MALARIA AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN NORTH SUMATRA, INDONESIA

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INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a hereditary abnormality in which the activity or stability of the enzyme G6PD is markedly diminished. Erythrocytes are most severely affected, and G6PD deficiency may result in haemolytic anemia after the administration of some kinds of drug: e.g. 8- aminoquinoline antimalarials (primaquine etc.). It is now known that G6PD deficiency is a sex-linked trait with a high degree of clinical, biological and genetic variations.The prevalence in numerous racial groups have been studied (WHO, 1969; Lie-Injo, 1969). In Indonesia there are some reports on the prevalence of G6PD deficiency (Lie-Injo and Poey-Oey, 1964; Mutoh and Ebisawa, 1974).

On the other hand, G6PD deficiency is thought to have conferred a selective advantage against infection of *Plasmodium falciparum* malaria because of the fact of high prevalence among population where malaria is endemic (Allison, 1960; Allison and Clyde, 1961). In addition, *P. falciparum* is found predominantly in the G6PD replete erythrocytes of girls heterozygous for the deficiency state (Luzzatto *et al.*, 1969).

Primaquine, which is gametocidal drug of *P. falciparum*, is widely used in malaria control, however, haemolytic crisis should be prevented. Therefore, the diagnosis of malaria and the detection of G6PD deficiency should be done at the same time in a place where malaria is endemic with a possibility of occurrence of G6PD deficiency and radical treatment is planned to clear gametocytemias. Several methods have been developed to screen the G6PD deficient individuals. However, some of these methods need equipment such as spectrophotometer and others (WHO 1969; Beutler *et al.*, 1979). We carried out the screening of G6PD deficiency by using a simple, low cost method (Fujii *et al.*, 1984) when malariometric survey was conducted in North Sumatra, Indonesia.

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MATERIALS AND METHODS

Among 11 prefectures in North Sumatra, we chose Nias and Asahan prefecture as malaria endemic areas, and Medan city as a non-endemic area. In Nias 281 blood samples were collected from 3 elementary schools; in Asahan 606 samples from 6 elementary schools; and in Medan 260 samples from outpatients of Medan National Health Laboratory. The investigations were carried out in January to June, 1984.

Blood samples were taken by finger prick at elmentary schools. The first drop was discarded, the second was used for thick film to examine malaria parasites, and the third was dropped on cellulose paper for G6PD test.

Thick films were stained with 4% Giemsa solution (pH 7.4) and examined in more than 200 microscopic fields under oil immersion.

The number of parasites per 1,000 white blood cells was counted and total parasite count estimated based on an assumption that white blood cell count was 8,000 cells per μl of blood.

G6PD test was done according to the method of Fujii *et al.* (1984). Before the blood was taken, the cation exchange cellulose paper (P81, Whatman, England) was saturated with 100mM Tris-HCl buffer, pH 6.5 containing 10mM $MgCl_2$, and dried. One drop of blood sample (=20μl) was absorbed onto the treated cellulose paper and dried. Then it was kept at 4°C until tested.

The gel was prepared by dissolving 20mg of glucose-6-phosphate (G6P, Boehringer Mannheim, West Germany), 4mg of nicotinamide adenine dinucleotide phosphate (NADP, Boehringer Mannheim), 4mg of 3 (4,5 dimethylthiazolyl 1-2) 2,5 diphenylterazolium bromide (MTT, Wako Chemical Co., Japan), 4mg of phenazine methosulfate (PMS, Sigma Chemical Co., U.S.A.) and 120 mg of Noble Agar (Difco Laboratories, U.S.A.) in 16 ml of 100mM Tris-HCl buffer, pH 6.5 containing 10mM $MgCl_2$ and 1mM sodium azide. The mixture was kept at 55°C and poured into Petri dish, 88mm in diameter. The gel thickness was 2.6mm. They were protected from the light and kept at 4°C until used. The cellulose paper with the dried blood was punched out in the size of 6mm in diameter and it was pressed onto the gel surface. The gel plates were protected from the light and kept at 37°C for 8 hours. When NADP is reduced to NADPH through the reaction of G6P, MTT is reduced to blue insoluble formazan in the presence of PMS. The blue ring of formazan appears around the circular paper. After 8 hours the diameter of each blue ring was measured. The ring size is directly propotional to the amount of G6PD. The sample whose diameter was less than 8mm was judged to be G6PD deficient. Deficient cases were further examined for anemia by measuring haemoglobin concentration or haematocrit level.

RESULTS

The result of G6PD test in 9 schools and Medan city is shown in Table 1. In 10 areas, the prevalence of G6PD deficiency in male ranged from 0 to 10.0%, the average being 3.9%. In malaria endemic area the prevalence of G6PD deficiency in male was 4.6% (21/458). In a non-endemic area the prevalence was 0.9% (1/110). Though the former figure was higher than the latter, the difference was not statistically significant ($\underline{X}^2 = 2.31$).

