The Human Genome and Malaria: Prevalence of Inherited Haemoglobin Disorders in Sokoto, Nigeria

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Abstract: Retrospective analysis of the results of haemoglobin genotyping of 1033 cases was conducted at the Haemoglobin Genotyping Laboratory in Sokoto, Nigeria. Haemoglobin genotype determination was performed by cellulose acetate electrophoresis. The results show that 666 (64.5%) were Hb AA, 241 (23.3%) Hb AS, 117 (11.3%) Hb SS, 5.0 (0.48%) Hb AC 2.0 (0.19%) and Hb SC 2.0 (0.19%). We conclude that the prevalence of genes for these haemoglobin variants could be explained on the basis of selection due to evolutionary pressure imposed by malaria in line with malaria theory and theory of balanced polymorphism. The implication of our findings on health care provision and planning in the region is discussed.

Keywords: Human genome, malaria, prevalence, haemoglobin disorders, Sokoto, Nigeria

INTRODUCTION

Malaria is an infectious disease caused by parasites of the genus *Plasmodium*. Each year an estimated 3 million people die from malaria and 500 million to 5 billion clinical episodes of the disease are recorded worldwide (Brennan et al., 2004; Snow et al., 2005). Sub-Saharan Africa bears the greatest burden with more than 150 million cases and about 1 million deaths annually mostly in children under the age of 5 years (WHO, 2005). Although four species of *Plasmodium* cause the disease, *Plasmodium falciparum* is responsible for the most severe form of the disease and is most extensive in prevalence and distribution (World Health Organization, 2003).

The human race and malaria parasites have had a long evolutionary host-parasite association (Wiesenberg, 1967; Cohuzi, 1999; Joy et al., 2003). Advances in molecular biology and bioinformatics (Hume et al., 2003; Hartl, 2004) in the past decades have provided evidence that malaria has been the strongest force for evolutionary selection in the recent history of the human race (Kwiatkowski, 2005). The presence of geographical pattern in the distribution of the sickle cell gene and its association with malaria has been well documented (Weatherall, 2001; Fauzi et al., 2003; Kwiatkowski, 2005). Different communities and ethnic groups in malaria endemic regions of the world have developed diverse genetic adaptations to malaria. These include diseases of the red blood cell such as sickle cell anaemia, sickle cell trait, thalassemia and glucose 6 phosphate dehydrogenase deficiency (Kwiatkowski, 2005).

The malaria hypothesis proposes a survival advantage for individuals with haemoglobin variants in areas of endemic *Plasmodium falciparum* malaria (Aarti et al., 2000). Epidemiological and in vitro support for the malaria hypothesis is best documented for the thalassemias and sickle haemoglobin (Hb S) (Fleming et al., 1979) because heterozygotes are protected against the lethal effects of *falciparum* malaria (Angustimotis et al., 1995). Haemoglobin C (Hb C) and haemoglobin E (Hb E) have also been found to be associated with a reduced prevalence of severe *Plasmodium falciparum*
malaria in heterozygotes (Ringelhann et al., 1976). The World Health Organization recently recognized sickle cell disease in Africa as a problem of major public health importance (Makano et al., 2007). It is therefore identified as a priority area for research. Current data on the prevalence of haemoglobin disorders is indispensable for health planning and policy. Against this background we sought to study the distribution of inherited haemoglobin disorders amongst the population in Sokoto, northern Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted at the Haemoglobin Genotype and Malaria Laboratory, Emergency Paediatric Unit, Paediatrics Department, Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto. Sokoto is located on latitude 13° 01'N and longitude 05°15' East. It is also located in the Sudan Savannah agro-ecological zone. The inhabitants are predominantly Hausas and Fulanis with some other ethnic groups like Igbo, Yoruba, Nupe, Ebira etc. They are mainly farmers, civil servants, traders and cattle rearers. There are three major tertiary health institutions in Sokoto; Usmanu Danfodiyo University Teaching Hospital, Specialist Hospital, Sokoto, Maryam Abacha Women and Children’s Hospital, in addition to scores of private hospitals and clinics. Sokoto is endemic for malaria. Transmission of malaria is intense all year round and reaches a peak after rains (September/October). Dry and wet seasons are distinct. Annual rainfall averages 300 mm.

Data Collection

A total of 1,033 cases were retrospectively analyzed from records of haemoglobin genotype test from January 2000- July 2005 at the Haemoglobin Genotyping Laboratory, Department of Paediatrics, Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria.

Cellulose-Acetate Paper Haemoglobin Electrophoresis

The haemoglobin genotyping was performed using cellulose-acetate paper electrophoresis as reported by White and Marsh (1991).

RESULTS

The results of the analysis of the hemoglobin genotyping reports are presented in Table 1. A total of 1,033 requests for haemoglobin genotyping requests were handled by the Laboratory from January 2000-July 2005. Analysis of the results shows that 666 (64.5%) were Hb AA, 241 (23.3%) Hb AS, 117 (11.3%) Hb SS, 5.0 (0.48%) Hb AC 2.0 (0.19%) and Hb SC 2.0 (0.19%).

