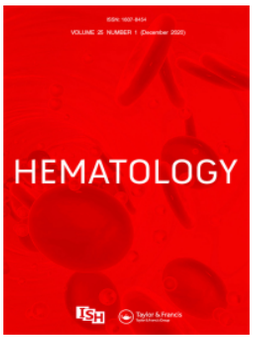

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RESEARCH ARTICLE

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Newborn screening for sickle cell disease in Kisangani, Democratic Republic of the Congo: an update

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ABSTRACT

Background: Neonatal screening is the first action necessary to identify children with sickle cell disease (SCD) and thus ensure their care. Using rapid tests to give an immediate result to families is a new resilient approach of great interest. These two aspects are essential for establishing an adequate health policy for this disease. This study was undertaken in Kisangani to update the current incidence of neonatal SCD.

Methods: Heel prick blood samples of 1432 babies born from different racial groups of parents living in Kisangani were collected at birth and screened using a point of care test, i.e. the HemoTypeSC™.

Results: The incidence at birth was 2.2% ($n = 31$; 95% CI: [1.5%−3.1%]) for HbSS homozygosity and 21% ($n = 303$; 95% CI: [19%−23%]) for HbAS heterozygosity. Compared to a previous study in 2010; the incidence at the birth of the HbSS form has doubled, while that of the heterozygous form HbAS remained almost unchanged. The inter-ethnic incidence of HbSS among the five top-represented ethnic groups was significant (<0.001).

Conclusion: The prevalence of homozygote form has doubled compared to the 0.96% reported in 2010. Setting up a neonatal screening program and an awareness unit is necessary to assess the need for care services correctly.

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Sickle cell disease; neonatal prevalence; HemoTypeSC; Kisangani

Introduction

Sickle cell disease (SCD) encompasses a group of blood disorders characterized by at least one hemoglobin S allele (HbS; p.Glu6Val in HBB) and a second HBB pathogenic variant resulting in abnormal hemoglobin polymerization. Hb S/S (homozygous p.Glu6Val in HBB) accounts for most SCD. Other forms of SCD result from compound heterozygosity for HbS with different specific pathogenic beta-globin chain variants (e.g. sickle-hemoglobin C disease [Hb S/C], sickle beta-thalassemia [Hb S/β+-thalassemia and Hb S/β0-thalassemia], Hb S/D, Hb S/O-Arab, Hb S/E) [1–3]. The homozygous hemoglobin S and C (HbSS & HbSC) genotypes result in sickle cell anemia (SCA). In contrast, heterozygous hemoglobin S and C genotypes (HbAS and HbAC) result in sickle cell trait (SCT) [1,2].

SCD is an autosomal recessive hereditary blood disorder that is heterogeneously distributed worldwide, yet more frequent and with a heavy burden in the African sub-Saharan region [3–5]. Multiple studies show that approximately 300,000 SCD children are born each year, 3/4 of them in Sub-Saharan Africa, with a prevalence of 0.12–7.7% for SS and 5.62% – 24.6% for AS [6]. SCD plays a considerable part in the morbidity and mortality of children under five years old [5]; nearly 50–90% of SCD children die before the age of five years [4,5]. As a hereditary disease, its prevalence may vary up and down with the ethnic composition of the inhabitants of a country. Immigration has been incriminated as a factor in the propagation of the gene of disease from region to region through anthropologic data [7]. Today, HbC, formerly the prerogative of West Africa [3], essentially of Burkina Faso, where it originated [8] is reported in Rwanda [9], Angola [10] and recently in Kindu (DRC) [11].

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During these three last decades, the Democratic Republic of the Congo (DRC) faced in its eastern part multiple displacement movements of people fleeing wars and ethnic conflicts [12]. Contingents from West and North Africa, alongside internal displacements towards Kisangani, may have changed epidemiological data on SCD. A previous study on the neonatal prevalence of SCD conducted in 2010 [13] to estimate blood transfusion service needs found a majority of 0.96% (5/520) SS and 23.3% (121/520) AS. Thus, this study aimed to update data on the neonatal prevalence of SCD within the current sociodemographic configuration of the inhabitants of Kisangani, the capital of the Tshopo province located in the northeastern part of the DRC, to evaluate the need for early childcare.

Methods

Study design and location

The study is part of doctoral research planned for three years, from March 2020 to April 2022 as prospective cross-sectional clinical study to screen newborns recruited in different maternities in the Health Zones (HZ) of Kisangani. It was planned to launch a systematic neonatal screening in many health facilities. However, because of COVID-19 breakthrough, the gradual recruitment of pregnant women as participants was processed only in the structures where the preliminary studies were carried out [14,15]. At launch, the COVID-19 pandemic spread quickly, reaching thousands of people per day with a relatively high number of deaths. The WHO declared a state of emergency on 10 March 2020. The DRC relayed this measure on the 19th of the same month with the closure of public places (schools, churches, restaurants, and bars). Preventive measures such as 'staying at home' and 'avoiding unnecessary outings' have been imposed on the population to prevent their movement and curb the spread of the disease.

