Glucose-6-phosphate dehydrogenase deficiency among newborn in Brunei Darussalam

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Abstract

Glucose-6-phosphate dehydrogenase deficiency is the most common enzyme disorder in the world affecting up to 400 million people. This X-linked defect predisposes affected persons to haemolyis caused by oxidative stress. Resistance to malaria is linked with G6PD deficiency. Its incidence in Brunei Darussalam was estimated with data obtained from routine screening of newborn children during the period 2007 to 2009 at the RIPAS hospital using a semi-quantitative fluorescent spot test.

Prevalence of glucose-6-phosphate dehydrogenase deficiency among infants in Brunei Darussalam over the three years was 3%. The prevalence among male infants (n = 5245) was 5% (95% CI: 4%, 5%) with 253 detected cases. The prevalence among females (n = 5338) was 1% (95% CI: 1%, 2%) with 69 detected cases. There was no association between Chinese or Malay ethnicity and the deficiency of glucose-6-phosphate dehydrogenase (P=0.775). These data are comparable with results from Malaysia which has a population with a similar ethnic composition. Comparison with data from Malaysia also suggests that a photometric assay-based quantitation of enzyme activity detects milder forms of G6PD deficiency that are missed by the fluorescent spot test.

Key Words: *Brunei, glucose-6-phosphate dehydrogenase deficiency, newborn*

Introduction

Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency is the most common inherited human enzyme deficiency in the world with as many as 400 million people carrying the defective gene. The defect is most prevalent in Africa (affecting up to 20% of the population), but is common also in the Mediterranean countries (4% - 30%) and Southeast Asia (0.5- 26%) [1-4]. It is an inherited disorder in which the G6PD gene on the X-chromosome is defective. G6PD is the first enzyme in pentose phosphate pathway that provides energy to the cells in the body, including erythrocytes, as well as maintaining the level of reduced nicotine adenine nucleotide phosphate (NADPH). NADPH detoxifies hydrogen peroxide through the glutathione pathway, thus neutralizing the oxidative stress to the cell. In patients with G6PD deficiency, this mechanism is impaired, making the cells more susceptible to oxidative damage. Red blood cells are prone to damage because of continuous change between deoxygenated and oxygenated states, which generates small amounts of superoxide anions. Exposure of blood to exogenous oxidizing agents, e.g. drugs such as primaquine and foods such as fava beans, and infections, can lead to haemolytic anaemia. Other clinical manifestations associated with G6PD deficiency are neonatal jaundice and sometimes chronic non-spherocytic hemolytic anemia. Management of G6PD deficiency involves early detection through screening of newborn and the avoidance of oxidative stress in deficient persons [1-4].

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The World Health Organization has classified the different G6PD variants according to the magnitude of the enzyme deficiency and the severity of hemolysis. Classes IV and V are of no clinical significance. Class I patients are rare, have severe enzyme deficiency (less than 10% of normal enzyme activity) and have chronic hemolytic anemia. Class II patients have severe enzyme deficiency but there is usually only intermittent hemolysis. Class III patients have moderate enzyme deficiency (10 to 60% of normal) with intermittent hemolysis that is usually associated with infection or drugs. Class IV patients have no enzyme deficiency or hemolysis and Class V patients have increased enzyme activity [4].

X-Chromosome inactivation and G6PD deficiency

G6PD deficiency is an X-linked disorder involving a gene located in the terminal region of the long arm of the X chromosome (Xq28). Red cells in heterozygous females with G6PD deficiency were found to be mosaic within two populations: one with normal G6PD levels and the other with deficient G6PD levels. Random inactivation of one of the X chromosomes in haemopoietic stem cells due to Lyonisation is the cause of the mosaicism. Carrier females with only 50% G6PD activity are also prone to haemolysis [1-4].

G6PD deficiency and protection against malaria

Despite the negative consequences of G6PD deficiency, defective genes have persisted in many parts of the world [1-4]. Different mutations giving rise to inactive or less active enzyme are found in different regions that are affected or have been affected in the recent past by malaria, suggesting strong evolutionary selection [1, 3]. The role of G6PD deficiency in protecting against *Plasmodium falciparum* was elegantly demonstrated by the preferential growth of the parasite in normal red cells, and not G6PD deficient red cells, in heterozygous female blood [5]. However field studies in Africa suggest that protection against *P. falciparum* was significant only in hemizygous males and not heterozygous females [6].

