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Prevalence of the Sickle Cell Trait in Gabon: A nationwide study

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ABSTRACT

Sickle Cell Disease (SCD) is an important cause of death in young children in Africa, which the World Health Organization has declared a public health priority. Although SCD has been studied at the continental scale and at the local scale, a picture of its distribution at the scale of an African country has never been given. The aim of this study is to provide such a picture for the Republic of Gabon, a country where precisely the epidemiology of SCD has been poorly investigated. To this effect, 4250 blood samples from persons older than 15 were collected between June 2005 and September 2008 in 210 randomly selected villages from the nine administrative provinces of Gabon. Two methods were used to screen Sickle Cell Trait (SCT) carriers: isoelectric focusing (IEF) and high-performance liquid chromatography (HPLC). SCT prevalence in Gabon was 21.1% (895/4249). SCT prevalence was significantly larger for the Bantu population (21.7%, $n = 860/3959$) than for the Pygmy population (12.1%, $n = 35/290$), ($p = 0.00013$). In addition, the presence of *Plasmodium* sp. was assessed via thick blood examination. Age was positively associated with SCT prevalence (odds-ratio for an increase of 10 years in age = 1.063, $p = 0.020$). Sex was not associated with SCT prevalence. The study reveals the absence of homozygous sickle-cell patients, and marked differences in SCT prevalence between the Gabonese provinces, and also between population groups (Bantu vs Pygmy). These findings could be used by the public health authorities to allocate medical resources and target prevention campaigns.

1. Introduction

Sickle Cell Disease (SCD) is an autosomal recessive genetic blood disorder that was reported for the first time by Herrick in the United States (Serjeant, 2001). SCD is characterized by the production of an abnormal hemoglobin and by red blood cells that display an abnormal sickle shape (Guberti et al., 1984; Jastaniah, 2011; Kumar et al., 2012). SCD is due to a mutation in the beta chain of the hemoglobin protein (HbA) called hemoglobin S (HbS), characterized by an adenine to thymine substitution in the 6th codon (GAG to GTG), resulting in a glutamic acid to valine substitution (Labie and Elion, 2010; Piel et al., 2010). Among the other variants, hemoglobin C (HbC) is common in West Africa and occasionally observed in Central Africa (Modiano et al., 2008).

SCD occurs in persons having two mutated alleles (HbS/HbS). In these persons, abnormal hemoglobin polymerizes into long fibers resulting in the distortion of the cells into a sickle shape. Compared to normal red cells, sickle-shaped cells are rigid and sticky (Serjeant, 2001). As a consequence, they tend to obstruct capillaries and restrict blood flow to organs, resulting in ischemia, pain, necrosis and often organ damage. Life expectancy of the affected persons is drastically shortened. When HbS is inherited from only one parent, the heterozygous (HbA/HbS) child is usually an asymptomatic carrier, although some symptoms may be present depending on the expression level of each allele (Serjeant, 2001).

SCD occurs mainly in people (or their descendants) living in tropical and subtropical areas but is also seen in people from other parts of the world (the Middle East, Central India, and countries bordering the Mediterranean Sea, especially Italy and Greece) where malaria is or was common. The allele frequency is thus

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higher in populations which historically have lived in low-lying and wet areas with a high prevalence of malaria, called the sicklemic belt (Lehman and Huntsman, 1974). It was indeed suggested that the allele responsible for this disorder was maintained at high frequencies within these populations because of a resistance against malaria of the heterozygous carriers (Haldane, 1949; Piel et al., 2010). The number of newborn affected by SCD is estimated to be 300,000 per year in the world (Komba et al., 2010; Makani et al., 2011) with 200,000 in Africa alone (Diallo and Tchernia, 2002). It is a major cause of child ill-health and death in Africa (Kumar et al., 2012). In West Africa, risk factors for death of children born with SCD include infections, low hemoglobin and fetal Hb (HbF), high white blood cell count and hemolysis (Leikin et al., 1989; Makani et al., 2011; Platt et al., 1994). Without treatments, which are rarely available in low-income, high-burden countries (Modell and Darlison, 2008), the vast majority of these children die before the age of five (Weatherall et al., 2006).

Regarding the origin of SCD, it was initially thought that the abnormal form of the beta gene spread all over the world from a single mutation event (Gelpi, 1973). Restriction-fragment length polymorphisms (RFLP) analysis has, however, shown that the HbS allele has actually arisen from mutations occurring several times at the same locus, resulting in different haplotypes (Kulozik et al., 1986; Ware, 2013).

