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Original Research

Prevalence of hemoglobin abnormalities in kindergartens of the city of Parakou (Benin) in 2013

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ABSTRACT:

Introduction: The hemoglobinopathy is a real public health problem in the world The aim of this study to épister of children with abnormalities of hemoglobin in schools, especially kindergartens in the city of Parakou Republic of Benin.

Methods: This is a descriptive cross-sectional study, conducted in kindergartens in the city of Parakou in Benin republic and having concerned 690 children aged 2 ½ to 5 years. The hemoglobin electrophoresis was done using alkaline pH hydragel and the quantification of haemoglobin fractions were performed with Hyrys densitometer; in some cases the medium is reduced for precipitation test.

Results: Five types of Hb were identified: A, S, M, C and K probably Woolwich. Qualitative hemoglobinopathy was found in 31.45% of the study population. The Hb-S was the most frequent (16.52%) followed by hemoglobin C (15.65%). Hereditary persistence of hemoglobin F was associated with phenotypes AA, AC and SS in 1.16% of cases. The hemoglobinopathies were found in all the major ethnic groups in Parakou with a clear predominance among *"Lokpa"* (53.3%) and *"Adja"* (37.5%).

Conclusion: The hemoglobinopathy is a real public health problem in Parakou, it is necessary to establish or to legislate for mandatory testing for hemoglobinopathies at birth.

Keywords:
Hemoglobinopathies, screening, Benin

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INTRODUCTION

Worldwide, each year more than 3,30,000 children are born with abnormalities in hemoglobin (83% with sickle cell anemia and 17% with thalassemia) (Modell and Darlison, 2008). According to the World Health Organization (WHO), sickle cell anemia affects over fifty million black children from Africa, Equator, America and India (Modell and Darlison, 2008). It is and a real public health problem in Africa. Indeed, the prevalence of sickle cell trait in the general population is 10% in Senegal and 30% or even 40% in the Democratic Republic of Congo (Beyeme-Owono and Chiabi, 2004).

Qualitative hemoglobin abnormalities are by far the most commonly seen in Africa with sickle cell disease in the lead followed by hemoglobin C (Diagne *et al.,* 2003).

In Benin, the prevalence of sickle cell trait S is 22.3% and that of hemoglobin C is 10.21%; it is estimated that about 4% of the population is affected by the homozygous SS and SC double heterozygosity (Latoundji *et al.,* 1991). As for the prevalence of beta thalassemia, it is estimated at 5%, based on estimates from neighboring countries such as Côte d'Ivoire and Nigeria (Zohoun, 1991).

The major hemoglobin abnormalities are responsible for the 3.4% of deaths of children under five around the world (Modell and Darlison, 2008). The mortality rate for major hemoglobin abnormalities is estimated at more than 9% in West Africa and 16% in some countries in the West African sub-region (Modell and Darlison, 2008). Before five years of age, the fatality rate of homozygous sickle cell disease varies from 50% to 80% in Africa (Modell and Darlison, 2008). Beyond five years, the survivors quickly develop degenerative complications causing a heavy morbidity and a shorter life expectancy (Tchokoteu, 2004).

Laboratory diagnosis of hemoglobin abnormalities are based on the analysis of the phenotype catagorization. In most cases several ways are existing but based on sickling tests, solubility, instability, Kleihauer-Betke, hemoglobin electrophoresis, isoelectric focusing, high performance liquid chromatography and even prenatal diagnosis by molecular biology techniques (Bardakdjian-Michau *et al.,* 2003; Trent, 2006) are helpful in the diagnosis of the disease.

In our present context, electrophoresis is used as the first line examination tool to detect many variants of hemoglobin, through the visualization of abnormal bands as compared to normal controls (Singuret and Andreux, 1997).

According to WHO, the ideal would be to detect the disease at the birth of a child (Modell and Darlison, 2008). Paradoxically, the diagnosis of sickle cell anemia and other hemoglobinopathies in Benin is still at a late stage, often not allowing proper care, especially early, the only guarantee of a reduction is morbidity and high mortality associated with these abnormal hemoglobin content (Rahimy, 1999). Despite high prevalence of hemoglobin abnormalities in Benin, there is virtually no early detection of structures, medical care and regular monitoring of affected children in the Northern Benin.