Study sample

As shown in Table 1, only twelve major health facilities were selected by convenience because of the COVID-19 breakthrough. The information concerning the health structures for recruiting newborns was described in previous publications [14,15]. All live babies born in the selected health facilities during the study and whose parents agreed to participate were eligible. In total, 1432 newborns were screened.

Genetic counseling

The project had constituted specific teams to provide genetic counseling targeting students, prenuptial meetings, antennal and postnatal consultations, and Visits to raise awareness of convenient measures and vaccination carried out in the maternities concerned. The participants were previously aware of being screened alongside their live babies within 72 h after childbirth [16]. However, not all mothers have been tested. The genotype of siblings depends on the father et mother crossing. The mothers were not screened before delivery, and only some whose babies were SCT or SCA were screened to support the result [17].

Screening method

The Screening was performed with HemoTypeSC (HT401RUO-USA). The material, procedures, and screening stages have been described elsewhere [18–20]. Once the box was opened, the kit was used within 30 days as defined by the manufacturer. A blood sample (1.5 µl) was taken in situ by the principal investigator, the midwife, or the nurse from the left heel of the newborn. The test result was immediately communicated to the parents (mother or father or both, as the case, could be) and then noted on the interview canvas and in the register in the office of the titular nurse. A small amount of blood was collected on filter paper (Whatman 903) to confirm the result, conducted at the Laboratory of Genetic Biochemistry of the University Hospital of Liège using the LC-MS (TQ5500) triple quadrupole (Sciex) source mass spectrometer system (AB Sciex, Njeuwerkerkaan den Ijssel) as described by Boemer F [21].

Data mining

The parents who accepted the Screening of their newborn were interviewed for 20 min to get their sociodemographic information and family history of SCD. The distribution frequencies of categorical, ordinate, and ratio variables observed in different health facilities were calculated. As a descriptive study, the distribution of newborn genotypes by health facilities and parents' ethnicity was calculated, but no correlative association was made.

Ethical considerations

The study was conducted according to the recommendations of STROBE GUIDELINES [22]. We obtained the approval of the University of Kisangani ethics committee (ref no UNIKIS/CER/005/2018). Informed verbal and written consent from the parents of the newborns was obtained beforehand. We guaranteed anonymity to the parents, and they were informed that they were free to stop participating in the study at any time without any coercion.

Table 1. Study sample size from health centers selected and global test-result.

Health Structures	Total births		Male child		Female child		HbSS	%	HbAS	%
	N	%	N	%	N	%	N	%	N	%
CS JAMAA	450	31.42	237	16.55	213	14.87	13	2.89	109	24.22
HGR/KABONDO	307	21.44	160	11.17	147	10.27	3	0.98	64	20.85
CS MATETE	194	13.55	79	5.52	115	8.03	2	1.03	30	15.46
HGR/MAKISO	186	12.99	103	7.19	83	5.8	4	2.15	49	26.34
NVDP	148	10.34	79	5.52	69	4.82	4	2.70	29	19.59
Others	147	10.27	79	5.52	68	4.75	5	3.40	22	14.97
TOTAL	1432	100	737	51.47	695	48.53	31	2.16	303	21.16

Genotype HbAA frequency is total minus HbSS and HbAS.
The other centers included: CS IMANI (50), HGR/MANGOBO (23), STANLEY (22), CS SAINTCAMILE (17), CUKIS (17), CS ALABUL (11), and HGR/TSHOPO (7).

Results

Coverage rate and participants

During the study period, 10,069 live births were registered, but only 1432 newborns were screened, indicating coverage of 14.22%. There were 51.40% (737/1432) male and 48.5% (695/1432) female newborns weighing 1.22–5.70 kg.

Prevalence of neonatal sickle cell disease

The neonatal prevalence was 2.2% ($n = 31$; 95% CI: [1.5% −3.1%]) for the homozygous HbSS, 21% ($n = 303$; 95% CI: [19%−23%]) for the heterozygous HbAS.

Table 1 presents the coverage disaggregated by study health centers. The majority of births (80%) occurred in five maternities, namely CS JAMAA, HGR/KABONDO, HGR/MAKISO, CS MATETE, and NVDP, where also most of SCD children were born. No HbSS was detected in CS Imani, Stanley, CS Saint-Camille, and HGR/Tshopo. Frequencies of HbAA genotype not indicated; they are total minus HbSS and HbAS frequencies.

Table 2. Neonatal Prevalence Frequencies of HbSS and HbAS in study newborns.

