Clinical, laboratory, and phenotypical features in Congolese sickle cell patients.

Profil clinico-biologique et phénotypique du drépanocytaire congolais.

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Résumé

La drépanocytose est l'hémoglobinopathie la plus répandue au monde. En RDC, l'influence du phénotype sur les variables hémato-biologiques n'est pas bien connue.
L'objectif de l'étude était de déterminer les caractéristiques hémato-biologiques en fonction du phénotype clinique de la maladie.
Il s'agit d'une étude transversale sur 140 patients drépanocytaires connus en période intercritique. Les patients ont été repartis en trois sous-groupes phénotypiques en fonction de la sévérité de la maladie: phénotype clinique bénin (PCB), phénotype clinique modéré (PCM) et phénotype clinique sévère (PCS). Cette classification est basée sur notre expérience locale de l'évaluation de la gravité de la maladie. L'état nutritionnel a été évalué par l'indice de masse corporelle (IMC).
Les moyennes statistiques entre les trois groupes ont été comparées par le test d'analyse de variance (ANOVA).
L'IMC et le taux d'HbF des patients avec le PCB étaient significativement supérieurs à ceux des patients de deux autres groupes phénotypiques, contrairement à leurs taux des GB et des protéines inflammatoires qui montraient des valeurs inférieures.
L'expression clinique du phénotype drépanocytaire en milieu congolais est influencée par certaines variables biologiques, l'environnement et la génétique comme la persistance héréditaire de l'HbF.

Mots clés: profil, clinique, biologie, phénotype, drépanocytose, RD Congo.

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Summary

Background: SCD is the most common single gene disorder worldwide. However, the influence of phenotype on the features of sickle cell disease has not been characterized.
Methodology: A cross-sectional study of data from 140 sickle cell patients living in Kinshasa, Democratic Republic of Congo was conducted, relaying on a built clinical phenotype score. Applied definitions were as following: Phenotype 1 or asymptomatic clinical phenotype (score ≤ 5), moderate clinical phenotype or phenotype 2 (score between 6 and 15), and severe clinical phenotype or phenotype 3 (score ≥ 16). ANOVA test were used to compare differences among categorical variables.
Results: The mean BMI value of the three groups was lower (<25 kg/m²) than the limit defining overweight. BMI of the subjects with phenotype 1 was significantly higher than those of phenotype 2 and 3 ($p< 0.05$). Sickle cell patient with phenotype 1 have a high mean steady-state hemoglobin concentration compared to those with phenotype 2 and 3 ($p <0.001$). The phenotype 3 group showed a significantely elevated baseline leukocyte count in this study ($p<0.001$), as well as a significant elevation of alpha 1 and alpha 2 globulins. The rate of HbF was significantly higher in phenotype 1 compared to the two others.
Conclusion. Our results strongly suggest the role of genetical factors such as the inherited persistence of fetal hemoglobin, and environmental determinants in our tropical environment on the clinical and biological phenotype of sickle cell disease in our milieu.

Key words: sickle cell disease, clinical phenotype, Fetal hemoglobin, Kinshasa, Democratic Republic of the Congo.

Introduction

Sickle cell disease (SCD) is an autosomal inherited structural disorder of hemoglobin, associated with an amino acid substitution of valine for glutamic acid at the sixth residue of the ß-chain. This genetic alteration yields an unstable RBC with a shortened survival that under stress (e.g. deoxygenation) becomes sickle-shaped (1).

Despite tremendous improvement in control measures in the last decade, SCD still remains a major public health problem in the world (2). SCD is the commonest genetic disease worldwide (1). The sickle cell genes occur commonly in areas of the world with intense malaria transmission. The annual number of newborns with SCD, was estimated to be 305,800 (CI: 238,400–398,800) globally in the world in 2010 (3). According to World Health Organization (WHO) global estimations in 2010, 79% (242,200 [CI: 194,500 (82%)–302,000 (76%)]) of newborns with SCD occurred in sub-Saharan Africa (3).

The highest frequencies of homozygous SCD in the world occur in sub-Saharan African where 3 to 4 % of populations are affected (4, 5).

In DRC, the prevalence of SCD is extremely high with 25 to 30% of sickle cell trait carriers in the general population. The HBB*S allele frequency in neonates varies in the country, from 0.96% to 1.4% (6, 7). This country has the highest population of SCD patients in the whole world after Nigeria and India (3). Every year, approximately 40,000 children are born with SCD (3). SCD has a wide spectrum of clinical manifestations which vary from an almost asymptomatic condition to severe illness despite the fact that all subjects with this disease have the same base change in their DNA. Some patients may experience vaso-occlusive crises leading to any organ involvment (1). However, phenotypic heterogeneity is characteristic of sickle cell anemia (8). Previous studies in western countries support the plausible notion that the actions of multiple genes determine the overall severity of SCD (9, 10).

