Prevalence Of Sickle Cell Disease In Kilamba Kiaxi Hospital, Luanda, Angola

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Abstract

Sickle-cell disease is one of the most important causes of severe anaemia in Africa. This study intend to determine the prevalence of sickle cell disease among children in Kilamba Kiaxi in Luanda, Angola. Hospital Divina Providência (Kilamba Kiaxi, Luanda, Angola) laboratory équipe, collected data for all the patients with diagnosis of sickle-cell disease during 2009 and 2010. More than 10,000 patients were investigated to determine those that will test positive for sickle cell disease. The result showed that sickle-cell disease affects 25% of population. Also the risk of severe anaemia and AS and SS genotypes accounted for the similar prevalence in the studied population. A positive diagnosis for the sickle-cell disease is not always confirmed by haemoglobin electrophoresis. This present pilot study could be a good beginning to create a drepanocytosis register in Luanda and to correlate drepanocytic patients with blood transfusion requirements.

Key Words: sickle cell disease, Angola, haemoglobin electrophoresis

.Introduction

The sickle-cell disease (SCD) is one of the most important causes of severe anaemia in Africa. It is characterized by the presence of haemoglobin S (HbS) in the erythrocytes [1,2]. Also Angola is affected by the presence of the gene HbS in its population and this is recognised world-wide as an adaptive mechanism to the presence of Plasmodium falciparum among Africans living in areas infested by this organism. Since the HbS protects against severe malaria [3,4,5].

The haemoglobin S (HbS) substitute the haemoglobin A1 (HbA1) normally present into the normal red blood cells and the HbS is responsible for structural alterations into the haemoglobin. These alterations cause a different conformation of the protein structure and this has serious effects in deoxygenated state. The deoxygenated state of haemoglobin S can lead the protein structure to a strong instability: when there is hypoxia erythrocytes lose their biconcave shape and become sickle-shaped. These erythrocytes are called drepanocytes. The sickle-shape condition is irreversible and it can lead to a drepanocytic crisis that can create a severe anaemic condition in the patient. Sometimes the anaemia is so severe that the patient needs blood transfusion [6,7].

In African hospitals having information concerned with sickle-cell disease is an important matter because the sickle-cell disease is one of the most important causes of severe anaemia in African children that are less than seven years old [7]. Furthermore it's useful to correlate drepanocytic data with blood transfusion data in order to know how many drepanocytic children received a blood transfusion and to know if drepanocytic children that received blood transfusion had severe anaemia due to malaria or due to a drepanocytic crisis, as suggested by other studies [8].

Aim of this study is to obtain data of prevalence of sickle-cell disease in Luanda suburb population and to differentiate this sub-population in homozygotes (HbS-HbS: SS) or heterozygotes (HbA-HbS: AS).

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Materials and Methods

Studied population: this study was conducted in a Kilamba Kiaxi hospital, a suburb located in the southern area of Luanda, capital of Angola. The hospital population is heterogeneous and almost all the children (< 14 years old) and all the pregnant women that come to this hospital are screened for drepanocytosis, as these two subgroups have a very high risk of severe anaemia. If the first screening test (sickle-cell disease test) is positive for the sickle-cell disease, another test is required in order to assess the genotype of the haemoglobin in the patient. Unfortunately it was not been possible to assess the genotype of all the patients that tested positive to the first screening test.

Ethical approval for this study was obtained from the ethics committee of Hospital Divina Providencia. This committee is composed by the general director and the clinical director. It was not possible to obtain informed consent from all participants involved in this study. This Ethic

Committee approved data utilization.

Sickle-cell disease test: 20 ì l of whole blood come in contact with 20 ì l of 2% sodium metabisulfite (diluted in distilled water), a reductive substance that captures erythrocyte oxygen. The oxygen reduction in absence of air causes a change in erythrocytic membrane structure and erythrocytes that have the HbS change their shape and become sickle-shaped. In order to quit air to allow oxygen reduction, the mixed solution of blood and sodium metabisulfite is collocated on a microscopy slide and it is hermetically closed by a cover slip with its edges sigillated by nail polish. The microscope slides are then collocated at 37ºC to accelerate the reduction reaction. After 30 minutes, the slides are ready to be read with a 40X objective of optic microscope: sickle-shaped red blood cells are easily distinguishable from normal red blood cells. The result of the SCD test is qualitative: it says if the specimen has or has not sickle-shaped erythrocytes [9]. This test allows physicians to receive the result (positive or negative) very quickly.

