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Detection of glucose-6-phosphate dehydrogenase G6PD enzyme deficiency in the field for treatment of malaria

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Malaria is the first priority health problem in Myanmar and early diagnosis with prompt and effective treatment is essential for reduction of morbidity and mortality due to the disease. Primaquine is the only effective drug to prevent relapses of liver form of *Plasmodium vivax* and *Plasmodium ovale* and can also be used to kill gametocyte form of *Plasmodium falciparum* and *Plasmodium malariae.* Primaquine can cause haemolysis in glucose-6-phosphate dehydrogenase G6PD deficient individual and prevalence of G6PD deficiency varies among different ethnic races. Malaria survey was done during 2001-2003 in Mon and Shan States and prevalence of G6PD deficiency was investigated by rapid screening method of Hirono using DEAE (Di-Ethyl Amine-Ethylene) and Sephadex mixture. In normal person the test shows orange ring due to the presence of G6PD enzyme which is absent in G6PD deficient person. Among 1079 samples tested, 47 (4.5%) was found to have severe type of G6PD deficiency by the test. In relation to the ethnic region, G6PD deficiency rate was 5.5% (29/338) among Burmese, 3.2% (6/191) among Chinese, 3.4% (5/146) among Indians, 3.3% (3/92) among Mons, 5.1% (3/59) among Shans and 6.7% (1/15) among Kayin races. This rapid test can detect severe G6PD deficiency in the field, thus primaquine can be prescribed safely to malaria patients.

INTRODUCTION

Malaria ranks as the top priority health problem in Myanmar and nearly 600,000 outpatients and 120,000 in-patients are recorded annually in public health facilities. Early diagnosis and prompt, effective treatment is essential for reduction of morbidity and mortality due to the disease. Among currently using various antimalarials, primaquine is the only effective drug to prevent relapses of the liver forms of *Plasmodium vivax* and *Plasmodium ovale* and can also be used to kill gametocytes of *Plasmodium falciparum* and *Plasmodium malariae*. However, primaquine can cause severe haemolysis in persons with glucose-6-phosphate dehydrogenase (G6PD)enzyme deficiency, whose prevalence varies among different races in malaria endemic countries. Routinely used methods for screening of G6PD deficiency [1, 2, 3] are costly or time-consuming, thus inconvenient for use in the field. *Hirano et al.* [4] have developed a rapid, single-step screening method for G6PD testing which requires only 5µl of blood, without expensive equipment and gives a test result within 40 minutes. This method was used to detect G6PD deficiency status among different races in Shan and Mon States together with malaria field surveys during 2001 to 2003. Some G6PD deficient blood samples were collected to identify the genetic pattern of deficiency to compare with those of other countries.

MATERIALS AND METHODS

Study area

Malaria endemic villages of Phar Auk and Kyaik Pun of Mudon Township, Mon State and Ho Peik and Mae Han of Lashio Township, Northern Shan State.

Study period

2001-2003

Method

Reaction mixture was prepared in a 1.5ml microcentrifuge tube as reported by Hirano *et al.* [4]. It contained 200µl each of DEAE (Di Ethyl Amine Ethylene) and Sephadex A 50Gel (Sigma Co. USA) equilibrated with 0.1Tris-HCl buffer pH 6.4 with 10mol. MgCl substrate mixture of 5 nM glucose-6-phosphateG6P), Boehringer, Germany, 0.4nM NADP (Nicotinamide Adenine Dinucleotide Phosphate) and 0.2% saponine.

Blood was collected from finger tip of malaria patients through a sterile single prick and 5µl of blood was mixed with 200µl of the above mixture in a microcentrifuge tube. The tube was kept in a dark plastic bag for 10 minutes and reading was done for a development of blue to purple ring (The ring was absent in G6PD deficient blood) (Fig 1 & 2). Heparinized blood of healthy volunteer with normal G PD activity and 0.9% saline were prepared for positive and negative controls respectively.