Currently five haplotypes are known: Senegal haplotype, Benin haplotype, Bantu haplotype, Cameroon haplotype and Asian (Arab-India) haplotype from Eastern Province of Saudi Arabia and Central India (11, 12). The pathophysiology of SCD is essentially similar in these different areas although the frequency and severity of complications may vary between areas and haplotypes (13).

In DRC, Bantu haplotype is predominant and is considered as a major risk factor associated to clinically severe form of SCD and organ damage due to low levels and activity of HbF (14-16). Fetal hemoglobin (HbF) is the major genetic modulator of the hematologic and clinical features of sickle cell disease (17-19). A recent paper shows that Congolese SCD patients displayed low levels of HbF and F-cells that contribute to the severity of the disease (20).

Clinical differences were observed among haplotypes due to fetal hemoglobin levels and possible familial clustering consistent with genetic factors (21).

SCD compromises host immune defense and predisposes to infections from several encapsulated bacteria (22). Infection promotes the production of inflammatory proteins, leukocytosis and hyperviscosity (23).

In DRC, neonatal screening program is partially introduced by private initiatives (7). Some aspects of clinical features have been previously described. Unfortunately, there is a lack of reports on the clinical course of sickle cell patient living in this country. The influence of phenotype on the features of sickle cell disease has not been characterized in our midst. Therefore, to improve our understanding of pathophysiological mechanisms of the severity of sickle cell disease and organs'involvement, the relationships between BMI, clinical and laboratory measures of disease expression need to be carefully screened.

Reliable data should be collected and assessed for development of accurate preventive measures and more efficient treatment strategies of SCD in low income settings such as the Democratic Republic of Congo.

Study area

The study has been conducted in Kinshasa, the capital of Democratic Republic of Congo with an estimated population of 10,000,000 inhabitants. The prevalence of Sickle cell trait is around 25% in adults and a prevalence rate of 1.4% is reported for homozygous SCD newborns

in this area (7). Kinshasa is located in an endemic malaria area with a stable transmission. The town is characterized by two distinct seasons, a rainy one with an average of 1350 mm of annual rainfall during the months of September to May and a second hot dry one from June to August.The town is located at 309 m of altitude, with an average temperature of 25.2°C. Mean relative humidity varies from 74% to 83%.

Study design

The cross-sectional study was conducted in the Sickle Cell Centre of Kinshasa (Centre de Médecine Mixte et d'Anémie SS de Yolo; CMMASS), between November and December 2012. This centre provides most of the sickle cell beds in DRC, reaching annually 6,500 consultations.

Study participants

All patients were free of pain for at least 15 days and have not been hospitalized or transfused for at least 100 days before enrolment in the study.

The samples were collected from patients regularly attending the CMMASS. The starting number was randomly chosen from the first three in the section roll call. Every third patient was taken until the assigned number was reached. We excluded all subjects with the following conditions: (i) initiated antibiotics treatment prior to seeking medical care; (ii) blood transfusion during the previous 3 months; (iii) current hydroxyurea treatment, or (iv) chronic transfusion program.

The following demographic, clinical and laboratory measures were analyzed: (i) age and gender (ii); BMI (iii); laboratory investigations including Hb, leukocytes, electrophoresis of hemoglobin and globulins and fetal hemoglobin levels.

Height (cm) and weight (kg) were measured for all patients. Body mass index was calculated according the following formula:

$$\text{BMI} = \left(\frac{\text{Weigth}}{\text{High}^2}\right) (\text{kg/m}^2) .$$

Data collection procedure and blood analysis

To obtain a detailed history about the disease, either the parents or the children when appropriate, were interviewed following an informed consent. The interview was relaying on a structured standard project questionnaire. A complete physical examination was then performed on all patients, focused on the determination of the phenotype.

Five ml of venous blood sample were drawn from each study participant into an EDTA tube, for determination of hemoglobin rate and leukocytes count. Hematologic parameters were analyzed at Institut National de Recherche Biomédicale (INRB) of Kinshasa using an automate Sysmex XS – 1000*i* (Lincolnshire, USA).

An additional EDTA tube of 5ml of venous blood was drawn for determination of hemoglobin electrophoresis and protein electrophoresis. Sickle cell screening was performed using semi-automated agarose gel electrophoresis technique with the Hydrasis II apparatus (SEBIA, France). Sickle cell disease was diagnosed in presence of production of mostly Hb S with no Hb -A.

Case definitions

The clinical phenotype used in this study relays on the combination of most relevant medical parameters resulting in a built score as indicated in the following table 1.

