ± 4.97	0.004
RBCs (×10^3)	3.78 ± 0.70	2.86 ± 0.73	0.001	1.71 ± 0.21	3.05 ± 0.78	0,001
Reticulocytes (%)	10.18 ± 6,43	16.63 ± 10.88	0.001	27.8 ± 12.38	15.23 ± 10.34	0.014
MCV (fl)	63.64 ± 4.93	83.71 ± 8.68	0.001	108.08 ± 5.03	79.59 ± 10.34	0.001
TCMH (g/dl)	31.53 ± 1.88	32.74 ± 1.46	0.001	32.56 ± 4.55	32.53 ± 1.49	0.607
Serum iron (µmol/l)	14.68 ± 9.19	17.95 ± 10.84	0.093	26.12 ± 11.07	17.18 ± 10.55	0.026

* : t Student test, ** : Wilcoxon test