ORIGINAL ARTICLE

GENETIC VARIATION IN THE GLUCOSE-6-PHOSPHATE DEHYDROGENASE GENE IN KELANTAN MALAYS WITH NEONATAL JAUNDICE

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Abstract

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked red blood cell enzymopathy common in malaria endemic areas. Individuals affected by this disease show a wide variety of clinical signs including neonatal jaundice. In this preliminary report we describe the heterogeneity of G6PD deficient gene in neonatal jaundice in the Malay population in Kelantan. Thirteen G6PD deficient Malay neonates with hyperbilirubinemia were subjected to mutation analysis of the G6PD gene for known candidate mutations. Molecular defects were identified in the 13 patients studied. Though all of these were mis-sense mutations, identified nucleotide changes were heterogeneous. Six patients were found to have a C to T nucleotide change at nucleotide 563 of the G6PD gene (C563T), corresponding to G6PD Mediterranean; three cases had a single nucleotide change at T383C (G6PD Vanua Lava), two cases had G487A (G6PD Mahidol) and two cases had G1376T (G6PD Canton). These findings suggest that there are heterogeneous mutations of the G6PD gene associated with neonatal jaundice in the Malay population in Kelantan.

Key words: *Glucose-6-phosphate dehydrogenase deficiency; Kelantan; Malaysia; neonatal hyperbilirubinemia; polymerase chain reaction (PCR)*

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) enzyme catalyses the first step in the hexose monophosphate pathway of glucose metabolism and generates nicotinamide adenine dinucleotide phosphate dehydrogenase (NADPH), which is

required for defence against oxidative stress. The production of NADPH by G6PD enzyme is of particular importance in red blood cells for the generation of reduced glutathione. G6PD deficiency is an X-linked enzymopathy common in malaria endemic regions and is estimated to affect approximately 400 million people worldwide. There is evidence that the high frequencies of deficient alleles have arisen because they confer a selective advantage against malaria infection.[1,2]

The majority of G6PD deficient individuals are asymptomatic, but exposure to fava beans, certain drugs such as the anti-malarial drug primaquine, or infections may trigger a variety of clinical manifestations ranging from mild acute haemolytic anaemia to severe manifestations of haemolytic anaemia. Neonatal jaundice also can be one of the manifestations. In some cases, the neonatal jaundice is severe enough to cause kernicterus resulting in death or permanent neurologic damage. In order to prevent the development of severe neonatal jaundince, for example, neonatal screening of G6PD deficiency has been carried out in Malaysia and Singapore where G6PD deficiency is common.[3]

G6PD deficiency is genetically heretogeneous. Studies on variants at the molecular level during the last ten years have disclosed at least 90 different point mutations and this list keeps on increasing. The mutations are almost exclusively mis-sense mutations, causing single amino acid substitutions. They are spread throughout the coding region of the gene. Different variants are unique to that population in different parts of the world where this disease is common. In Thailand G6PD Mahidol is the most common variant,[4] whereas G6PD Canton is common in Singapore Chinese.[5] Hence, it is necessary to determine molecular background of G6PD deficiency in each population.

A mutation screening method of the G6PD gene called Multiplex PCR using Multiple Tandem Forward Primers (MPTP) and a common reverse primer have recently been described.[6] The significant advantage of MPTP is that all sequence variations in a target region can be localised to a narrow region after two PCR steps. MPTP has been applied to localise mutations of the G6PD gene of G6PD deficiency patients in Singapore and Philippines.[6,7]

Malaysia is one of countries where G6PD deficiency is common. However, no study correlating mutations of the G6PD gene and neonatal jaundice has been done on Kelantan Malay population. G6PD deficiency is a common cause of neonatal jaundice in Kelantan where the birth incidence rate is about 2.5%. Kelantan has the south of Thailand as its northern border. The majority of population here are Malays and Chinese and Indians are minorities. In this study we analysed mutations of the G6PD gene in G6PD deficient cases complicating neonatal jaundice. Our objective was to study the molecular variants of G6PD gene known to be associated with neonatal jaundice in the Kelantan Malays.