Both *P. falciparum* and *Plasmodium vivax* are common parasites in Southeast Asia. Investigations on the relationship between the Mahidol mutation, a G6PD deficiency gene with an allele frequency of 12% in Thailand, and the *Plasmodium* parasite density in Thailand showed that it was significantly associated with reduced *P. vivax* but not *P. falciparum* density in blood [7]. It was hypothesized that *P. vivax* preferentially infects reticulocytes which have a high level of glutathione, an antioxidant tripeptide, to protect it from oxidative stress. On the other hand, *P falciparum* has no preference to reticulocytes, indicating that it is less sensitive to oxidative stress. Therefore, a person with reduced G6PD activity would be prone to oxidative stress and this would have a greater effect on *P. vivax* than *P. falciparum* [7]. It is also reported that G6PD deficient red cells parasitized by *P. falciparum* are more readily phagocytosed to provide a degree of protection against malaria [8]. The relative protection provided by G6PD deficiency against *P. falciparum* and *P. vivax,* and indeed the other species of human malaria parasites, is therefore not well established and is confounded by the fact that the prevalence of the two major parasite species has changed over time in different parts of the world.

G6PD deficiency assessment in Brunei

All newborn children are screened for G6PD deficiency to allow early detection and medical intervention. However there is little published data on the prevalence and incidence of G6PD deficiency in Brunei. Ethnic differences are possible because of differential exposure to malaria parasites among the distinct founding communities over the centuries and therefore a differential selection pressure. In addition, the traditional food preferences are not the same among the different communities in Brunei Darussalam. The screening method used in Brunei is a semi-quantitative fluorescence spot assay for G6PD activity based on the formation of NADPH after the addition of G6P and NADH to blood spotted on filter paper, followed by illumination with 340nm UV light. The fluorescence is determined visually with samples showing NADPH fluorescence termed normal and those not showing fluorescence termed G6PD deficient. In principle, this method, which was described initially by Beutler and Mitchel [9], can have a sensitivity of 100% and specificity of 99% in homozygous females and hemizygote males [10]. In heterozygote females however the sensitivity and specificity are reported to be 32% and 99% respectively [10].

The incidence of G6PD deficiency among newborn in Brunei Darussalam estimated with data in the period 2007 to 2009 is presented in this report.

Materials and Methods

This was a retrospective study on all babies born in RIPAS, among whom were babies confirmed to have G6PD deficiency through the routine neonatal screening tests for the period 2007 to 2009. The tests were carried out by the clinical chemistry laboratory at RIPAS. The data collected from birth records included the gender and ethnicity. Details of every newborn baby diagnosed with G6PD deficiency were recorded together with the total newborn population into a database. A list of code numbers, rather than names, was created to safeguard privacy. This study had the approval of the Ministry of Health Ethics Committee.

Statistical analysis

Data was entered and analyzed using the SPSS version 16.0 statistical software.

Laboratory test for G6PD deficiency

All samples were routinely tested by RIPAS hospital staff using a semi-quantitative fluorescent spot method for detection of deficient types using a kit made by Randox Laboratories (Antrim, UK). This method is recommended by the International Committee for Standardization in Hematology as a simple method of screening for G6PD deficiency and is stated by the manufacturer to have high sensitivity in high to moderate G6PD deficiency but low sensitivity in mild G6PD deficiency.

Results

Table 1. Percentage prevalence of G6PD deficiency amongst neonates in the period 2007-2009

Year	Variable		Prevalence among males (95% CI)	Prevalence among females (95% CI)	Total prevalence (95% CI)
2007	Ethnicity				
		Malay	5 (3, 6)	1 (0, 2)	3 (2, 4)
		Chinese	6 (0, 13)	2 (- 2, 5)	3 (0, 6)
		Others	9 (2, 16)	4 (0, 8)	5 (2, 8)
2008	Ethnicity				
		Malay	4 (3, 5)	1 (1, 2)	3 (2, 3)
		Chinese	1 (- 1, 3)	1 (- 1, 4)	2 (0, 4)
		Others	9 (4, 13)	2 (0, 4)	4 (2, 6)
2009	Ethnicity				
		Malay	6 (5, 7)	1 (1, 2)	3 (3, 4)
		Chinese	2 (0, 6)	3 (0, 7)	3 (1, 6)
		Others	3 (0, 5)	1 (0, 2)	1 (0, 3)

