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BIOCHEMICAL CHARACTERISTICS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE VARIANTS AMONG THE MALAYS OF SINGAPORE WITH REPORT OF A NEW NON-DEFICIENT ($Gd^{Singapore}$) AND THREE DEFICIENT VARIANTS

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Summary Biochemical characteristics of one non-deficient fast G6PD variant ($Gd^{Singapore}$) and six different deficient variants (three new, two Mahidol, one each of Indonesian and Mediterranean) were studied among the Malays of Singapore. The $Gd^{Singapore}$ variant had normal enzyme activity (82%) and fast electrophoretic mobilities (140% in TEB buffer, 160% in phosphate and 140% in Tris-HCl buffer systems respectively). This variant is further characterized by normal K_m for G6P; utilization of analogues (Gal6P, 2dG6P; dAmNADP), heat stability and pH optimum. The other six deficient G6PD variants had normal electrophoretic mobility in TEB buffer with enzyme activities ranging from 1 to 12% of Gd^{B+}. The biochemical characteristics identity them to be 2 Mahidol, 1 Indonesian and 1 Mediterranean variants and three new deficient variants.

Key Words G6PD, electrophoresis, deficiency, new variants, kinetics, inhibition

INTRODUCTION

Red cell glucose-6-phosphate dehydrogenase (G6PD) in man is an X-linked enzyme. G6PD deficiency is one of the common inherited metabolic disorders in man and prevalent in almost all the populations of south-east Asia. A remarkable extent of biochemical heterogeneity with about 370 genetic variants of the enzyme have been reported in the literature (Yoshida *et al.*, 1971; Beulter and Yoshida, 1973; Yoshida and Beutler, 1978, 1983; Beutler and Yoshida, 1988). Several new variants at the G6PD locus have been described among the populations of south-east Asia and they have been reviewed recently by Panich (1982, 1986). However, most of the studies have been carried out on G6PD deficient samples because

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of the ease of screening the test. Population genetics of red cell G6PD in African and Asian populations suggest that the prevalence of non-deficient alleles is not uncommon (Nakatsuji and Miwa, 1979; Samuel *et al.*, 1981; Saha, 1984; Panich, 1986; Saha and Samuel, 1991). As such, probably the earlier studies based on the enzyme deficient samples grossly underestimate the degree of genetic heterogeneity of the *Gd* locus in man.

There have been no studies on the biochemical characterization of red cell G6PD among the populations of Malaysia and Singapore although the enzyme deficiency is quite common in the region (Lie-Injo and Chin, 1964; Lie-Injo and Ti, 1964; Lie-Injo *et al.*, 1964; Saha and Banerjee, 1971; Saha, 1984; Panich, 1986). Most extensive studies on biochemical characterization of the G6PD variants have been carried out in Thailand, Papua New Guinea, Indonesia (Bali) and China (Guangdong province) and Japan, exploring a very high degree of heterogeneity at the Gd locus in these populations (Miwa *et al.*, 1977; Nakatsuji and Miwa, 1979; Panich, 1981; Panich and Nakorn, 1980; Panich *et al.*, 1972; Cockkalingam, 1982a, 1982b; Du *et al.*, 1988).

The multiracial population of Singapore comprising the Chinese, Malays and Indians offers a unique opportunity to study the biochemical characteristics of G6PD variants in light of only sketchy information available on these populations in general. In this communication we report on the biochemical characterization of G6PD variants detected among the Malays of Singapore.

MATERIALS AND METHODS

Ten ml of blood samples were collected from nine male individuals previousyl identified as having G6PD variants and twelve male individuals with common Gd^{B+}. The initial screening was carried out by starch-gel electrophoresis in Tris-EDTA-Borate (TEB) buffer of pH 8.6 and the dye-decolouration screening test for red cell G6PD. The partial purification of the G6PD variants was carried out as outlined by WHO (1967). Electrophoretic mobility of the purified variants was estimated in TEB, pH 8.6 buffer for all samples; and in phosphate (PO4) and Tris-HCl buffer (TRIS) for some of the samples. Biochemical characterization includes relative enzyme activity, relative electrophoretic mobility, K_m for glucose-6-phosphate (G6P) and nicotinamide adenine dinucleotide phosphate (NADP); percentage utilization of analogues *eg.* galactose-6-phosphate (Gal6P); 2-deoxyglucose-6-phosphate (2dG6P); deamino NADP (dAmNADP); heat stability at 45°C and pH optimum. The criteria for identification of G6PD variants was based on the above biochemical parameters in comparison to tabulations reported in the literature (Yoshida *et al.*, 1971; Beutler and Yoshida, 1973; Yoshida and Beutler, 1978, 1983; Panich, 1986; Beutler and Yoshida, 1988; Du *et al.*, 1988).

RESULTS AND DISCUSSION

The biochemical characteristics of seven G6PD variants are presented in Table 1. All the subjects were clinically healthy with haemoglobin concentrations within normal range.

Group I. $Gd^{Singapore}$: A non-deficient Gd variant with a relative enzyme activity of 82% has been detected. The relative mobility was 140% in TEB and Tris buffers while it was 160% in PO4 buffer. The other characteristics (K_m G6P), utilization of analogues (2dG6P, dAmNADP), heat stability and pH optimum were within normal range while K_m for NADP (23 μM) and % utilization for Gal6P were higher (11%) than that in Gd^B. $Gd^{Singapore}$ is a new non-deficient variant unreported in the literature. Recently a new hyper-active variant ($Gd^{Khartoum}$) of slow mobility, prevalent in Sudan and Saudi Arabia has been characterized (Saha and Samuel, 1991).

