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Research Article

Neonatal Screening of Sickle Cell Disease in Gabon: A Nationwide Study

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ABSTRACT

Gabon is a country in Central Africa with about 2.5 million inhabitants; it is one of the most under populated countries in the world. Its location on the equatorial plane and its tropical climate makes a favorable environment for the development of sickle cell disease. There are about 21% of people living with sickle cell trait throughout the country. Given the number of inhabitants, this percentage is worrying as it could lead to the birth of children sickle cell disease. This study aims at assessing the rate of births affected by sickle cell disease in Gabon, maintaining and expanding the routine neonatal screening program for the management of sickle cell disease, and controlling sickle cell disease at a national level. A total of 3,957 blood samples were collected on Guthrie paper between January 2007 and September 2010. Abnormal hemoglobin presence was detected by the Isoelectric Focusing (IEF) method and confirmed by High-Performance Liquid Chromatography (HPLC). The results of this work revealed that 17.13% (678/3,957) of the children tested were carriers of the sickle cell trait (HbAS) and 1.34% (53/3,957) had sickle cell disease (HbSS).

Keywords: Sickle cell disease; Screening; High-performance liquid chromatography; Hemoglobin

Abbreviations: HPLC: High-Performance Liquid Chromatography; IEF: Isoelectric Focusing; SCD: Sickle Cell Disease; HbS: Sickle Haemoglobin; WHO: World Health Organization; CE: Capillary Electrophoresis; IAEA: International Atomic Energy Agency; HbA: Hemoglobin A, HbS: Hemoglobin S: HbC Hemoglobin C; SCT: Sickle Cell Trait.

INTRODUCTION

In resource-limited settings, are born each year 280,000 children with Sickle Cell Disease (SCD) [1]. A neglected chronic, multi-system disorder disease, sickle cell disease is of major importance in the global health context [2,3]. Sickle Haemoglobin (HbS) is one of the most important single gene disorders of humans and results from the substitution of glutamic acid for valine at position 6 on the β-globin molecule (βGlu6Val/βs-gene) [4]. This variant form of haemoglobin, known as sickle haemoglobin or HbS, polymerizes reversibly under low oxygen tension to alter the shape and rheological properties of red blood cells, a phenomenon that is central to the pathophysiology of SCD [5]. Sickle cell disease is referred to when inheritance of HbS is in homozygous state (HSS) or coinheritance of HbS with other mutations of the HBB gene, most notable among them being a second structural haemoglobin variant, HbC, and β-thalassaemia [5]. SDC although it is a non-communicable disease, it is growing with high morbidity and mortality rates [6]. One approach that developed a geo-statistical mapping model based on the frequency of the Hemoglobin S (HBS) allele and population data has been suggested that one factor associated with the high incidence of SCD in tropical Africa is the protection against Plasmodium malaria associated with having the SCT [7,8]. Estimates from developed countries indicate that genetic diseases already constitute up to 40% of the requirements for chronic care in pediatric practice widely unknown to millions of Africans, Sickle Cell Disease still presents a health problem, and it has been more than a decade since the World Health Organization (WHO) identified it as a major public health problem [4]. A majority of children born with SCD in low-income developing countries die before the age of 5 due to lack of early diagnosis and