The objective of this study is to detect carrier kids of hemoglobin abnormalities in the kindergartens of the city of Parakou.

MATERIALS AND METHODS

Ethics

This study received approval from the institutional review board.

Framework

This study was a part of the selected topics in the biochemistry laboratory of the Centre Hospitalier, Parakou Départemental Borgou in Benin Republic for sample manipulation.

Equipments

The apparatus consisted of an electrophoresis system (Brand Scan Power generator 300 tank Sebia® K20), a brand centrifuge Rotofix 32, a water bath Sélecta

P and a densitometer Hyrys 2 Version 2.50 (Sebia®).

The reagents used were dipotassium hydrogen phosphate (K_2HPO_4), anhydrous ammonium sulfate $(NH_4)_2SO_4$, pure saponin sodium dithionite ($Na_2S_2O_4$) laboratory acquired Prolabo, and electrophoresis kit for hemoglobin experiment (Sebia®) (HydraGel hemoglobin (e) K20).

Type and period of the study

We conducted a descriptive cross-sectional study with prospective data collection, from 2 May, 2013 to 16 August, 2013.

Study Population

These were children aged 2 ½ to 5 years, with no history of blood transfusion within three months prior to the survey, attending public and private kindergartens in the city of Parakou. They were selected after getting consent from their parents and informed, read and approved properly.

Sampling

Sampling was probabilistic. The cluster sampling technique with two degrees WHO-type has been used as a cluster unit school. In total, 30 clusters have been identified and located throughout the city of Parakou.

We have randomly selected the first degree; 30 Clusters among the exhaustive list of kindergartens in the municipality. Then in the second degree selected school children were admitted in the first degree. A total of 690 children of both sexes were selected.

Study Variables

The variables studied were the electrophoretic profile, phenotype hemoglobin, the proportion of each hemoglobin fraction percentage and ethnicity.

Data collection

Data were collected using a survey form developed for this purpose and the collection of venous blood.

Realization samples

The children selected were subjected to the collection of venous blood samples (3ml) on EDTA tubes. The blood samples thus obtained were transported immediately to the laboratory at room temperature and biological examinations were carried out on the same day.

Technical handling of blood samples

Electrophoresis of hemoglobin

According to the manufacturer's instructions, the blood samples obtained were centrifuged at 5000 rpm for five minutes and the sera was removed. The resulting pellets were washed twice with ten volumes of serum salt 0.9% and 10 µl of washed red cells were incubated with 130 µl of haemolyzing solution for five minutes. Then 10 µl of the hemolysate were deposited on the agarose gel with an applicator. The migration was carried out in buffer tris barbital pH = 9.2 ± 0.5 (165V; 7 ± 2 mA, 15 minutes). Bands were stained with amidoschwarz L a proportion of the different fractions of hemoglobin was determined by densitometer Hyrys 2 v 2.50 (Sebia®).

Precipitation test

It was performed as previously described (Assoumanou *et al.,* 2010) on the samples identified by electrophoresis in an alkaline medium as having hemoglobin S in order not to confuse other migrants at the same level of S.

Data analysis

Data were analyzed using SPSS software version 19.0. The results were presented as proportions. To test whether the prevalence of phenotypes observed in our population is consistent with that expected according to the Hardy-Weinberg, we applied a mathematical model to determine the frequency of alleles A, S, C and K calculated from the phenotypes according to the formula:

$p + q^2 + ^2 + 2pq\ 2pr\ 2QR + + r^2 = 1 + 2pt$

Where as; $P = A$; $q = O$; $r = C$; $t = K$.