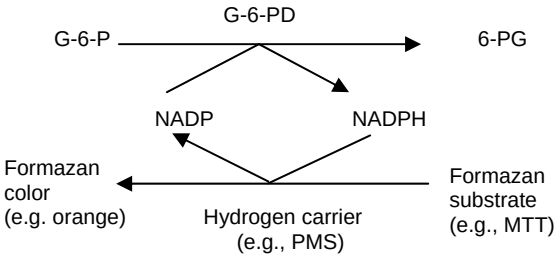

Fig.1. Mechanism of orange ring formation in normal person having glucose-6-phosphate enzyme in the blood

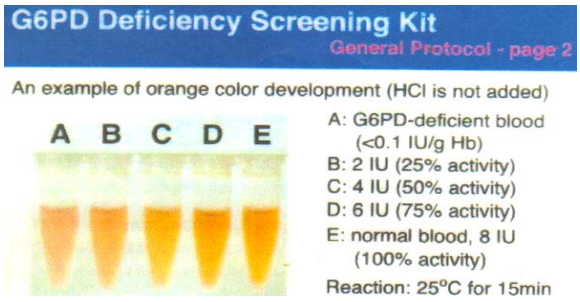

Fig. 2. Appearance of different intensities of orange colour depending on the level of G6PD enzyme in the blood

Inclusion criteria

Clinically suspected malaria patients of all ages and both sexes were included in the study for malaria microscopy and rapid G6PD testing.

RESULTS

The study showed that among 1079 samples tested for G6PD deficiency in Myanmar, 47 samples (4.4%) were found to have severe deficiency. Regarding to the respective races, Bamar (5.3%), Shan (5.1%) and Kayin (6.7%) were above the national value of 4.4%, while Chinese (3.2%), Indian (3.2%) and Mon (3.3%) were below the national value. It showed that severe G6PD deficiency varies widely among the different races of Myanmar (Table 1).

Table1. Prevalence of severe G6PD deficiency (%) among different races

SN	Races	Number of blood sample tested	Severe G6PD deficient samples	G6PD deficiency status (%)
1	Bamar	550	29	5.3
2	Chinese	191	6	3.2
3	Indian	146	5	3.2
4	Mon	39	3	3.3
5	Shan	59	3	5.1
6	Kayin	15	1	6.7
7	Others	26	0	-
	Total	1079	47	4.4

DISCUSSION

Glucose - 6 - phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy, affecting over 400 million people around the world. Vulliamy *et al.* [5] with the highest prevalence in the tropical Africa, the Middle East, tropical and subtropical Asia, parts of the Mediterranean and in Papua New Guinea. It was estimated as G6PD deficiency rate of 0.1% in Japan and Northern Europe, 25-30% in Africa and Asia [6]. In Myanmar, it was reported as 4-14% among various ethnic groups [7] and 15-17% among populations residing in the malaria endemic areas [8].

The gene encoding the G6PD enzyme displays X linked inheritance, implying that hemizygous males and homozygous females are the most likely to show clinical manifestations of the disease. G6PD deficient persons are prone to acute haemolytic anaemia, usually triggered by exposure to a variety of oxidants including antimalarials such as primaquine or quinine (or) by certain foods (fava beans) or most commonly by infections.

Primaquine is currently the single available drug, effective for the elimination of liver hypnozoites of *Plasmodium vivax* and *P. ovale* and also for killing of gametocyte forms of *Plasmodium falciparum* and *P. malariae.* Administration of primaquine to severe G6PD deficient person can give rise to acute severe haemolysis which is undesirable for malaria patients. Having G6PD deficiency status of malaria patient in the field is quite essential for radical treatment and successive implementation of malaria control strategies.

Routinely used G6PD tests are quite expensive and inconvenient to be used in the field. Rapid screening for G6PD test, as introduced by Hirono *et al.* [4] is quite suitable for malaria endemic countries especially in the field, where other G6PD tests are not suitable.

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