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comprehensive care [9,10]. As a consequence, SCD is responsible for an increasing proportion of overall childhood mortality in Sub-Saharan Africa, reaching 6% or more in a number of countries within the region [1,9,11]. The high mortality rates in Sub-Saharan Africa are influenced by multiple factors including limited resources leading to poor access to care, and lack of comprehensive SCD management programs [12]. Survivors suffer from repeated acute events and develop rapid progressive organ damage which reduces their life expectancy. Cultural background, lack of (medical) education and limited health care facilities are probably the major factors contributing to such high childhood mortality [13]. Effective management of SCD revolves around genetic counseling, neonatal screening and early diagnosis [14]. Universal newborn screening, in combination with early intervention for affected infants, has nearly eliminated early childhood mortality due to Sickle Cell Disease (SCD; HbSS) in high-income developed countries [15,16]. Since the introduction of newborn screening throughout much of Europe and North America, the majority of children born with SCD in these regions are diagnosed early, placed on life-long care, and can expect to live to middle-age and beyond [17]. The decrease in morbidity and mortality among SCD children in low-resource countries is partly attributed to the presence of comprehensive care programs that include immunizations and vaccinations, prophylaxis therapy, vitamin supplements and patient and caregiver empowerment through education [13]. In Central Africa, the prevalence of this pathology appears high (1.65%) [18,19]. Improving the care management and quality of life of patients with SCD requires establishing a reliable diagnosis feasible in resource-limited countries [20,21]. Currently, two or three gold standard approaches are validated for first-level neonatal screening and routine diagnosis: High Performance Liquid Chromatography (HPLC), Capillary Electrophoresis (CE) and Isoelectric Focusing (IEF) with sensitivity and specificity are close to perfect to detect normal and common abnormal hemoglobin variants [22-24]. A Previous nationwide newborn screening study reported an overall sickle cell trait prevalence of 21%, and that about 770 children were born with sickle cell disease [25]. In this state, about 30.4% of sickle-cell children die from multiple infections such as malaria and it is estimated that 2.2% of the population has homozygous patients [26]. Despite the deleterious effects of sickle cell disease, little is done in Gabon to improve the healthcare system and the lives of patients suffering from this debilitating condition. The overall objective of this global study is to maintain and expand the routine neonatal screening program for the management of sickle cell disease, control sickle cell disease at the national level, and assess the impact of sickle cell disease on the public health care system in Gabon through neonatal screening.

METHODOLOGY

Study area and sampling

When the project was implemented, all regional hospitals and a few medical centers, hospitals and maternity hospitals in the nine provinces of Gabon had been presented as pilot sites for this study (Figure 1). Indeed, Gabon is a central African country and is administratively divided into nine provinces (Estuaire, Haut-Ogooué, Moyen-Ogooué, Ngounié, Nyanga, Ogooué-Ivindo, Ogooué-Lolo, Ogooué-Maritime and Woleu-Ntem) with regional hospital centers in each province. Between January 2007 and September 2010, a total of 3,957 blood samples were collected on Guthrie paper. The information of the mothers was obtained in the delivery room.

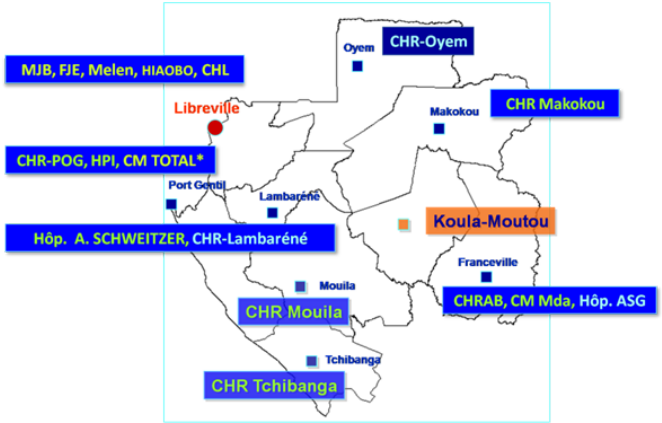

Figure 1: Sample collection pilot sites. Libreville Hospital Center (CHL), Josephine Bongo Maternity (MJB), Melen Hospital (Melen), Jeanne Ebori Foundation (FJE), Regional Hospital Centers (CHR), Centre hospitalier de Moanda (CM Mda).

Ethics statement

This project is funded by the International Atomic Energy Agency (IAEA). Consent was obtained from all participants. Our study received the approval of the Gabonese Ministry of Health.

Blood collection and processing

After obtaining the mother's consent, the removal of cord blood in 2 EDTA tubes or on Guthrie paper was carried out. After Identification of the samples, 4 drops of the EDTA blood tubes were deposited as a spot on paper and dried at room temperature and then attached to an individual fact sheet. The samples and information sheets thus collected were sent once a week to the CIRMF Sickle Cell Laboratory in Libreville for hospitals located outside Libreville and every day for others. All dried blood spots sample received kept at -80°C until analysis (Figure 2). Red blood cell samples were then processed for screening of abnormal hemoglobin. Abnormal hemoglobin presence was as doubtful by the Isoelectric Focusing (IEF) method by which proteins are separated according to their isoelectric points. For samples with an abnormal profile at IEF, or those with unreadable Hemoglobin A, newborns less than 33 weeks gestational age and neonatal baby samples with a weight of less than 1500 g were analyze by High-Performance Liquid Chromatography (HPLC) by using of "Sickle cell short program" to identify the exact variant: Hemoglobin A (HbA), Hemoglobin S (HbS) or Hemoglobin C (HbC); according to the protocol described in manufacturing protocol.