G6PD deficiency and other categorical variables

Categorical variables were analysized using Chi-square to determine any association with G6PD deficiency (Table 2)

Table 2. Factors associated with G6PD deficiency in the period 2007 to 2009

Variable	***n***	**G6PD deficiency *n* (%)**	**Normal *n* (%)**	**χ^2 statistic (*df*)**	***P* value**
Gender					
Male	5245	253 (4.8)	4992 (95.2)	111.8 (1)	<0.001
Female	5338	69 (1.3)	5269 (98.7)		
Ethnicity					
Malay	11178	332 (3.0)	10846 (97.0)		
Chinese	561	15 (2.7)	546 (97.3)	0.561 (2)	0.775
Others	870	29 (3.3)	841 (96.7)		

**Missing for gender = 2026; n = numbers ;*

The detected incidence among males was significantly higher than in females (P<0.001) as would be expected of an X-linked recessive gene. However, the incidence for Malay, Chinese and Others (others were Indians, Caucasians, and indigenous Bruneians) were not significant different (P = 0.775). Malays, Chinese and Others account for 73.8%, 14.8% and 1.4% of the population of Brunei respectively [11].

Discussion

Sensitivity of the screening test

The incidence of G6PD deficiency among newborns in RIPAS hospital may be taken as an approximate indication of its prevalence among the population of Brunei - Muara district, which is the major catchment area for births at RIPAS hospital. Brunei – Muara district is the most populous in Brunei, with approximately 66% of the total population of the country [11] and therefore the prevalence may also be taken to be approximately indicative of the whole country. The results show that the Randox test is detecting a proportion of females heterozygous for defective G6PD genes. The prevalence of G6PD deficiency reported here for Brunei is similar to the 3.3% detected in Malaysia using the same method [12]. However, the prevalence of G6PD deficient mutants in Myanmar (0-10.8% in different ethnic groups), Laos (7.2%) Indonesia (6%) was higher when direct DNA-based analysis for mutants was carried out [13].

A comparison was made in Malaysia between the efficiency between the Randox semi-quantitative fluorescent spot test and quantitative enzyme assay for detecting G6PD deficiency [12]. The semi-quantitative fluorescent spot test is based on the fluorescent appearance of reduced pyridine nucleotide (NADPH) when activated by UV light. Appearance of fluorescence indicates sample is normal whereas if fluorescent is not seen, the sample is said to be G6PD deficient. Enzyme assay is a quantitative test involves lysis of red cells followed by incubation of the lysates with substrate and the cofactor NADP, and subsequent photometric measurement of NADPH formation at 340 nm [12]. The prevalence of G6PD deficiency with the semi-quantitative fluorescent test and enzyme assay were found to be 3.3% and 7.2%, respectively [12]. Therefore the semi-quantitative Randox test failed to detect 3.9% of neonates with G6PD deficiency in that study. This may also be the case in Brunei Darussalam. Use of the more sensitive photometric enzyme assay test will help detect the milder form of G6PD deficiency, be helpful in management of potential neonatal jaundice, and identifying all of the deficient individuals for studies on types and prevalence of mutations at the DNA level. Furthermore when a G6PD deficiency based on enzyme assay was used to test protection against clinically uncomplicated malaria caused by *P. falciparum,* African females were found to be significantly protected [14] in contrast to genetic typing of females carrying mutant G6PD [6]. This further highlights the importance of using the appropriate assay for G6PD deficiency in epidemiological studies.

G6PD variants in Southeast Asia

There are numerous types of G6PD variants in Southeast Asia that are related to the severity of hyperbilirubinemia as well as the onset of jaundice. G6PD mutations and their G6PD activity were identified through genetic analysis and enzyme assays, respectively. The three most common alleles G6PD Viangchan, Mediterranean and Mahidol were reported to cause at least 80% of G6PD deficiency in Malaysian Malays [15] and G6PD Mahidol and Viangchan are present elsewhere in Southeast Asia [13]. Investigation of these and other alleles in Brunei and elsewhere in Borneo island may yield useful information pertaining, for example, to population migrations, habitat in relation to present and previous malaria exposure, and preferences for traditional medicines and foods.

Competing Interests

The authors declare that they have no competing interests.

Author Contribution

RR conceived the project and wrote the manuscript. PUT supervised clinical aspects of the project and LKCA collected and analysed data.

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