Identification of point mutations in Glucose-6-Phosphate Dehydrogenase gene in Timor Island people: A preliminary report

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Abstract

Glucose 6-phosphate dehydrogenase (G6PD) deficiency is common in malaria endemic region, however no molecular study has been performed on G6PD deficiency in Timor Island, Indonesia, a malarial hyperendemic area which Proto Malay is the majority of the people in that island. To observe the frequency and molecular type of mutations in G6PD deficient Proto Malay people, 118 native people were screened using formazan ring test. Mutation in the G6PD gene were determined by MPTP (Multiple PCR using Multiple Tandem Forward primers and a common reserve Primer) and confirmed dilakukan dengan automatic sequencer. This study shows that three males have lower G6PD activity. Using MPTP method, a point mutation could be indicated in the two cases. Sequencing of the amplified products in 2 G6PD patients disclosed mutations of T383C in exon 5 and C592T in exon 6 in respective case. Our results documents point mutations in exon 5 and exon 6 in the G6PD gene of two Proto Malay people in Timor. These mutations are common in Asia region. (Med J Indones 2001; 10: 210-3)

Keywords: mutations, G6PD, Proto Malay.
G6PD deficiency, especially developing countries. One hundred eighteen native Proto Malay people were screened using formazan ring test. Two unrelated patients of G6PD deficiency were studied for its nucleotide change in exons 5 and 6 of the G6PD gene by MPTP. Here, we demonstrate two mutations T383C and C592T of the G6PD gene in Proto Malay Indonesians.

METHODS

Blood samples from 118 independent Proto Malay were randomly collected on filter in July 1998 in sub district Insana, district Kefamenanu, Timor Island, Province of Nusa Tenggara Timur.

G6PD deficiency was screened by using the Formazan ring method. Mutation scanning was performed in the G6PD gene using MPTP developed by Shirakawa et al. MPTP was carried out by the hot start PCR method. Ten microlitres of solution containing 8 μl of master mixture (10 mM Tris-HCl, pH 8.3; 10 mM KCl; 250 umM dNTPs; 2.5 mM MgCl2 dan 5% DMSO), 1 ul of the primer mixture, 1 μl of the enzyme mixture (0.9 μl of distilled water and 0.1 μl of 5 U Ampli taq DNA Polymerase Stoffel Fragment Perkin Elmer Co, USA) were preheated to 85°C. PCR was then carried out with the following cycles: 25 cycles at 95°C and 72°C and O°C for 30 second each. The products were run in 4% agarose gel electrophoresis (MetaPhor/ Seaken HGT 3:1) containing 0,5 μg/ml ethidium bromide and the gels were photographed using a UV transiluminator. All exons of the G6PD gene were then amplified from DNA samples and was subjected to direct sequencing using a dye terminator kit (Perkin Elmer Co), an automatic DNA sequencer (AB PRISM 310), Genetic Analyser (Perkin-Elmer Co).

RESULT

G6PD deficiency is common in malaria endemic regions and is estimated to affect upwards of 400 million people world-wide. To date, more than 300 different G6PD variants have been distinguished on the basis of biochemical parameters, suggesting a vast genetic heterogeneity. So far, however, only a small portion of this heterogeneity has been confirmed at the DNA level through the identification of about 60 different mutations in the G6PD coding sequence. A mutant G6PD enzyme may be different from person to person, mutation can be in the form of point mutation or can range from one to several base pair deletions as well as replacements in the DNA. Different populations have different types of mutations, but within a specific population, common mutations are usually shared. In Southeast Asia mutation in exon 5, 6 and 9 are common.

Two patients with G6PD deficiency and one patient with low enzyme activity were detected from 118 Proto Malay individuals by the formazan ring test. We examined mutations in exon 5 and exon 6 of the G6PD gene in two patients. One patient was found to have a T > C substitution at nucleotide position 383 in exon 5 (Fig 1). This mutation corresponds to the variant G6PD Banks and Torres (Vanua lava). The other patient was found to have a C > T substitution in exon 6 at position 592 and corresponds to the variant Coimbra (Fig 2) respectively. These substitutions were confirmed by the nucleotide sequencing procedure. However, we could not identify any mutation in the patient with low enzyme activity.

DISCUSSION

Our result indicated that mutation screening in the limited number of exons of the G6PD gene has succeeded to identify mutations in the Proto Malay...
Indonesians, even though Proto Malay has been considered as an isolated population in the NTT region. This suggested that Proto Malay is not isolated but mixed up with other Indonesian, since both G6PD Banks and Torres (Vanua Lava) and G6PD Coimbra have been identified in other parts of Indonesia. However, it needs further study to get a conclusion. G6PD Coimbra is included in the “Mediterranean Type” which are characterized by decreased Michaelis constant (Km) for both G6P and NADP and low substrate specificity.10

Our result showed that MPTP scanning mutation in exons 5 and 6 are useful to characterized molecular background in Proto Malay G6PD deficiency. The usefulness has been confirmed not only in Singapore but also in Philippines.11,12 Since MPTP needs only PCR machine, this technique could be employed in South East Asia countries to characterized mutations. Another method using SSCP (Single-Strand Conformation and Sequence Analysis) had been used to identify genetic heterogeneity in Calabria.13

Among 118 Proto Malay Indonesians in Timor Island, two males were found to have a point mutation. One of them has a point mutation in exon 5 corresponding to the variant G6PD Banks and Torres and the other in exon 6 corresponding to the variant Coimbra.

Further studies are planned in the same region this year. A bigger population would be investigated, which also includes malaria cases. The need for utilizing screening measures for early detection of the genetic defect is immediately apparent, once consideration is taken of the high probability of association of G6PD deficiency with other hemoglobinopathies, such as sickle cell anaemia, which may lead to a more serious condition. In addition, there is a compelling need for introducing measures such as genetic counselling and public health education as part of the overall health and welfare services in the area.

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Figure 1.

Sequencing of one patient with G6PD deficiency. He was found to have a $T > C$ substitution at nucleotide position 383 in exon 5. This mutation corresponds to the variant G6PD Banks and Torres (Vanua lava).
Mutations in G6PD gene in Timor island people

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Figure 2.

Sequencing of one patient with G6PD deficiency. He was found with a C > T substitution in exon 6 at position 592 and corresponds to the variant Coimbra.

REFERENCES