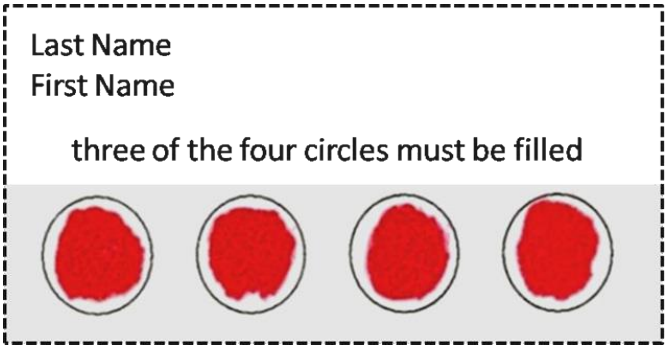

Figure 2: Example of dried blood spot sample with information on the identity of the newborn.
At least three of the four circles must be filled.

Statistical analysis

All statistical analyses were performed using the statistical package STATA 14. First, continuous variables were described using summary statistics such as mean, median, minimum, maximum and quartiles. Where deemed necessary, we also added box plots and histograms. Categorical variables were described using proportions (percentages). Grouping the results of the screening tests into HbAS and HbSS, the incidence rate over the period was derived as the number of cases with a specific test result divided by the number of children tested. Multiple regression analyses were then used to explore the potential impact of the child hemoglobin status at conception, on his/her weight at birth or on his/her duration of the pregnancy, controlling for other factors such as the year of birth/test and the region.

RESULTS

During the study period, 3,957 blood samples were collected with an average of nearly 25% of samples per year. The most represented province was the Estuary province with 1,893 (47.8%) births, followed by Haut-Ogooue province with 1352 (34.2%) births. The least represented regions/provinces were the Moyen-Ogooue, the Nyanga, the Ogooue-Ivindo and the Ogooue-Maritime provinces with respectively 38 (0.9%), 75 (1.9%), 161 (4.1%), and 436 (11.0%) births recorded (Table 1).

Table 1A: Per year: In the period from 2007 to 2010, a sample of 3,957 was constituted with an average of almost 25% of subjects per year.

Year	Frequency	Percent	Cum.
2007	950	24.01	24.01
2008	951	24.03	48.04
2009	989	24.99	73.04
2010	1,067	26.96	100
total	3,957	100	

Table 1B: Per provinces: Births in 6 (Estuaire, Haut-Ogooué, Moyen-Ogooué, Nyanga, Ogooué Ivindo, Ogooué-Maritime) of Gabon's 9 administrative provinces were tested. The percentage of births tested is reported here by study province.

Region	N	Percentage
Estuaire	1,893	47.80%
Haut-Ogooue	1,352	34.20%
Moyen-Ogooue	38	0.90%
Nyanga	75	1.90%
Ogooue-Ivindo	161	4.10%
Ogooue-Maritime	436	11.00%
Total	3,955	100%

The average duration of a pregnancy was 39 weeks and did not vary with regions/provinces. Over 50% of pregnancies lasted at least 39 weeks; however, we also registered pregnancies as short as 18 weeks and as long as 49 weeks. These characteristics of the duration of pregnancy did not vary from year to year over the study period (Table 2 and Figure 3).

Table 2: Length of pregnancy by province: Variation in the average length of pregnancies of patients received in the study areas during the study period.