PARAMETERS	N	%	N	%	N	%	*p*-Value
Newborn sex							
Female	695	48.5	14	2	140	20.1	0.3746
Male	737	51.5	17	2.3	162	22	
Family SCD history							
Known	334	23.3	20	6	209	62.6	<0.001
Unknown	1098	76.7	11	1	94	8.6	
Family ethnics							
Lokele	265	18.5	4	1.5	57	21.5	<0.001
Topoke	218	15.2	9	4.1	44	20.2	
Mukusu	114	7.9	0	0	19	16.7	
Tolombo	76	5.3	1	1.3	18	23.7	
Basoko	75	5.2	1	1.3	20	26.7	
Mbole	54	3.8	1	1.9	9	16.7	
Tetela	50	3.5	1	2	10	20	
Ndande	48	3.4	0	0	10	20.8	
Mongando	47	3.3	0	0	9	19.1	
Rega	46	3.2	2	4.3	10	21.7	
Mbuza	42	2.9	1	2.4	12	28.6	
Others	396	27.7	11	2.8	85	21.5	
Total sample	1432	100	31	2.2	303	21.2	

Genotype HbAA frequency is total minus HbSS and HbAS.
The other ethnics included: Luba (37), Boa (35), Mukongo (35), Minyamituku (35), Mukumu (30), Hema Logo (27), Mobangobango (27), Mongo (26), Budu (23), Ngwandi (21), Musonge (20), Genia (17), Mobali (17), Zande (15), Mongelema (12), Momanga (12), Mushi (6), and Tanzanian (1).

Table 2 presents the coverage disaggregated by sex, family history of SCD, and parents' ethnicities. No significant statistical differences were observed between female and male newborns. However significant differences appeared on family history of SCD and ethnicities. The parents belong to different ethnic groups, of which the most representative included 265 (18.5%) Lokele, 218 (15.2%) Topoke, 114 (7.9%) Mukusu, 76 (5.3%) Tolombo, and 75 (5.2%) Basoko. However, the inter-ethnic prevalence of HbSS varied from zero to 13.04%. One case of Tanzanian citizen, foreigner to DRC was found AS type.

Table 3 gives an overview detailing the data on the similarities and differences between this study and the one previously carried out in the same city to explain differences between the present and the previous

Table 3. Comparative factors backing differences between present and previous study.

INDICATORS	PRESENT STUDY	PREVIOUS STUDY [13]
1. Incidence at birth		
Sample size	1432	520
HbAS	303 (21.1%)	121 (23%)
HbSS	31 (2.2%)	5 (0.96%)
2. Screening methods		
Type	HemoTypeSC	Isoelectric focusing
Confirmation test	LC-MS and	Isoelectric focusing
Result delay	Parents'	After several days
Blood collection	Hb	Cord blood on filter
Parent acceptance	Bed side	paper
	Heel prick	(100%)
	(40%)	
3. Major ethnicities included		
Lokele	(23%)	(19%)
Topoke	(24.2%)	(26.3%)
Mbole	(18.5%)	(33,3%)
Basoko	(28%)	(20.0%)
Tolombo	(25%)	(0%)
Budu	(30.4%)	(23.5%)
Mobangobango	(11.1%)	(27%)
Genia	(41.1%)	(0%)
Rega	(26.0)	(16.6%)
4. Study health centers		
Maternities number	CS Alabul	Hôpital Général
	Cs Imani	Provincial
	Cs Jamaa	Hôpital Général de
	Cs Matete	Référence
	Cs Saint Camile	Center de Santé de
	Cukis	Boyoma,
	Hgr/Kabondo	Center de Santé Saint-
	Hgr/Makiso	Joseph
	Hgr/Mangobo	Center de Santé de
	Hgr/Tshopo	Makiso
	Nvdp	
	Stanley	

prevalence. The two studies differed in sample size (1432/520), screening method (HemoTypeSC/isoelectric focusing), ethnics number (large/small), and number of study health centers (12/5).

Discussion

This study showed a low screening coverage, i.e. 14.22% (1432/10069) of cases. A survey by Pare NI [23] in Mali observed 25% (1982/7923), while Shongo MYP et al. [24] reported 56.3% in Lubumbashi (DRC). In both cases, the main reason cited was the parents' refusal to have their newborns screened. In our series, the grounds were the COVID-19 pandemic which justified early discharge from maternity before the time required for screening. Newborns' mothers feared being infected with COVID-19 and having their newborns vaccinated against COVID-19 as circulated by fake news [25,26]. These rumors made the population believe that the DRC was selected among the countries where the anti-COVID-19 vaccine was to be tested [14], joining the experience already noted with the vaccine against Ebola in the North-East of the country [25]. During this last situation, the cause of the refusal by the population was the false rumor reporting a vaccine having the capacity to reduce the reproduction of the Congolese and negatively affect mental faculties [25]. These reasons explain many cases of home birth in our community [26] because the correct information is still not yet received on this subject.