Other six different deficient variants of G6PD have been characterized as follows:

Previously reported variants (Groups II–IV)

Group II. Two cases of this variant have been characterized. The biochemical characteristics suggest this variant to be similar to the $Gd^{Mahidol}$ variant described earlier in the Thai, Cambodians and Chinese (Panich *et al.*, 1972; Panich, 1986; Du *et al.*, 1988). One Thai Muslim of southern Thailand, an ethnic group related to the Malay was also reported to have $Gd^{Mahidol}$ by Panich and Na-Nakorn (1980).

Group III. One sample having biochemical characteristics similar to the $Gd^{Indonesia}$ variant (normal electrophoretic mobility, severe enzyme deficiency (3%), normal K_m NADP and K_m G6P and slightly reduced heat stability) has been identified (Kirkman and Lie-Injo, 1969).

Group IV. One example of $Gd^{Mediterranean}$ with severe enzyme deficiency (2%), normal K_m NADP (9 μM), low K_m G6P (38 μM) and higher utilization of Gal6P (57%), 2dG6P (62%) and dAmNADP (296%) has been found. The stability of the enzymes was low (40%) while pH optimum was biphasic.

New deficient variants (V–VII)

Group V. Two samples of these variants have been characterized. This variant is characterized by low enzyme activity (5–9%), normal electrophoretic mobility in TEB buffers, very low K_m G6P (26, 32 μM), higher K_m NADP (9, 11 μM) and raised utilization of Gal6P, 2dG6P and dAmNADP. This G6PD variant appears similar to the $Gd^{Corinth}$ variant (Beutler and Yoshida, 1988) excepting heat stability and pH optimum. They can be differentiated from the Indonesian variant by normal 2dG6P utilization, biphasic pH optimum and higher heat stability.

Table 1. Biochemical characteristics of glucose-6-phosphate dehydrogenase variants among male Malays of Singapore.

G6PD variants	Hb g/dl	Enzyme activity (IU/gHb)	Relative enzyme activity (% of Gd^{B+})	Electrophoretic morbility (% of Gd^{B+})		Utilization of analogues						Remarks
					K_m NADP (μM)	K_m G6P (μM)	Ga16P (% of G6P)	2dG6P (% of G6P)	dAmNADP (% of NADP)	Heat stability (45°C)	pH optima	
I	15.4	5.00	82%	TEB 142 PO4 161 TRIS 142	73	75	11	5	55	100%	Normal	*Gd*Singapore Class IV
II	14.7	0.71	12%	TEB 100	20	92	2	5	51	88%	Normal	Mahidol
II	15.2	0.73	12%	TEB 100	18	71	2	4	49	95%	Normal	Class III
III	15.3	0.20	3%	TEB 100	4	67	0	0	66	69%	Normal	Indonesia Class II
IV	14.9	0.11	2%	TEB 100	9	38	57	62	296	40%	Biphasic (6, 8)	Mediterranean Class II
V	15.2	0.58	9%	TEB 100	11	32	11	16	64	0%	—	New Class II
V	16.4	0.30	5%	TEB 100	9	26	13	17	67	0%	Normal	New Class II
VI	14.7	0.08	1%	TEB 100	9	31	0	36	47	75%	Peak at 8	New Class II
VII	14.4	0.18	3%	TEB 100	15	194	10	22	64	78%		New Class II
Control Gd^B (n=12)		6.12 ±0.86		TEB 100	4.85 ±3.64	80.08 ±13.45	4.33 ±1.41	6.00 ±1.41	41.50 ±5.09	87.50 ±5.89	Normal (Truncate)	

Group VI. This variant is similar to the Bangkok or Salata-like variant with the exception of being non-anaemic (Class II), more heat stable (75%), and having a low K_m G6P (31 μM) and normal K_m NADP. However, the relative utilization of 2dG6P of the variant is much higher (36%) than that of the Bangkok variant (8%) (Talalak and Beutler, 1969).

Group VII. This variant is possibly a new variant with very high K_m G6P (194 μM), high utilization of 2dG6P (22%) and dAmNADP (64%) and slightly raised K_m for NADP (15 μM). No other variant with Gd^B-like electrophoretic mobility with such a high K_m G6P and 2dG6P has been reported earlier. A similar variant (Viangchan) in Laotian could be differentiated by a much lower K_m G6P (105 μM) and dAmNADP utilization (45%) (Poon *et al.*, 1988).

It is seen from the above that the heterogeneity at the Gd locus is quite large in the Malay population of Singapore. The non-deficient variant ($Gd^{Singapore}$) is quite unique and new. The groups II–IV are similar to earlier reported variants *eg.* $Gd^{Mahidol}$, $Gd^{Indonesia}$ and $Gd^{Mediterranean}$, respectively. The incidence of classical Mediterranean variant is very low in this population. This is ptobably due to past Arab contact of the present Malay population. Groups V–VII appear to be new G6PD variants belonging to class II of WHO classification.

In conclusion, we present evidence for a new non-deficient G6PD variant ($Gd^{Singapore}$) as well as three new deficient G6PD variants observed in the Malay population of Singapore. In addition, 2 Mahidol, and one each of Indonesia and Mediterranean variants were also identified in the present study. The presence of the Mahidol variant is expected as it is the most common G6PD variant in the south-east Asian region (Panich, 1986), while the origin of the Malays from Indonesia and the past Arab influence are consistent with the presence of the $Gd^{Indonesian}$ and $Gd^{Mediterranean}$ respectively. However, a great deal of possible inter-laboratory differences in the results of kinetic studies and incomplete information on some variants are some of the limitations of identification of G6PD variants. Further, recent report of the presence of same mutations at DNA level (563 C→T and 637 G→T) in several biochemically distinct protein variants at the Gd locus (Beutler, 1991) should be considered in interpretation of the G6PD variants.

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