Provinces duration	mean	med	min	max	N
Estuaire	38.76	39.00	27.00	49.00	1,720
Haut-Ogooue	38.87	39.00	30.00	47.00	320
Moyen-Ogooue	38.50	39.00	34.00	42.00	26
Nyanga	38.18	39.00	32.00	45.00	39
Ogooue-Ivindo	38.90	39.00	30.00	43.00	146
Ogooue-Martime	38.74	39.00	18.00	44.00	381

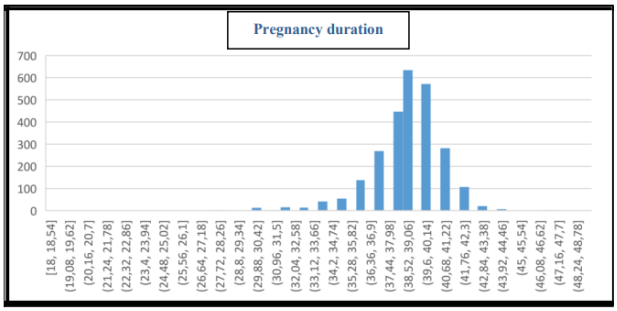

Figure 3: Length of pregnancy by province: Variation in the average length of pregnancies of parturients received in the study areas during the study period.

The average birth weight was 3.1 kg and varied very little with years and provinces during the study period. Newborns with less than one kilogram and almost 5 kgs were also recorded (Table 3). We have consolidated the results of the screening tests as AS and SS. Of the 3,957 children tested during the study period, 678 were heterozygous Haemoglobin S (HbAS), with an incidence of 17.13% and 53 children homozygous Haemoglobin S (HbSS), with an incidence of 1.34% (Table 4).

Table 3: Birth weight by year (A) and provinces (B): Variation in average birth weight by region during the study period and by provinces.

A					
Year	Mean(g weight)	Med(g weight)	Min(g weight)	Max(g weight)	N(g weight)
2007	3132.09	3120	1213	4710	939
2008	3121.08	3150	820	4520	916
2009	3083.53	3075	1180	5900	596
2010	3061.14	3045	1120	4890	350
B					
Provinces duration	Mean(g weight)	Med(g weight)	Min(g weight)	Max(g weight)	N(g weight)
Estuaire	3125.25	3130	820	4710	1,826
Haut-Ogooue	3129.03	3120	1500	4520	331
Moyen-Ogooue	3050.33	3075	1870	3870	30
Nyanga	2830.99	2850	1250	4080	71
Ogooue-Ivindo	3002.59	3000	1500	4410	145
Ogooue-Maritime	3113.39	3100	1120	5900	397

Table 4: Hemoglobin variants in the study population: Results of the sickle cell screening test performed on newborns in the study areas and during the study period.

Group	Frequency	Percent	Cum.
A*	3,226	81.53	81.53
AS	678	17.13	98.66
SS	53	1.34	100
Total	3,957	100	

Note: (A*) A allele.

These incidence rates varied slightly by region, but were not statistically significant. These rates also varied slightly with the date of birth, without being statistically significant (Figures 3 and 4).

We have also tried to explore the potential impact of the status of the child (distinguishing AS, SS from others) on the child's birth weight over the duration of the pregnancy, taking into account the year of birth/test, the region, using statistical models of multiple regression. With respect to the duration of pregnancy, these analyses indicated that individuals with the HbSS genotype have a slightly longer duration of pregnancy (on average 0.5 kg more) than the rest of the children in the other groups and this were statistically significant at 10%. However, the same analyses indicate that the heterozygous AS did not have duration of pregnancy significantly different fromthe rest. All of this after we control the potential effects of the year and the region/province (Table 5).

With regard to birth weight, these analyses indicated that the group has no impact on the child's birth weight. All of this after checking the potential effects of the year and the region/province. In the Moyen-Ogooue and Nyanga regions, on the other hand, birth weights are slightly lower than the rest of children, and these differences were statistically significant (Table 6).

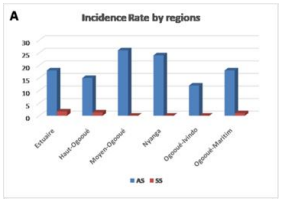

Figure 4: A and B Incidence of each hemoglobin phenotype by regions, C and D Incidence of each hemoglobin phenotype by year. On B and D, gr2 mean AS group; gr3 mean SS group.

Table 5: Pregnancy duration among haemoglobin state: Duration of pregnancy according to the status of the screened child (AS or SS) each year during the study period and study areas.