In Africa, where SCD-related mortality and morbidity are severe childhood health problems, epidemiological studies are poorly developed, usually conducted in health centers, and record the prevalence of SCD only in hospitalized patients. Attempts to improve the medical care of patients with SCD in Africa have been scarce, reflecting the lack of concern from the African medical community and, hence, from the politicians (Diallo and Tchernia, 2002). Such a lack of interest may have several grounds: child mortality is currently attributed to factors identified long ago such as malaria, malnutrition, or bacterial and parasitic infections, and the aggravating role of SCD is not identified (Diallo and Tchernia, 2002).

Today, SCD is a major public health problem in the Republic of Gabon with a proportion of heterozygous HbS/HbA, hereafter called Sickle Cell Trait (SCT) carriers, estimated to be between 5% and 40% (Diallo and Tchernia, 2002). The aim of the present study was to accurately determine the frequency of abnormal hemoglobin in the Gabonese population and its distribution across the administrative provinces of Gabon as well as its variation according to age and sex.

2. Methods

2.1. Study area and sampling

Located in Central Africa, Gabon is crossed by the equator and about 80% of its 267,667 km^2 area is covered by dense forest. Gabon population is estimated to be around 1.5 million inhabitants (5.6 inhabitants/km^2), 73% of whom live in urban areas. Gabon is administratively divided into nine provinces with 2048 villages located mainly along roads and rivers. Few villages have more than 300 inhabitants. The main activities are subsistence farming, hunting, gathering and fishing (Njouom et al., 2012).

A total of 4250 blood samples were collected between June 2005 and September 2008 during a project focused on the Ebola virus in Gabon (Becquart et al., 2010). In brief, the investigation covered 210 randomly selected villages from the nine administrative provinces of Gabon (Estuaire, Haut-Ogooué, Moyen-Ogooué, Ngounié, Nyanga, Ogooué-Ivindo, Ogooué-Lolo, Ogooué-Maritime et Woleu-Ntem) with 8–35 villages per province (Fig. 1). In these villages, all healthy volunteers over the age of 15 who had been residing in the village for more than one year were recruited for the study. During enrollments, several informations were collected from each person: age, sex, membership to Pygmy vs Bantu populations.

2.2. Ethics statement

Written consent was secured from all participants. In the case of minors, consent was obtained from at least one parent. Our study received the approval of the Gabonese Ministry of Health, with a research authorization Nb. 00093, March 15, 2005.

2.3. Blood collection and processing

Blood samples were usually collected in the village health care center into 7-ml vacutener tubes containing EDTA (VWR International, France). The tubes were transported daily to the field laboratory for centrifugation (10 min, 2000*g*). Plasma, Buffy coat and red blood cells were stored separately. Samples were preserved at −20 °C until the end of the field mission and then transferred on dry ice at the Centre International de Recherches Médicales de Franceville (CIRMF) and kept at −80 °C until analysis. Red blood cell samples were then processed for screening of abnormal hemoglobin.

The presence of abnormal hemoglobin was ascertained by the isoelectric focusing (IEF) method by which proteins are separated according to their isoelectric points. When an abnormal protein was detected, high-performance liquid chromatography (HPLC) was used to identify the exact variant: hemoglobin A (HbA), hemoglobin S (HbS) or hemoglobin C (HbC) (Ingram, 1956; Siguret and Andreux, 1997), according to the protocol described in Tatu et al. (1997). Thick and thin blood films were stained with 20% Giemsa and examined for malaria parasites by two experienced microscopists. A sample was considered negative if no parasites were seen in 100× magnification oil-immersion fields (Nkoghe et al., 2011).

2.4. Statistical analysis

All statistical analyses were performed with the R software (R core team, 2013). Several explanatory variables were considered: province, population group (Bantu vs Pygmy), age of individuals and gender. Univariate associations were assessed by Chi-squared tests for the qualitative variables, and by logistic regression for age. A logistic model including all the studied covariates was also fitted, but no interaction was put in evidence, and since it gave very similar results than the univariate models, it will not be discussed further.

3. Results and discussion

3.1. Prevalence of SCT in Gabon

Hemoglobin genotypes were obtained for 4250 volunteers. The HbC allele was found in a unique individual who was a migrant from Ghana, and was excluded in all subsequent analyses, leaving 4249 samples (Table 1). This is in agreement with other studies showing that the common allele of abnormal hemoglobin responsible for SCD in central Africa is the Bantu genotype (HbS/HbS) (Kéclard et al., 1996; Ojwang et al., 1987; Oner et al., 1992).

The global SCT prevalence in Gabon was 21.1% (895/4249) in persons older than 15 years of age. This result is consistent with that observed in malaria transmission areas of central Africa where SCT prevalence ranges from 5% to 40% according to Diallo and Tchernia (2002) or from 19% to 25% according to Piel et al. (2010).