One hundred and five cases (10.1%) of the individuals of age group 0-11 months (babies/infants) were found to carry a normal haemoglobin genotype (AA) whilst 77 (7.4%) were found to have at least one form of haemoglobin variant. Amongst individuals within the age group 12-23 months (toddlers), 737 (7.0%) were found to carry the normal haemoglobin whilst 410 (4.0%) were found to carry at least one form of haemoglobin variant. Amongst individuals of age group 24-48 months (small children), 126 (11.6%) had normal haemoglobin genotype (AA) whilst 55 (5.3%) were found to carry a haemoglobinopathy. 108 (10.4%) of the juveniles (49-60 months) carry normal haemoglobin and

Table 1: Frequency of haemoglobin variants among study subjects in Sokoto, Nigeria

<table>
<thead>
<tr>
<th>Haemoglobin genotype</th>
<th>Hb AA</th>
<th>Hb AS</th>
<th>Hb SS</th>
<th>Hb AC</th>
<th>Hb SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>666 (64.5)</td>
<td>241 (23.3)</td>
<td>117 (11.3)</td>
<td>2.0 (0.19)</td>
<td>2.0 (0.19)</td>
</tr>
</tbody>
</table>
Table 2: Age specific distribution of haemoglobin disorders in Sokoto, Nigeria

<table>
<thead>
<tr>
<th>Age (Months)</th>
<th>Haemoglobin type, No. of individuals (%)</th>
<th></th>
<th>Variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-11 (Infants/Babies)</td>
<td>105 (10.1)</td>
<td>77 (7.4)</td>
<td></td>
</tr>
<tr>
<td>12-23 (Toddlers)</td>
<td>73 (7.1)</td>
<td>41 (4.0)</td>
<td></td>
</tr>
<tr>
<td>24-48 (Small children)</td>
<td>120 (11.6)</td>
<td>55 (5.3)</td>
<td></td>
</tr>
<tr>
<td>49-60 (Juveniles)</td>
<td>108 (10.4)</td>
<td>66 (6.4)</td>
<td></td>
</tr>
<tr>
<td>&gt;60 (Others)</td>
<td>260 (25.2)</td>
<td>128 (12.3)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>666 (64.5)</td>
<td>367 (35.5)</td>
<td></td>
</tr>
</tbody>
</table>

66 (6.4%) had at least a form of haemoglobin variant. 250 (25.2%) of those above 60 months of age including adolescents and adults) were found to have normal haemoglobin genotype and 128 (12.3%) were found to possess at least one form of the variant (Table 2).

**DISCUSSION**

Analysis of the data collected for this study shows that haemoglobin disorders are common amongst individuals in Sokoto and the finding is therefore of public health significance. The prevalence of the haemoglobin disorders among individuals seems not to differ significantly from that carried out in a Nigerian national survey (extended, involving 16,000 randomly selected) of adults greater than fifteen years of age, covering 30 states, in mid nineties (Angastiniotis et al., 1995) and the sickle cell genotyping in Abia state, eastern Nigeria (Mba and Amadi, 2004). Present study recorded higher prevalence of haemoglobin disorders than the previous survey reported by Angastiniotis et al. (1995).

This was due to difference in the survey design we employed. Present data was obtained from a tertiary level hospital laboratory which also serves as a referral centre and regularly holds a sickle cell clinic. We may therefore have overestimated the actual prevalence. Despite this shortcoming the data may serve as base-line upon which future studies could be anchored. The data is important because it derives from micro mapping of the community. Our study, however, recorded lower frequencies for HbAC and HbSC but recorded the same estimates for HbAS than the previous study which was based on random selection of adults’ population. Our prevalence rate for the carrier status (HbAS) is consistent with a World Health Organization’s Report (WHO, 1989) and is therefore significant.

Each year about 300,000 infants world-wide are born with a major haemoglobinopathy, representing a global birth rate of over 2.0/1000 (Angastiniotis et al., 1995). Almost three-quarters of the affected births are in Africa where up to 2% of all children are born with SCD (Modell and Butzynhenkov, 1988). In Nigeria, over 30 million people carry the sickle cell trait and 2% of infants born each year have sickle cell disorder (Osegbeue, 2006). Among African countries, Nigeria carries the greatest burden of the disorder and therefore occupies a special place and may serve as a good model for haemoglobin disorders for the rest of Africa.

