Original Article/นิพนธ์ต้นฉบับ

Improvement for Diagnosis of G6PD Deficiency Using an In-House Spectrophotometric Assay

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Abstract

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common red cell enzyme defect found in Thai population. Accurate diagnosis is essential for counseling.

Objective: To establish a G6PD enzyme assay and reference values.

Methods: G6PD deficient Thai individuals and healthy volunteers were recruited. Identification of *G6PD* mutations and G6PD enzyme assay were performed in all subjects. The cut-offs for classification of residual enzyme level were identified using Receiver Operating Characteristics (ROC) curves.

Results: Eighty-eight subjects were divided into three groups according to their *G6PD* genotype: Group 1, Wild-type (n = 35); Group 2, Carrier (n = 27) and Group 3, Deficiency (n = 26). Median G6PD level (interquartile range) of Group 3 was significantly lower than that of Group 2 and Group 1, 0.6 (0.3 to 1.5) vs 5.3 (4.6 to 6.7) vs 9.3 (8.0 to 10.3) IU/gHb; $P < 0.01$). G6PD level of ≤ 2.9, > 2.9 - 6.7, and > 6.7 IU/gHb were found to be optimum for classification of residual G6PD enzyme into deficiency, intermediate and normal. These cut-offs resulted in 87% sensitivity and 97% specificity for correct classification of enzyme level according to genetic diagnosis. The enzyme level of 78% of subjects in Group 2 were precisely classified as intermediate deficiency. *G6PD* Viangchan (871G > A) and Canton (1376G > T) are the two most prevalent mutations found.

Conclusions: The established G6PD enzyme assay and its cut-off values provided high sensitivity and specificity for classification of individuals into G6PD deficiency, intermediate and normal.

Keywords: G6PD enzyme assay, G6PD assay cut-offs, G6PD deficiency

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) is a critical enzyme in the redox chemical reaction of all aerobic cells. It catalyzes the first and the rate-limiting step of pentose phosphate pathway, leading to synthesis of reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) as an end product.[1] The –SH groups of several enzymes and of the β-globin chain are exceptionally susceptible to oxidative stress. The role of NADPH to protect red blood cells (RBCs) against reactive oxygen species is mediated by glutathione. Reduced glutathione (GSH) can reverse oxidized -SH groups and buffer reactive oxygen species by becoming itself oxidized (Oxidized glutathione, GSSG) via glutathione peroxidase.[2] Continuous production of NADPH is required for regeneration of GSH, therefore G6PD deficient RBCs are sensitive to cellular oxidative damage. G6PD deficiency, inherited in an x-linked recessive pattern, is the most common RBC enzyme defect, affecting 400 million individuals worldwide and 5 to 18% of Thai population.[3-7] Affected individuals usually present with moderate to severe hemolytic anemia after a few days of exposure to certain biochemical agents including fava beans, antibiotics with sulfa-groups and anti-malaria drug “primaquine”. Damaged hemoglobin becomes unstable and form aggregates, which can be visualized using brilliant cresyl blue staining of RBCs and are known as Heinz body. Diagnosis made soon after birth as a part of jaundice work-up is rather common since neonates born with this condition are at increased risk of neonatal jaundice by unclear mechanisms.[8, 9]

Screening test for diagnosis of G6PD deficiency can be performed using commercialized fluorescent spot test (FST). World Health Organization (WHO) gold standard tests for diagnosis, however, include Heinz body detection, G6PD enzyme assay and DNA-based genotyping. FST was originally used as a point-of-care G6PD screening in *Plasmodium vivax* endemic, where primaquine therapy was often required.[10-12] It is widely used in the vast majority of hospitals worldwide owing to its uncomplicated technique and cheap price. Nevertheless, accuracy of FST for the diagnosis of *G6PD* mutation carrier, in which G6PD enzyme is intermediately deficient, can be as low as 30%.[13, 14] This is a rather important issue for counseling provided to the patients and their family. Not only individuals with G6PD severely deficient, but also with intermediately deficient are at risk of having severe hemolysis following exposure to certain biochemical agents mentioned earlier.

DNA-based genotyping is the most reliable method to confirm diagnosis of G6PD deficiency and its carrier state, however, it is too costly and time-consuming to be performed in every suspected case. A G6PD enzyme assay using spectrophotometric method provides quantitative measurement of residual enzyme level and is a more feasible test as compared to DNA-based genotyping. This study aims to establish an easy-to-follow G6PD enzyme assay and to provide cut-off values based on *G6PD* genotype for determination of G6PD-normal, -intermediate and -deficient individuals. The assay also enable us to classify patients into class I to V according to their residual enzyme activity (WHO classification) for genetic counseling.[15]

Methods

Study Design and Population

This cross-sectional study was performed at Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University from October 2016 to September 2017. Healthy Thai volunteers and patients, who ever experienced neonatal jaundice or at least one acute hemolytic episode and who were identified using qualitative G6PD FST as having G6PD deficiency, were included in the study. Following written informed consent, 1.75 mL of peripheral blood specimens were collected in venipuncture vacuum tubes containing 250 µL of anticoagulant citrate dextrose (ACD) for determination of G6PD enzyme level. Additional 2 mL of blood from each individual was transferred to Ethylene Diamine Tetra Acetic Acid (EDTA) anticoagulant tube and store at 4 °C for DNA extraction and subsequent *G6PD* mutation

identification. Manual reticulocyte count was performed using brilliant cresyl blue staining in every sample and those with reticulocyte count > 3% were excluded from the study. G6PD enzyme level were measured in parallel with identification of *G6PD* mutations.