Ideally, screening was planned to occur after 72 h of birth, but with the advent of COVID-19, compliance with this measure would not allow any screening. Waiting for 72 h in the maternity ward was impossible for those who gave birth. However, it was essential to respect the deadline provided by the manufacturer because the prevalence of the disease depends on it [17–20]. In Africa, it is recognized that many families do not have readily identifiable addresses or permanent addresses. This results in difficulty locating families of affected babies to provide diagnosis and care for an affected child [27].

Concerning the difference from the point of view of the screening test applied in Kisangani, Agasa et al. [13] used the IEF with the shortcoming of the prolonged delay in the result, making it challenging to find the parents to inform them of the diagnosis of the affected child. In addition, umbilical venous blood samples were collected on filter paper. In this case, the risk of giving false results either by mixing maternal and fetal blood or when this has not been well preserved is high. It couldn't be the case with the HemoTypeSC, which uses capillary blood suitable for neonatal screening and whose sensitivity and specificity are around 100% [18–20]. Also, parents who received genetic counseling quickly planned to consider care for their children within three months, depending on the results after confirmation [17]. The HemoTypeSC gives the result at the patient's bedside and promotes the conduct of genetic counseling [10,15]. It also makes it possible, as it is cheaper, to analyze the Hb of the parents to confirm the neonate SS status and to persuade them to accept themselves as the affected child's parent and adhere to the Treatment [15,17].

As shown in the results section, the current HbSS prevalence is two times higher than that reported twelve years ago [13] and also surpassed that reported for Kinshasa (DRC) [28], but was lesser than 3.47% (6/173) written by Shongo MPY et al. [24] in Lubumbashi (DRC). Various studies in the sub-Saharan Africa region show that the prevalence varies from 0.8% to 3% for SCA and 7 to 24% for SCT [3,4], consistent with our findings. Comparatively, in our central region, the current neonatal SCA prevalence in Kisangani is higher than that reported recently in Uganda [29], Tanzania [30] and the Republic of Congo [8]; similar prevalence rates have been observed in West African countries, including Benin, Ghana, Burkina Faso and Nigeria [3], all of which are in the sicklemic belt where malaria is endemic [8].

To explain the increasing rates of SCD incidence, everyone knows that Africa, by its socioeconomic backwardness, is greatly affected by malaria [31]. People living in the same biotope as the Plasmodium falciparum vector (heterozygous) have developed a mutation associated with resistance against severe forms of Plasmodium falciparum malaria [31]. This explains the prevalence of the S or C gene in regions with a high prevalence of Plasmodium falciparum malaria because of its protective effect [1,6]. This disease is rife in its endemic form in Kisangani [32]. The role of malaria, whose epidemiology is currently superimposable on SCD, should not be overlooked.

Alongside malaria infection, the literature has also reported the impact of migration on the propagation of pathological genes [7]. As mentioned in the introduction, HbC, formerly the prerogative of West Africa, has recently been detected in Rwanda [9], Angola [10] and recently in Kindu (DRC) [11]. Even though HbC has not been found in Kisangani, the search for this HbC will have to continue because its migration towards the center of Africa has been demonstrated [9].

Not withdrawing the effect of sample size and the screening methods used, the population growth and ethnic mixings may bark the differences. The ethnic group composition of 2010 is different from the one in the current study, e.g. two ethnic groups, Tolombo and Genia, were absent in 2010, regarding the reasons for this high prevalence in the identified ethnic groups. Agasa et al. [13] mentioned kinship

due to the high frequency of endogamous marriage in the ethnic groups concerned. In Tshilolo et al. [28], some differences occurred in certain tribes because of endogamous marriage. In these tribes, Father and mother could be from different ethnicities. However, culturally we only considered the father's race for couples or the mother's if she was single.

Conclusion

Compared to the previous situation, the neonatal prevalence of SCT has remained stationary while that of the SCA is growing and taking on a new epidemiological figure. The modification of the epidemiological data effectively indicates the impact of the migratory movements of the population towards the city of Kisangani following the rural exodus. Awareness is essential to increase the use of maternity services to cover all newborns in neonatal screening. Setting up a neonatal screening program and an awareness unit is necessary to assess the need for care services for this disease correctly. Prenuptial tests must be compulsory in the DRC.

Limitations

COVID-19 posed a severe threat to the completion of this study, explaining low screening coverage and low community buy-in to the process. The other obstacle is the diversity of ethnic groups with the possibility of a crossover, making it difficult to interpret the results about ethnicity, as highlighted in the previous articles.

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