Two major theories have been advanced to explain the origin of the sickle cell gene. According to the single mutation theory the sickle cell gene arose, in a single event, in Equatorial Africa. But the multiple mutation theory, supported by recent development in molecular genetics, argues that the gene originated from both the Arabian Peninsula and Equatorial Africa as a consequence of five independent mutation events 70,000-150,000 years ago. Strong evidence has accumulated to support the thesis that the high frequency of the sickle cell and thalassaemia genes has been maintained by exposure of populations to malaria. People with sickle cell trait are somewhat more resistant to malaria (Aarti et al., 2000). This agrees with the fact that, this region is endemic for malaria. The widely accepted theory is that sickle cell trait offers selective protection against Plasmodium falciparum malaria probably because of induction of sickling even at physiological oxygen tension by P. falciparum followed by sequestration of parasitized red cells deep within reticulo-endothelial system where the microenvironment is hostile for parasite growth (Friedman, 1978; Carlson et al.,
The protection of sickle cell trait against *Plasmodium falciparum* applies almost entirely to infants. Infants with sickle cell trait become infected with *Plasmodium falciparum* but their infections occur less frequent and are milder than those in AA infants (Ringelhann et al., 1976). Thus, people with sickle cell trait would have a better chance of surviving an outbreak of malaria and passing their genes (sickle and normal haemoglobin) to the next generation when they have children. The remarkable stability of sickle gene in Africa which allows it to remain at a relatively constant level in a population without being eliminated is thought to be because of the most widely accepted theory of balanced polymorphism (Allison, 1954; Friedman, 1978). Present findings are supportive of and confirmatory to these theories.

About 1.5% (570,000) of all babies born to indigenous African parents every year would be with sickle cell anaemia. This translates to birth rate of 48/1000 of the total population. This prevalence is high only at birth and progressively decreases through late childhood, adolescence and adulthood. This rate is very high compared to other races. The distribution of indigenous sickle cell disease coincides with the present or past distribution of endemic *Plasmodium falciparum* malaria.

Conversely, classical population genetics has shown that eradication of falciparum malaria by eliminating the reproductive advantage of HbAS carriers predictably leads to gradual dilution of the sickle gene pool within a population. The situation in South Africa provides an interesting example. In South Africa and Southern Mozambique, both of which lie within the temperate non-malarious zone of sub-Saharan Africa, the frequency of the sickle cell gene is so low (0.3%) such that Sickle Cell Anaemia (SCA) is not perceived as a public health problem (Bernstein, 1969). In contrast, the frequency is higher in the Bantu people in northern Mozambique and in countries lying north of the Zambezi river (Altman, 1949; Bernstein, 1969). The 2000 year-old-migration of Bantus from western to malaria-free South Africa is thought to have led to the remarkably low sickle cell gene frequency among the South African Bantus. The same argument is advanced for the lower S gene frequencies in African Americans (AS 8%) and African West Indians (10%) (Luzzato, 1975). Thus the ultimate control of the S gene within a population is linked to the eradication of malaria in that population. Luzzato (1975) estimated that even if malaria were controlled it will still take some 300 years for the gene frequency to decrease to half.

Ironically, individuals with sickle cell disease (Hb SS), already moderately-to-severely anaemic cannot survive a malaria environment. Invasion and destruction of their red blood cells simply push the anaemia to critical levels. They are thus less fitted for survival in the hostile environment.

The red cell in patients with sickle cell disorders has a higher propensity to assume a sickled configuration when blood is oxygenated (Bunn, 1997). Although patients may adapt to their anaemia quite well, their illness is interspersed with acute episodes, or crises, which include episodes of sequestration of blood into the lung, liver or spleen, or the occlusion of cerebral vessels with resulting stroke. Patients with sickle cell disorders are particularly prone to infection in early childhood and at all ages leading to high morbidity (Dover and Platt, 1998). They also require blood transfusions. Since red cells are required, the blood has to be processed and also screened for hepatitis B and C viruses, Human Immunodeficiency Virus (HIV) and in many countries malaria parasites, in addition to measures to prevent iron overload, basic treatment of disorders of haemoglobin is very costly. The cost of diagnostic technology, medical staff, hospital admissions for complications and other items add to the burden of management. Hb SC disease, although milder than sickle cell anaemia, is associated with complications such as aseptic necrosis of femoral or humoral heads, haematuria, proliferative retinopathy and thrombotic tendency which, particularly in pregnancy and the puerperium, may lead to massive pulmonary thrombo-embolic disease and death (Serjeant, 1992).

The public health significance of our study is that it has supplied data that could be used, not only for comparative purposes between regions and countries, but also for planning and as guide for allocation of resources for care of patients with haemoglobin disorders. There have been very few
attempts to quantify the economics of managing the haemoglobin disorders. World Health Organization has made a very approximate estimate (WHO, 1989), but due to variation in costs of health care between countries the data offer only the broadest guidelines. Although it is difficult to estimate the cost of the future management of haemoglobin disorders in Africa, there can be no disagreement that as the number of people with haemoglobinopathy who survives to reproductive age continues to increase more burden is placed on the already over stretched, inefficient and ill-equipped African health system. To mitigate this problem the least that could be done is to make accessible effective treatment of malaria cases and other infectious diseases, provision of adequate and reliable diagnostic facilities, effective community education programmes and provision of safe blood for transfusion.

Studies in economically advanced countries have shown that the prognosis for patients with sickle cell anaemia may be improved considerably by establishing neonatal screening programmes augmented by the early use of prophylactic oral penicillin and the effective management of intercurrent infections (Ballas, 1998). As the world is becoming a global village due to advances in technology, Africa would certainly have to learn and do more for its population living with haemoglobin disorders.

REFERENCES