RESULTS AND DISCUSSION

Qualitative abnormalities

In our study, a total of 690 children were subjected of which 217 were with qualitative abnormal

hemoglobin. The prevalence of qualitative abnormalities in hemoglobin was therefore 31.45%. This proves that qualitative hemoglobinopathies in Benin and especially in Parakou were a real public health problem. This result is similar to those reported in Burkina Faso, which were 30.17% and 30.08% respectively (Simpore *et al.,* 2002 and 2003). By cons, lower prevalences were reported in Tunisia (Mseddi *et al.,* 1999) and Mauritania (Deyde *et al.,* 2002), which were 9.4% and 16.6% respectively. Table 1 shows the different hemoglobin phenotypes found in the study population.

Table 1: Hemoglobin phenotypes in pre-school children of the city of Parakou in 2013

	Effect	Percentage
AA	473	68.55
AS	101	14.64
AC	99	14.35
SC	7	1.01
SS	6	0.87
CC	2	0.29
AK probable	2	0.29
Total	**690**	**100.00**

The prevalence of Hb S abnormality, most represented in the study population was 16.4%. In Burkina Faso against the Hb S was the anomaly least represented (Simpore *et al.,* 2002 and 2003). The prevalence rate in our study population is similar to that found in Togo (16.70%) after the retrospective analysis of 5028 hemoglobin electrophoresis performed at the Campus University Hospital (Segbena *et al.,* 2002).

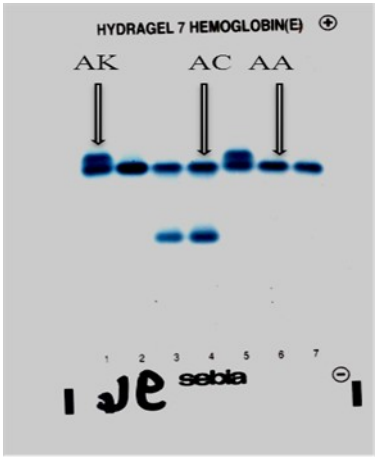
Our work has found a high prevalence of hemoglobin C 15.6% as is the case in Burkina Faso (Simpore *et al.,* 2002 and 2003) and Mali (Toure, 2006) with rates of 21.47%, 21.52% and 13.1%. This finding is consistent with the literature data showing that hemoglobin C is common in West Africa at the voltaic plate represented by the following countries: Ivory Coast, Ghana, Burkina Faso, Togo, South Mali, West bank of the Niger, Benin, with 15 to 40% of carriers (Piel, 2013).

Of the 217 cases of qualitative abnormalities in hemoglobin identified, over 90% are related holders; they therefore play an important epidemiological role. The sickle cell trait AS was majority with 46.54% followed by the heterozygous Hb AC with 45.62% (Table 2). A similar order of frequency was reported in 2006 in Mali, including: AS (54.41%), AC (19.42%), SS (12.40%) and CC (2.20%) (Toure, 2006). This high prevalence of stroke subjects relatively carrying sickle cell leads us to advocate the introduction of screening before enrolling children in kindergartens.

Hemoglobin K is very rare, and not described in Benin. It was found in two children of the same family, of *"Nagot"* ethnic group (Figure 1). According to the literature, this type of hemoglobin is the specificity of *"Akan"* ethnicity in Ghana and Côte d'Ivoire (Cabannes *et al.,* 1980; Molez *et al.,* 1989). An investigation into the origin of the grandparents of these children with establishing their family tree could elucidate this phenomenon. This could suggest a common origin of the peoples of the subregion despite their ethnic differences. Indeed, some communities of *"Nagot"* (Republic of Benin) have a Ghanaian origin. This is the case of the inhabitants of Okounfo Gbédé and villages in the district of Kaboua (Republic of Benin) which is originally

Table 2: Frequency of abnormal phenotypes in qualitative hemoglobin abnormalities in children of kindergartens in the city of Parakou in 2013

	Effect if	Percentage
AS	101	46,54
AC	99	45,62
SC	7	3,23
SS	6	2,77
CC	2	0,92
AK probable	2	0,92
Total	**217**	**100,00**



Legend: 1 and 5: AK, 3 and 4: A, 2, 6 and 7: AA

Figure 1: Electrophorogram showing three phenotypes of hemoglobin in children of nursery schools in the city of Parakou in 2013

derived from the father of the children screened in this study.