Table 1. Clinical criteria and severity score based on phenotype

Clinical criteria	Score (points)
Days of hospitalization /year	
≤1	0
2 – 7	2
≥ 8	5
Severe vasoocclusive crisis/year	
0	0
1 – 2	2
≥ 3	5
Blood transfusion/year	
0	0
1 – 2	2

Clinical criteria	Score (points)
≥ 3	5
Hip disease	
Absent	0
Present	5
Hepatobiliary complications	
Absent	0
Cholecystectomia	2
Present	5
Neurologic events	
Absent	0
Present	5
Renal disorders	
Absent	0
Present	5
BMI	
19 – 27	0
<19	2
Total	
≤ 5 : ACP (1)	
6 – 15 : MCP (2)	
≥ 16 : SCP (3)	

*ACP = asymptomatic clinical phenotype; MCP = moderate clinical phenotype; SCP=severe clinical phenotype

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

Data management and analysis

Results were manually entered into a microcomputer and analyzed using the Excel Version 2002 (CDC). 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 (%). ANOVA test were used to compare differences among categorical variables. Statistical significance level was set at p=0.05.

Results

Characteristics of the subjects

A total of 140 patients in steady state, 58 with ACP, 52 with MCP and 30 with SCP were recruited over the study period.

The group included 89(63.6%) females and 51 (36.4%) males.

The sex-ratio female to male was respectively 2.2:1, 1.45:1 and 2:1, in ACP, MCP and SCP.

Age of patients

Overall median age was 24.62 ± 12.34 years. The mean age of the patients was 21.6 (SD = 11.8) for patients with ACP, 19.8 (SD = 9.7) for patients with MCP and 24.0 (SD = 11.2) for patients with SCP.

Relationship between phenotype and BMI

The mean BMI of the patients was 20.3 (SD = 4.7) for patients with ACP, 18.2 (SD = 3.5) for patients with MCP and 16.8 (SD = 4.0) for patients with SCP (Table 2) (p = 0.016).

Table 2. BMI and hematologic features according to clinical phenotypes.

Variables	ACP (n=58)	MCP (n=52)	SCP (n=30)	p-value
BMI (kg/m^2)				0.016
Mean	20.3	18.2	16.8	
SD	4.7	3.5	4.0	
Hb (g/dl)				0.001
Mean	9.0	7.2	6.4	
SD	1.1	0.8	0.3	
WBC (10^9WBC/L)				.001
Mean	8.4	11.7	14.4	
SD	1.2	1.9	1.0	
Hb F (%)				0.001
Mean	15.7	2.9	0.0	
SD	7.4	4.6	0.0	

*ACP = asymptomatic clinical phenotype; MCP = moderate clinical phenotype; SCP=severe clinical phenotype

Relationship between phenotype and Hb values

The mean steady-state Hb concentration was 9.0 (SD = 1.1), 7.2 (SD = 0.8) and 6.4 (SD =0.3) in sickle cell patients with ACP, MCP and the SCP, respectively ($p < 0.001$).

Relationship between phenotype and leukocytes

Table 2 shows that the mean leukocytes level was significantly low in sickle patients with ACP ($p < 0.001$). Details of comparative value are given in Table 3.

Table 3. Electrophoresis of proteins according to the clinical phenotype

Variables	Phenotype-1 n=58	Phenotype-2 n=52	Phenotype-3 n=30	p-value
Albumin (%)				0.002
Mean	56.8	54.0	46.1	
SD	7.5	8.3	10.8	
α_1 (%)				0.003
Mean	2.5	2.7	3.8	
SD	0.9	0.9	2.4	
α_2 (%)				0.005
Mean	7.4	7.6	9.9	
SD	1.8	2.2	4.0	
β (%)				0.506
Mean	11.1	11.2	12.7	
SD	3.1	4.0	5.3	
γ (%)				0.074
Mean	22.2	24.5	27.5	
SD	7.0	7.2	7.9	

Relationship between phenotype and Hb F

The mean Hb F was significantly higher in sickle cell patients wih ACP ($p < 0.001$). Details of comparative value are given in Table 3.

Relationship between phenotype and Albumin

Albumin was analyzed for the case and control groups. The mean albumin levels was significantly higher in sickle cell patients with ACP ($p = 0.002$). Details of comparative value are given in Table 3.

Relation between phenotype and Globulins

As a all, patients with phenotype 1 had lower level of globulins compared to the two others. . However they were not statistically significant for the value of beta and gamma globulins.

Ratio of albumin to globulins shows that the values increase from ACP to SCP.

Discussion

Environmental, nutritional, and infectious factors in many parts of Africa make it difficult to distinguish the role of haplotype in modulating the course of disease. This study was designed to determine the relationship between BMI, Hb, leukocytes, HbF, albumin, globulins and clinical phenotype of SCD patients at the Sickle cell Center of Yolo at Kinshasa. These parameters were evaluated for the first time in Sub-Saharan Africa.