Source	SS	df	MS			
Model	52.39691	10	5.239691	Number of obs=2,632		
Residual	11707.83	2,621	4.466934	F(10, 2621)=1.17		
				Prob>F=0.3041		
				R-squared=0.0045		
				Adj R-squared=0.0007		
Total	11760.23	2,631	4.469871	Root MSE=2.1135		
	Duration	**Coefficient**	**Std. Err.**	**t**	**P>t**	**[95% Conf. Interval]**
SS	0.634647	0.356182	1.78	0.075	-0.06378	1.333074
AS	-0.109255	0.106691	-1.02	0.306	-0.318463	0.099953
Year						
	2008	-0.019854	0.101841	-0.19	0.845	-0.21955	0.179843
	2009	0.214841	0.144166	1.49	0.136	-0.067849	0.497532
	2010	0.168086	0.195291	0.86	0.389	-0.214854	0.551026
Province						
	Haut-Ogooué	0.145898	0.130915	1.11	0.265	-0.110809	0.402605
	Moyen-Ogooué	-0.201441	0.421508	-0.48	0.633	-1.027964	0.625082
	Nyanga	-0.700088	0.383656	-1.82	0.068	-1.452387	0.05221
	Ogooué-Ivindo	-0.036384	0.21214	-0.17	0.864	-0.452362	0.379594
	Ogooué-Maritime	-0.183789	0.169864	-0.17	0.279	-0.516871	0.149293
	_cons	38.74231	0.078552	493.21	0	38.58827	38.89634

Table 6: Birth weight among haemoglobin state: The influence on birth weight of the child according to the sickle cell phenotype or not (AS or SS).

Source	SS	df	MS			
				Number of obs=2,800		
Model	8.357033	10	0.8357	F(10, 2621)=3.21		
Residual	725.544	2,789	0.26014	Prob>F=0.0004		
				R-squared=0.0114		
				Adj R-squared=0.0078		
Total	733.9011	2,799	0.262201	Root MSE=0.51004		
Kg	**Coefficient**	**Std. Err.**	**t**	**P>t**	**[95% Conf. Interval]**	
SS	-0.04217	0.08258	-0.51	0.61	-0.2041	0.11976
AS	-0.0216779	0.02497	-0.87	0.385	-0.07063	0.02728
			Year			
2008	-0.00815	0.0239	-0.34	0.733	-0.05502	0.03872
2009	-0.02287	0.03372	-0.68	0.498	-0.08898	0.04324
2010	0.00687	0.04663	0.15	0.883	-0.08456	0.09829
			Province			
Haut-Ogooue	0.00156	0.03101	0.05	0.96	-0.05925	0.06236
Moyen-Ogooue	-0.07364	0.09486	-0.78	0.438	-0.25964	0.11235
Nyanga	-0.30715	0.07437	-4.13	0	-0.45297	-0.16132
Ogooue-Ivindo	-0.11523	0.05085	-2.27	0.024	-0.21494	-0.01551
Ogooue-Maritime	-0.01023	0.03997	-0.26	0.798	-0.0886	0.06815
cons	3.13646	0.01828	171.56	0	3.10061	3.17231