One case of G6PD deficient female was found in Perupuk subvillage III, Asahan prefecture. The formazan ring size was 6mm in diameter. However, it could not be decided that she was homozygous or heavy heterozygous.

The prevalence of malaria in the 9 schools are shown in Table 2. Thev ranged from 0 to 19.2% (average 9.0%). No relation was observed between the prevalence of malaria and the prevalence of G6PD deficieny. *Plasmodium vivax* (56%) and *P. falciparum* (48%) were detected in this investigation.

The prevalence of *P. falciparum* in G6PD normal group and in G6PD deficient group was 4.1% and 9.5% respectively (Table 3). The rate in G6PD normal group was lower than that in G6PD deficient group. However there was no statistically significant difference between them ($\underline{X}^2 = 0.41$). The number of parasite was counted in positive slides of *P. falciparum*. In G6PD normal group (18 cases) the parasite counts ranged from class I (less than 100 parasites per μl) to class IV (401-800 per μl). The parasite density index (Bruce-Chwatt, 1958) was 1.8. In G6PD deficient group (2 cases) the parasite counts

Table 1

Pervalence of G6PD deficiency in North Sumatra, Indonesia.

Location	Male		Female	
	No. examined	No. G6PD deficient (%)	No. examined	No. G6PD deficient (%)
Nias prefecture				
Afia	53	2 (3.8)	28	0 (0)
Hilianaa	40	4 (10.0)	42	0 (0)
Boto Hilitano	58	3 (5.2)	60	0 (0)
(sub total)	151	9 (6.0)	130	0 (0)
Asahan prefecture				
Perupuk, sub-village II	141	8 (5.7)	139	0 (0)
Perupuk, sub-village III	33	2 (6.1)	36	1 (2.8)
Perupuk, sub-village V	38	0 (0)	35	0 (0)
Perupuk, sub-village XI	24	1 (4.2)	27	0 (0)
Durian	37	1 (2.7)	36	0 (0)
Guntung, sub-village II	34	0 (0)	26	0 (0)
(sub total)	307	12 (3.9)	299	1 (0.3)
Medan city	110	1 (0.9)	150	0 (0)
Total	568	22 (3.9)	579	1 (0.2)

Table 2

Prevalence of malaria in school children in North Sumatra, Indonesia.

Location	Male				Female			
	No. exam.	No. P.v.	P.f	Positive total (%)	No. exam.	No. P.v	P.f	Positive total (%)
Nias prefecture								
Afia	53	0	1	1 (1.9)	28	0	1	1 (3.6)
Hilianaa	40	0	0	0 (0)	42	0	1	1 (2.4)
Boto Hilitano	58	7	5	12 (20.7)	60	3	1	4 (6.7)
(sub total)	151	7	6	13 (8.6)	130	3	3	6 (4.6)
Asahan prefecture								
Perupuk, sub-vil II	141	7	8*	15 (10.6)	139	10	10*	20 (14.4)
Perupuk, sub-vil III	33	1	1	2 (6.1)	36	0	1	1 (2.8)
Perupuk, sub-vil V	38	4	3*	7 (18.4)	35	3	4	7 (20.0)
Perupuk, sub-vil XI	24	0	0	0 (0)	27	0	0	0 (0)
Durian	37	0	0	0 (0)	36	0	0	0 (0)
Guntung, sub-vil II	34	6	2	8 (23.5)	1	1	0	1 (3.8)
(sub total)	307	18	14	32 (10.4)	299	14	15	29 (9.7)
Total	458	25	20	45 (9.8)	429	17	18	35 (8.2)

* one mix *p.v.* and *p.f.*
p.v. = *Plasmodium vivax*
p.f. = *Plasmodium falciparum*.

Table 3

Prevalence of malaria in G6PD normal and G6PD deficient males.

Location	No. G6PD normal	No. malaria pos. %	P.v.	P.f.	No. G6PD def.	No. malaria pos. %	P.v.	P.f.
Nias	142	12 (8.5)	7	5	9	1 (11.1)	0	1
Asahan	295	30 (10.2)	17	13*	12	2 (16.7)	1	1
Total	437	42 (9.6)	24 (5.5)	18* (3.7)	21	3 (14.3)	1 (4.8)	2 (9.5)

* Two cases (0.4%) mixed *P.v* and *P.f.*

Table 4

Haematocrit or haemoglobin concentration in G6PD deficient males.

Location	Case No.	Age (years)	Haematocrit (%) or Haemoglobin (g/dl)	Parasite
Perupuk,	1	7	39	negative
Sub-village II	2	7	40	negative
	3	9	37	*P. vivax*
	4	13	37	negative
	5	13	37	negative
	6	13	36	*P. falciparum*
	7	14	40	negative
	8	13	41	negative
Durian	9	9	35	negative
Medan city	10	30	16.9 g/dl	----------

Test of anemia in G6PD deficient was not done in Nias and Perupuk sub-village III and XI.

classified as class I. The parasite density index was 1.0.