Haemoglobin electrophoresis: the sickle-cell disease test is not able to assess if positive patients to this test are homozygotes or heterozygotes for HbS gene. Haemoglobin electrophoresis gives us this information. Haemoglobin electrophoresis is performed using cellulose acetate electrophoresis technique. A small quantity of venous blood (500 ì l) is placed in a tube, washed with physiological solution and mixed with 500 ì l of water to lyse red blood cells and 1000 ì l of chloroform to pack cell residues. With the aid of an applicator the hemolysate is placed on the cellulose acetate strip and electrophoresis was performed for 50 minutes at 280 mV in Tris buffer solution. Haemolysates from blood sample known to be homozygotes or heterozygotes for HbS gene was run as control. The electrophoresis results are given as AS (heterozygote) or SS (homozygote) [9].

Statistical analysis: statistical analysis was done using Chi-Square test to determine statistical significance at p value of < 0.05.

Results

Table 1 shows absolute numbers and prevalence of results of sickle-cell disease test during 2009 and 2010. The total population of patients seen at

Table 1: absolute numbers and their prevalence of SCD test in patients during 2009 and 2010.

Month	Total number of patients	-ve SCD nº (%)	+ve SCD nº (%)	Total
Jan. 2009	2,917	377 (72.9)	140 (27.1)	517
Feb. 2009	2,974	356 (77.6)	103 (22.4)	459
Mar. 2009	3,142	392 (74.7)	133 (25.3)	525
Apr. 2009	3,340	395 (80.4)	96 (19.6)	491
May 2009	2,938	329 (74.6)	112 (25.4)	441
Jun. 2009	3,146	291 (74.0)	102 (26.0)	393
Jul. 2009	3,017	314 (77.3)	92 (22.7)	406
Aug. 2009	3,365	304 (76.4)	94 (23.6)	398
Sep. 2009	3,118	316 (77.3)	93 (22.7)	409
Oct. 2009	3,184	258 (78.9)	69 (21.1)	327
Nov. 2009	3,105	326 (70.9)	134 (29.1)	460
Dec. 2009	3,360	390 (76.0)	123 (24.0)	513
	37,606	**4,048 (75.8)**	**5,339 (24.2)**	**5,339**
Jan. 2010	2,978	332 (73.6)	119 (26.4)	451
Feb. 2010	3,294	376 (86.2)	60 (13.8)	436
Mar. 2010	3,459	420 (78.5)	115 (21.5)	535
Apr. 2010	4,419	373 (79.5)	96 (20.5)	469
May 2010	4,582	378 (73.8)	134 (26.2)	512
Jun. 2010	4,356	402 (77.6)	116 (22.4)	518
Jul. 2010	5,094	291 (72.9)	108 (27.1)	399
Aug. 2010	5,098	264 (67.0)	130 (33.0)	394
Sep. 2010	5,725	356 (76.9)	107 (23.1)	463
Oct. 2010	4,005	246 (79.4)	64 (20.6)	310
Nov. 2010	4,177	202 (72.7)	76 (27.3)	278
Dec. 2010	4,492	278 (76.0)	88 (24.0)	366
	51,679	**3,918 (76.4)**	**1,213 (23.6)**	**5,131**

Table 2: absolute numbers and their prevalence of haemoglobin electrophoresis in patients during 2009 and 2010.

Month	Total number of electrophoreses	Total number of AS	AS in children n° (%)	Total number of SS	SS in children n° (%)
Jan. 2009	21	13	9 (64.3)	8	5 (35.7)
Feb. 2009	26	14	9 (47.4)	12	10 (52.6)
Mar. 2009	12	9	5 (62.5)	3	3 (37.5)
Apr. 2009	38	25	17 (56.7)	13	13 (43.3)
May 2009	24	13	12 (54.5)	11	10 (45.5)
Jun. 2009	40	21	18 (51.4)	19	17 (48.6)
Jul. 2009	39	23	19 (54.3)	16	16 (45.7)
Aug. 2009	46	23	16 (42.1)	23	22 (57.9)
Sep. 2009	53	25	13 (31.7)	28	28 (68.3)
Oct. 2009	38	16	12 (36.4)	22	21 (63.6)
Nov. 2009	22	8	5 (33.3)	14	10 (66.7)
Dec. 2009	34	23	16 (59.3)	11	11 (40.7)
	393	**213**	**151 (47.6)**	**180**	**166 (52.4)**
Jan. 2010	34	14	9 (34.6)	20	17 (65.4)
Feb. 2010	47	22	21 (47.7)	25	23 (52.3)
Mar. 2010	31	19	14 (56.0)	12	11 (44.0)
Apr. 2010	31	16	12 (46.2)	15	14 (53.8)
May 2010	58	37	25 (58.1)	21	18 (41.9)
Jun. 2010	35	21	15 (51.7)	14	14 (48.3)
Jul. 2010	80	52	27 (55.1)	28	22 (44.9)
Aug. 2010	61	40	16 (5.7)	21	19 (54.3)
Sep. 2010	94	80	14 (51.9)	14	13 (48.1)
Oct. 2010	141	120	10 (41.7)	21	14 (58.3)
Nov. 2010	68	52	13 (52.0)	16	12 (48.0)
Dec. 2010	54	38	4 (25.0)	16	12 (75.0)
	734	**511**	**180(48.8)**	**223**	**189 (51.2)**