DISCUSSION

The two most represented provinces are those in which the Interdisciplinary Center for Medical Research laboratories are located (Estuaire and Haut-Ogooue), which suggests a well-established procedure for communication, monitoring and sample collection. It will also be necessary to add to this the number of partner hospital centers as part of the project's implementation, which are more numerous in the Estuaire and Haut-Ogooue provinces. The low birth rates observed in other provinces can therefore be explained by the low sampling rate in those provinces and further disturbances in the transport of blood samples from one province to the other, taking into account existing regulations which could lead to a decrease or loss of motivation by health care personnel involved in the project (Table 1A). Looking for the best birth conditions some women do not hesitate to travel to larger cities with better health care system. However, an analysis of the collection of samples made each year showed that the number remains more or less the same (Table 1B). Although the study was conducted in different regions, we did not notice any significant difference in birth weight and this observation is also true when we consider the results of each year (Table 3 and Figure 5). Not all regions are affected in the same way in terms of Sickle Cell Trait (SCT) or Sickle Cell Disease (SCD) status. Indeed, the Moyen-Ogooue province had 26% SCT and SCD prevalence very close to zero, followed by the province of Nyanga a prevalence of SCT of 24% of sickle cell disease very close to zero. This value of sickle cell disease prevalence very close to zero was also observed in the Ogooue-Ivindo region where 11% of tested blood samples were carriers of the sickle cell trait. In the provinces of Estuaire, Haut-Ogooue and Ogooue-Maritime, we observed respectively 18%, 15% and 18% of sickle cell trait carriers and 1.6%, 1.2% and 0.1 newborns with sickle cell disease. Sickle cell disease is an autosomal recessive hemoglobinopathy. It affects up to 2% of newborns in some countries of sub-Saharan Africa [27].When we consider the population in relation to the different provinces, we find that the prevalence of sickle cell trait carriers ranges from 11% to 26%. Our findings are in agreement with Sickle Cell Trait prevalences of (5% and 40%) and (10–20%) reported respectively in sub-Saharan African countries of the literature which assesses and the Democratic Republic of the Congo [28,29]. The highest prevalence of Sickle-Cell Trait (SCT) in Africa occurs between the latitudes of 15° North and 20° south, where the prevalence ranges between 10% and 40% of the population [30]. The prevalence of the SCT in Cameroon, the Democratic Republic of Congo, Gabon, Ghana, and Nigeria ranges from 20% to 30% [31].

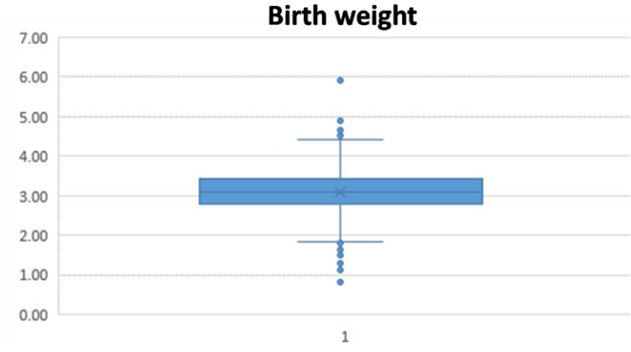

Figure 5: The most observed average birth weight.

Worldwide sickle cell anemia or Sickle Cell Disease (SCD) is estimated that 75-85% of children born with SCD are born in Africa [7]. Because many births occur outside of hospitals and many children die before diagnosis with SCD, there are limited statistical data on the incidence of SCD in Africa [6,32]. The results obtained in this study on the prevalence of sickle cell disease are consistent with those already published in some African countries such as Tunisia (1.89%), Cameroon (1.7%) and the Democratic

Republic of Congo (1.6%) [27]. However, the prevalences reported in the current study may be under estimated due to the fact that the inclusion of newborns requires the consent of the parents. It would be important for this screening to become a compulsory examination, especially in countries with a high prevalence of malaria, as it is the case for Gabon. Neonatal screening is strongly recommended to set up an early healthcare network organized around the child and doctors [27].

There is no real difference in the weight of these newborns when considering the sampling areas. However, it can be observed that the provinces of Nyanga and Moyen-Ogooue have newborns whose low birth weight was significantly different from the rest of the study regions (weight which may be less than 1500 g). We might be tempted to explain this low birth weight by the prematurity of the newborns, which is not the case because it was not reported in the birth register. But the unfavorable conditions in which these children's mothers live may partly explain the observed weight.

CONCLUSION

The prevalence of sickle cell disease of newborns is comparable to that reported in the literature, i.e., 1.34%. These incidence rates vary slightly by region and with the test/birth year, without these variations being statistically significant. Sickle cell status did not influence the birth weight of the child. The particularity here is that we will be able to offer a management and a follow-up of sickle cell births from this data and generate a SCD map.

AUTHOR CONTRIBUTIONS AND ACKNOWLEDGEMENTS

A.G, E.R, M.GH, B.SH put together the article content. F.A and V.GH analyzed the data. The manuscript was drafted by E.R, M.GH, A.G. All authors have read and approved the final manuscript.

SOURCES OF FUNDING

This study has been approved by the ethics committee of Mashhad University of Medical Sciences (code: IR.MUMS.MEDICAL.REC.1400.089). Furthermore, confirming that informed consent was obtained from all subjects and their legal guardian.

CONFLICT OF INTEREST

None

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