The examination of haemoglobin concentration or haematocrit levels were performed in 10 out of 21 G6PD deficient cases (Table 4). There was no severe anemic case.

DISCUSSION

In malaria control there is a possibility to administer antimalarials to parasite carriers, especially to gametocyte carriers in order to intercept malaria transmission. Chloroquine for asexual form and primaquine for gametocyte is generally employed for this purpose. Primaquine, however, tends to provoke haemolytic crisis in individuals with G6PD deficiency. We intended, first of all, to know the prevalence of G6PD deficiency in North Sumatra, Indonesia. The overall result was 3.9% (Table 1). These individuals who are judged to be G6PD deficient by Fujii's

method are considered to have less than 50% normal enzyme activity. They belong to class I to class III (Yoshida *et al.*, 1971) which have a risk of drug induced haemolysis. Therefore a careful consideration should be taken before using primaquine. For example, in treatment of malaria of G6PD deficient individuals, primaquine should not be given or it should be administered under careful supervision with close attention to the dosage and duration of treatment (Clyde, 1981).

We carried out the diagnosis of malaria and the detection of G6PD deficiency at the same time from two drops of the blood. Children cooperated well during medical examination in this survey. After examination chloroquine and primaquine were administered to parasite carriers with normal G6PD according to the routine regimen and three-day schedule. Only chloroquine was given to the parasite carriers with G6PD deficiency. In this study, no haemolytic crisis was observed.

Fujii's method employed this time is useful when there are a lot of samples to be examined. Hundreds of samples can be tested in one day. The cost of examination is rather low: $0.05 for one sample. Commercialized test kit is easy to use but expensive: $0.65 for one sample. Beutler's method (Beutler *et al.*, 1979) is cheaper than that of Fujii's: $0.01 for one sample, however, ultraviolet light and much experience are needed. Fujii's method needs no special equipment except an incubator. The procedure is simple and reading is easy. This method is suitable for field surveys.

In Indonesia Lie-Injo and Poey-Oey (1964) had already reported 1.1% of G6PD deficiency in Jakarta, Java island; Mutoh and Ebisawa (1974) reported 3.7% in Dumai, Sumatra island. Our findings are similar to them. In the other countries in Asia, the prevalence of G6PD deficiency was 5-12.7% in the Philippines, and 7-33% in Thailand (WHO, 1967). Allison (1960) also reported high prevalence of G6PD deficiency (15-28%) in malaria endemic areas in East Africa. The prevalence of G6PD deficiency in Indonesia seems lower than that in these countries. However, the prevalence is different in races and geographical areas. Since there are many ethnic races in Indonesia, more investigations are needed to estimate the prevalence of G6PD deficiency in this country.

In West Africa it was reported that the prevalence of G6PD deficiency in malaria endemic areas was higher than that in non-endemic areas (Allison, 1960; Allison and Clyde, 1961). Our studies showed that the prevalence of G6PD deficiency in malaria endemic areas was higher than that in a malaria non-endemic area. However, there was no significant difference between them, and the sample size was too small for definite comparison.

It has been reported that enzyme deficient subjects do not have any greater resistance against *P. falciparum* malaria than normal subjects (Kruatrachue *et al.*, 1962; Bienzle *et al.*, 1972). Our result are in accordance with these reports. In this study, there was no difference between the prevalence of *P. falciparum* malaria in G6PD deficient group and that in normal group. If G6PD deficiency offers any selective advantage to malaria, there should be difference in severity or prevalence of malaria. Gilles *et al.*, (1967) observed in Nigeria that severe *P. falciparum* malaria cases (over 100,000 parasite counts per μl) in G6PD deficient children were significantly lower that in G6PD normal children. In our result the parasite counts of *P. falciparum* in G6PD deficient group tended to be lower than those in G6PD normal group. The number of cases was, however, not large enough for analysis.

Chronic haemolytic anemia sometimes occurs in G6PD deficient individuals. In our

study there was no case of chronic haemolytic anemia. Their haematocrit levels were within the limits for normal children. On the other hand, it has been indicated that severe anemia tends to give "false deficient" result because red blood cells are few in number and the activity of G6PD appeared to be a low level as a whole. No severe anemic patient was found in our series, thus there was no false case in G6PD deficiency.

SUMMARY

A screening test for glucose-6-phosphate dehydrogenase (G6PD) deficiency was carried out in North Sumatra, Indonesia by using a simple agar plate method. The prevalence of G6PD deficiency in male was 6.0% (9/151) in Nias prefecture, 3.9% (12/307) in Asahan prefecture and 0.9% (1/110) in Medan city (average 3.9%). The prevalence of malaria was investigated at the same time in Nias and Asahan. It was 8.6% (13/151) and 10.4% (32/307) in males. The parasite rate of *Plasmodium falciparum* in normal and G6PD deficient groups was 4.1% and 9.5%, respectively. There was no statistical significance between them. The usefulness of the system of detecting malaria and G6PD deficiency at the same time was discussed in relation to malaria control.

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