The *"Lokpa"* followed by *"Adja"* had the relative prevalence of highest qualitative abnormalities in hemoglobin among the ethnic groups studied with respective frequencies of 53.3% and 37.5%. These two ethnic groups are therefore more risk of having qualitative abnormalities in hemoglobin. This suggests the implementation of a socio-anthropological medical study to confirm or refute this finding and to elucidate the associated factors.

Quantitative abnormalities

Of the 690 children included in the study, 1.16% had Hereditary Persistence of Fetal Hemoglobin (HPFH). This rate was lower than that reported in Guinea (1.4%) (Loua, 2011). This was associated with HPFH profiles AA (0.58%), AC (0.15%) and SS (0.43%). Apart from the association to the AC profile described in our study, in Guinea the same associations to other profiles have been reported: AA (0.6%) and SS (0.8%) (Loua, 2011). That fetal hemoglobin is more beneficial in the SS subjects because it is a potent inhibitor of polymerization of HbS (Davies and Gilmore, 2003).

In our study on 469 children with normal electrophoretic pattern AA, 146 (31.13%) had a proportion of hemoglobin A2 with 3.5% greater than the normal (Trent, 2006). Its prevalence in the study population was 21.16%. This finding requires further exploration in order to find their anomaly and appreciate its true scale.

Frequency calculation p, q, r and t of the alleles A, S, C and K

p = (101 x 473+ 1/2 + 1/2 + 1/2 x 99 x 2) / 690 = 0.832 for allele A

q = (1/2 x 101 1/2 x + 6 + 7) / 690 = 0.087 for the S allele

r = (1/2 x 99 1/2 x + 7 + 2) / 690 = 0.080 for allele C

t = (1/2 x 2) / 690 = 0.0015 for allele K

Calculation of the expected theoretical numbers and their different phenotypes

AA = p^2 x 690 = $(0.832)^2$ x 690 = 477, 6

AS = x 690 = 2pq (2 x 0.832 x 0.087) x 690 = 99, 8

SS q = ^{2} x 690 = $(0.087)^2$ x 690 = 5.2

AC = 2pr x 690 = (2 x 0.832 x 0.080) x 690 = 91.8

SC = 2QR x 690 = (2 x 0.087 x 0.080) x 690 = 9.6

CC = r^2 x 690 = $(0.080)^2$ x 690 = 4.4

2pt AK = 690 x = (2 x 0.832 x 0.0015) x 690 = 1.7

The observed prevalence of the electrophoretic profile of the children in our study comply with prevalences according to Hardy- Weinberg. The same conclusion was found in the literature (Simpore *et al.,* 2003).

Quality and validity of the study

This population-based study with a sampling technique used was that of a random cluster probabilistic two-stage recommended by WHO which enables us to say that the work results can be extrapolated to the entire population of children nursery schools of the city of Parakou.

The technique of hemoglobin electrophoresis on agarose gel in alkaline buffer used was not possible to differentiate Hb S, Hb D and Hb C, Hb E. It should be associated with the completion of the electrophoresis at acidic pH which allows this differentiation. Use of the

environment reduces precipitation test enabled to differentiate Hb S and Hb D; in that Hb D presents no precipitation. No test was used to differentiate Hb C and Hb E as Hb E is found in South-east Asia (Singuret and Andreux, 1997) and not in Africa. The results obtained in our work could therefore be valid.

CONCLUSION

After this study, it appears that the hemoglobin abnormalities are a public health problem in Parakou. Indeed, a child each in three kindergartens in the city of Parakou possess qualitative abnormal hemoglobin. More than nine out of ten children with abnormal hemoglobin are carriers. The study found a very rare hemoglobin, hemoglobin K Woolwich, never described in Benin. This has given the magnitude of the observed anomalies, it is necessary to establish or to legislate for mandatory newborn screening for hemoglobinopathies.

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