Materials and methods

Patient selection – Blood samples from Malay neonates diagnosed as neonatal hyper-bilirubinemia (defined as serum bilirubin level of 15 mg/dl or more) at the Hospital Universiti Sains Malaysia and the Kota Bharu Hospital, Kelantan, were collected and screened for G6PD enzyme deficiency using ultraviolet fluorescent spot test. For this test, blood samples were blotted on to filter paper and repeated twice using the standard recommended method. Thirteen Malay babies with neonatal hyper-bilirubinemia with G6PD deficiency were identified.

Biochemical analysis – Five to ten mililitres of venous blood was collected in bottles containing heparin. Part of this blood was taken for biochemical assay which was performed using the G6PD activity determination kit (Sigma-Aldrich Corporation, St. Louis, MO, U.S.A.). No neonate was diagnosed with ABO incom-

patibility. Four patients were subjected to phototherapy. In one patient, blood exchange transfusion was recommended but refused by the parents. Fortunately no neurological sequelae developed in this case.

DNA analysis – Part of the blood sample that was collected in heparin bottles above was further subjected to DNA extraction using standard phenol chloroform method. In order to screen mutations of the G6PD gene, Multiplex Polymerase Chain Reaction using Multiple Tandem Forward Primers (MPTP) was employed.[6] Amplification of the G6PD exons and DNA sequencing were performed as reported elsewhere.[6,7] All the deficient samples were first tested for the polymorphic variants in exons 2, 5, 6, 10, 11 and 12 of the G6PD gene. In cases noted to have a mutation of the G6PD gene by MPTP scanning, mutations were confirmed by sequencing of amplified product from the G6PD gene using mutation specific primers for the known mutations as described above.[6,7]

Results and discussion

Biochemical assay – Of the samples analysed the mean level of G6PD enzyme activity was 0.41 + 0.5 U/gHb. This accounted for 3.5% of residual activity of normal. Thus most patients were in class II of the Beutler's classification of G6PD variants.

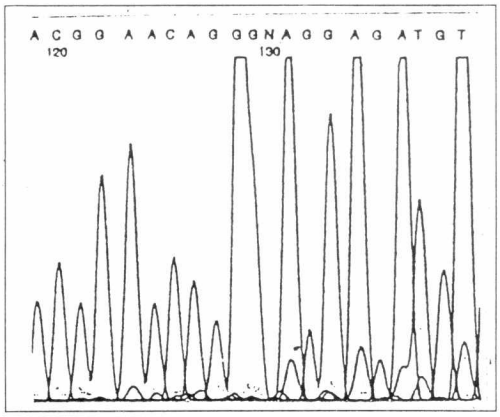
Testing for known polymorphic variants. DNA samples of 13 Malay neonates diagnosed as G6PD deficiency were subjected to mutation analysis of the G6PD gene as described. From the results, 13 cases were identified to have a mutation of G6PD gene (Table I). In six cases, a C to T nucleotide change at nucleotide 563 (C563T) was identified (Fig. 1). This mutation has been shown to result in amino aid substitution of Ser to Phe at 188th codon and this mutation has been described as G6PD Mediterranean.[8] Three cases had a T to C nucleotide change at 383nt (T383C) and this mutation corresponded to G6PD Vanua Lava. Two had a G to A nucleotide change at 487 (G487A), corresponding to G6PD Mahidol.

Table I. Summary of mutations identified in male Malay G6PD deficiency neonates complicating jaundice in Kelantan