the hospital laboratory during 2009 and 2010 was 89,285. Among these patients 10,470 sickle-cell disease tests were required: of these, 7,966 (76.1%) were negative and 2,504 (23.9%) were positive.

Table 2 shows absolute numbers and prevalence of results of haemoglobin electrophoresis during 2009 and 2010. Monthly distribution of required sickle-cell disease tests and the number of required haemoglobin electrophoresis shows that electrophoresis requests are sensibly less numerous then positive sickle-cell disease tests. The number of monthly required electrophoresis has no relationship with the number of positive sickle-cell disease tests ($p>0.10$).

Discussions

In all sickle-cell disease tests done, the prevalence of positive tests is mostly constant during all the months of the two years. That indicates that sickle-cell disease test request –that normally anticipates an anaemia suspicion- is independent from malaria epidemiology, as mentioned in other studies [10]. If it is thought that most of patients for whom sickle-cell disease test is required are pregnant women or children younger then 5 years old a prevalence mean of positive sickle-cell disease test near 25% is high. This implies that more or less a quarter of the population at high risk of severe anaemia (children and pregnant women) is drepanocytic. It was not possible to differentiate sickle-cell disease tests by age because data are fragmented but from a pilot study (data not shown) we can affirm that 85% of required sickle-cell disease tests are required in children.

The most important data in Table 2 is the prevalence of SS and AS genotypes undergone to electrophoresis. In total during these two years, 686

electrophoresis out of 1127 were performed in children. Among these, 331 (48.3%) had AS genotype while 355 (51.7%) had SS genotype. Of all the electrophoresis done in children, the prevalence of SS genotype and AS genotype is almost the same: SS genotype occurs in a little higher rate then AS genotype but this difference is not statistically significant. So we can say that the two genotypes AS and SS are equally distributed in the examined population. Among analyzed children, homozygosis rate is almost equal to heterozygosis rate and this proportion is stably maintained during all the months of 2009 and 2010. This could be an intriguing data because as there is no relationship between the number of positive sickle-cell disease tests and the number of haemoglobin electrophoresis required, it can be deduced that physicians ask for a haemoglobin electrophoresis only when it is possible to correlate result with clinical practice, which is in the most serious cases. Surprisingly, SS genotype has not a higher rate then AS genotype and this means that both sickle-cell disease types have the same possibility to undergo to a severe anaemia.

The same conclusion is also confirmed by a pilot study (data not shown) that takes into account the number of blood transfusions that we have done in drepanocytic children; both AS and SS children have the same probability to receive blood transfusion. Above all, among analyzed drepanocytic children, the possibilities of receiving a blood transfusion are higher if anaemia is due to a drepanocytic crisis than severe malaria. As earlier suggested in previous studies [6, 10], this could be explained if it is thought that drepanocytic children are more protected against severe malaria then children with no sickle-cell disease.

Conclusions

Even with the partial data that our hospital has produced during 2009 and 2010, data we discussed here suggest that sickle-cell disease is one of the causes of severe anaemia that leads children to receive blood transfusion. Scientific literature is not very rich concerning this clinical field and present reports suffer for the same data missing that this study possesses [8, 11]. As recently suggested [6, 12], it should be very useful to investigate more in order to collect epidemiological data of sickle-cell disease because by now we know very few about sickle-cell situation in Africa.

Authorship

We declare that all the authors, Cristina Lussiana, Ivan Alejandro Pulido Tarquino and Isabel Paulo, has contributed substantially and equally to this manuscript and no one of them has conflict of interests in the publication of this work.

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