From the 1950s, many researchers have reported an almost total absence of adults suffering from SCD among Africans, especially in Congolese adults (24).

SCD potentially affects growth leading to wasting and stunting. Patients are usually characterized by lower BMI values compared to ethnic-matched population (25). In our study, the mean BMI value of the three groups was lower (<25 kg/m^2) than the limit defining overweight. This situation is due to the increased resting energy expenditure caused by the increased erythropoietic and cardiac activities as reported by Barden *et al.* (25). In addition, this may be due to the fact that National Center for Health Statistics standards (NCHS) were developed in the United States from composite data involving various races, and social classes and the standard of living in the United States of America are higher than in DRC. As such it is not surprising that a random selection of Congolese had poorer growth characteristics. The current report confirmed previous observations reported in Nigeria (26).

BMI of the subjects with ACP was significantly higher than those of MCP and SCP, suggesting

that severity of disease is associated with the increased resting energy expenditure.

The relevant laboratory tests for predicting severity and outcome measurement of hemoglobin levels and white-cell count are readily available in the literature (27).

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 (28).

There was a significant difference in the leukocytes-distribution of patients'phenotypes. An elevated baseline leukocyte count is associated with SCP in our study. This finding suggests that leukocytes may play a major role in phenotypic severity of the disease. This is in concordance with some previous reports (29, 30). Leukocytes contribute to the pathogenesis of sickle cell disease by releasing cytotoxic enzymes. As neutrophils and mononuclear cells (MC) are important producers of cytokines and are activated during infection and inflammation (31, 32). Cytokines may participate in several mechanisms that contribute to vaso-occlusive pathogenesis in SCA, including vascular endothelial activation, induction of red cell and leukocyte adhesion to vascular endothelium (33). The adverse effect of neutrophils on adhesion to vascular endothelial cells by mediation of adhesion molecule under inflammatory conditions was previously described (33).

In this report, the rate of HbF was higher in patients with ACP compared to two others phenotypes. The degree of fetal hemoglobin (HbF) expression appears then as a major determinant of phenotypic severity of sickle cell disease (SCD). High concentrations of HbF dilute the amount of HbS, but importantly, by failing to be incorporated into the HbS polymer, HbF inhibits deoxyHbS polymerization. This situation decreases risk of hemolysis of red cells and the frequency of vaso-occlusive crisis in sickle cell patients (34).

Data indicate that endothelial cells of sickle cell anemia patients may have abnormal inflamematory and adhesive properties even outside of the chronic inflammatory and vaso-occlusive environment of patients. Chronic inflammation and endothelial cell activation promote vaso-occlusion in SCD (35). In our series, a significant elevation of inflammatory proteins (alpha 1 and alpha 2) in patients with severe clinical phenotype was observed in comparison with ACP and MCP (Table 3). Sickle cell disease is intimately linked to a pathophysiologic condition of multiple sources of pro-oxidant processes with consequent chronic and systemic oxidative stress. This situation leads to production of inflammatory proteins (36).

Previous studies indicate that pro-inflammatory proteins could be used as related markers for assessing disease severity, and consequently therapeutic intervention (35, 37).

The SCD population in this study lives in a tropical environment, heavily exposed to continous contact with infectious agents such as parasites, bacteria and virus (38, 39). They are such prone to a continouos stimulation of their immune. The hypergammaglobulinemia observed in sickle cell disease as in the present study, is taught to be related to impaired immunity and to the early loss of splenic function in these patients. Hyperstimulation of the humoral immunity could also be considered as well as the result of chronic reticulo-endothelial stimulation by chronic hemolysis, as recently described in SCD patients living in tropical environment (40).

The ACP had lower plasma concentrations of gammaglobulin (Table 3). However, there was no significant difference between the three groups.

Conclusion

Clinical differences observed in the present study could be linked to fetal hemoglobin levels and possibly to familial clustering consistent with genetic factors as hereditary persistence of

fetal hemoglobin. HbF is the major genetic modulator of the hematologic and clinical features of sickle cell disease. In this report, the nutritional status of patients with sickle cell disease is strongly influenced by the clinical severity of the disease. Determining the electrophoretic profile of serum proteins has allowed us to better understand the role and influence of inflammation in the variability of the clinical expression of the disease in our tropical environment.

Competing interests

The authors declare that they have no competing interests.

Authors'contributions

TMM, JMMM and PTL conceived, designed, deployed and directed the case–control study at the Department of Paediatrics at Kinshasa University Hospital and wrote the manuscript. TMM carried out patient recruitment and follow-up, sample collection, storage and transport. JMMM, PTL brought some precious corrections. TMM and MNA analyzed data. MNA edited the English and made corrections. All authors read and approved the final manuscript.

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