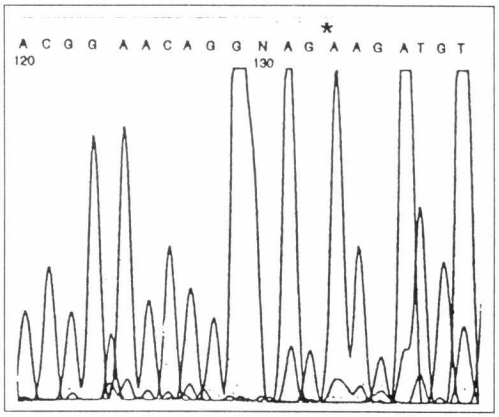
No.	Mutation	G6PD Variant	C133T*	Phototherapy
1	C563T	G6PD Mediterranean	Present	Not done
2	C563T	G6PD Mediterranean	Not present	Not done
3	C563T	G6PD Mediterranean	Not present	Not done
4	C563T	G6PD Mediterranean	Not present	Not done
5	C563T	G6PD Mediterranean	Not present	Not done
6	C563T	G6PD Mediterranean	Not present	Not done
7	T383C	G6PD Vanua Lava	Not present	Done
8.	T383C	G6PD Vanua Lava	Not present	Done
9	T383C	G6PD Vanua Lava	Not present	Not done
10	G487A	G6PD Mahidol	Present	Done
11	G487A	G6PD Mahidol	Not present	Not done
12	G1376T	G6PD Canton	Not present	Done
13	G1376T	G6PD Canton	Not present	Not done

* C133T – Silent mutation associated with the variant identified

**Fig. 1. Partial nucleotide sequences of the normal and mutated G6PD cDNA.
The sequence of mutated cDNA from the patient is compared with that of control.
The single base substitution of C->T at position 563, G6PD Mediterranean is indicated by an asterisk.**



Normal



Patient

Another two cases had a G to T nucleotide change at 1376nt (G1376T), corresponding to G6PD Canton.

The silent mutation causing a single base substitution from C to T at nucleotide 1331 (C133T) was also studied in these patients and out of these, two cases had a single base substitution from C to T at nucleotide 1331 (C133T), but this mutation is known not to affect the codon. This silent mutation (C133T) was found together with mutations of the gene in G6PD Mediterranean and G6PD Mahidol (Table I). Therefore, C133T did not correlate with mutations of the G6PD gene as previously thought.

This is the first study to show that G6PD Mediterranean and G6PD Vanua Lava are the two most prevalent G6PD mutations in the Malays. G6PD Mediterranean was first described in the people of Mediterranean countries[8] but since then it has been described in several parts of the world, like Brazil.[9] Since G6PD Mediterranean has never been reported as a major mutant in Southeast Asian countries, our findings suggests that the Malays in Kelantan have a different genetic profile when compared to the Malays in Indonesia.[10] G6PD Mahidol was also identified in two cases (Table I). Since G6PD Mahidol is a predominant variant in Thailand,[4] this could be due to migration of people due to the close proximity of Kelantan to Thailand. As for G6PD Canton, this variant has been described in the Chinese[11,12] and perhaps it might be related also to the migration pattern.

In this report, 13 cases were identified to have G6PD mutations. In future we would like to study more cases in order to characterise their mutations and also in cases who had no identifiable mutation.

A striking feature of neonatal jaundice in association with G6PD deficiency is the wide variation in its frequency and severity in different populations. Even though these cases showed neonatal jaundice, severity of jaundice was variable. The cause of this variability is incompletely understood. Four of our reported cases were managed by phototherapy and they were identified to have the following mutations of the G6PD gene: two G6PD Vanua Lava, one G6PD Mahidol and one G6PD Canton (Table I). G6PD Vanua Lava was first described in the Southwestern Pacific Vanuatu Archipelago and Papua New Guinea.[13] In view of the fact that

Melanesians might have migrated to Vanuatu from South East Asia via Papua New Guinea[14] it is interesting to note that we have found G6PD Vanua Lava in our samples and this needs to be looked into further. G6PD Canton could have been introduced from China; in the Chinese population in Taiwan the frequency of this mutation is 50%.[15] Of interest are those cases with G6PD Mediterranean mutation, none of whom required any treatment to reduce the serum bilirubin level. Another possible cause of neonatal jaundice is superimposed ABO incompatibility between mother and infant, but in our study no ABO incompatibility was present. Hence it is difficult to predict the development of jaundice by only associating with the gene mutation. We are currently increasing the number of G6PD deficiency cases studied to correlate the molecular epidemiology of neonatal jaundice in Kelantan Malays.

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