G6PD Enzyme Assay

Assay for quantitative measurement of G6PD level was spectrophotometric rate determination method originally described by WHO scientific group.[15] A 2 mL mixture of whole blood and ACD was centrifuged at 3000 rpm for 5 minutes. Plasma was removed and remaining packed red cells were washed with 3 mL of normal saline solution (NSS) for a total of three times. To avoid inaccuracy of G6PD level measured in patients with various level of hemoglobin (Hb), in this study Hb content of each sample was adjusted of to 15 g/dL by adding drops of NSS to the washed red cells. Subsequently, 50 µL of adjusted washed red cells and 950 µL of distilled water (DW) were thoroughly mixed prior to snap freezing of the mixture at -20 °C for 5 minutes. At this stage, a sample hemolysate was obtained after centrifugation of the mixture at 5000 rpm for 3 minutes.

G6PD enzyme level can be determined when a sample hemolysate is added to an assay buffer containing a certain amount of substrate glucose-6-phosphate (G6P) and its cofactor NADP. An assay buffer was prepared from a mixture of 0.1 M Tris-HCl pH8.0, 0.01 M $MgCl_2$, 0.2 mM NADP and 0.6 mM G6P.

For each G6PD enzyme assay, diluent buffer, hemolysate, and substrate were sequentially added into cuvettes as follow:

Solution	Volume, µL		
	Blank	Test	Standards*
Initial DW	850	-	-
Assay buffer	-	850	850
Sample hemolysate	50	50	50
Additional DW	100	-	-
G6P	-	100	100

* Standards: G6PD Tri-level control was purchased from Trinity Biotech, USA.

NADPH generation was determined, using a temperature-controlled spectrophotometer (Shimadzu, Japan), as changes in absorbance rate at 340 nm of the enzyme reaction maintaining at 37 °C for 10 minutes in UV cuvettes (Quartz, 10 mm Light Path). G6PD enzyme level was calculated from the following formula and reported as IU/gHb:

$$\text{G6PD level (IU/gHb)} = \frac{(\Delta OD/min) \times 6.44 \times 10^3}{Hb(g/dL)}$$

G6PD Mutation Identification

The ten most prevalent *G6PD* mutations found in Thai population were identified using Multiplex Amplification-Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) technique as previously described.[16] These *G6PD* mutations include Canton (1376G > T), Chinese3 (493A > G), Chinese4 (392G > T), Chinese5 (1024C > T), Coimbra (592C > T), Gaohe (95A > G), Kaiping (1388G > A), Mahidol (487G > A), Union (1360C > T) and Viangchan (871G > A). For those previously diagnosed G6PD deficient patients, who do not carry the ten most prevalent mutations, additional five mutations, including

Aures (143T > C), Bangkok (825G > C), Bangkok Noi (1502T > G), Chatham (1003G > A) and Songklanagarind (196T > A) were identified using the same technique.

Statistical Analysis

Data were recorded to and analyzed in SPSS version 20 (IBM SPSS statistics for Window, Version 20.0 Armonk, NY: IBM Corp; 2011). Overall and group-specific median and range of G6PD enzyme level were determined. To explore correlation between demographic factors, such as *G6PD* mutations and G6PD enzyme level, independent t-test, chi-square and descriptive tests were used as appropriate. Cut-off values of G6PD enzyme level were determined using Receiver Operating Characteristic (ROC) curves, for which 95% confidence intervals (CI) were calculated. Sensitivity and specificity for selected G6PD level cut-off values were identified.

Ethical Considerations

Ethical approval for the study was provided by Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, No. MURA2016/590. This study was also conducted in accordance with the Declaration of Helsinki.

Results

Characteristic of Enrolled Subjects

A total of 88 Thai individuals (44 males, 50%), whose median age was 17 years (range 6 months - 43 years), were recruited. These included 26 previously diagnosed, using FST, as G6PD-deficient patients (22 males, 85%) and 62 healthy individuals (22 males, 35%). None of those previously diagnosed as having G6PD deficiency shows clinical phenotype of chronic non-spherocytic hemolytic anemia (CNSHA). Enrolled subjects were then divided into three groups according to *G6PD* genotype; Group 1) Wild-type, 2) Carrier, and 3) Deficiency.

Group 1 comprised 35 healthy subjects (21 males, 60%). Heterozygous *G6PD* mutations (Group 2) were identified in 27 all-female individuals. Group 3 composed of 23 hemizygous males and three compound heterozygous females (summarized in Table 1). All except one of previously diagnosed G6PD deficient subjects were classified into Group 3. The remaining female subject, however, was found to harbor heterozygous *G6PD* mutation, therefore was classified into Group 2. Type and frequency of mutated alleles in Group 2 and 3 are shown in Table 2.

Table 1 Characteristic of Enrolled Subjects

Characteristic	No. (%)	
	Male	Female
Enrolled subjects (n = 88)*	44 (50)	44 (50)
Previous clinical diagnosis		
Healthy individual (n = 62)	22 (35)	40 (65)
G6PD deficiency (n = 26)	22 (85)	4 (15)
Genetic diagnosis		
Group 1: G6PD wild-type (n = 35)	21 (60)	14 (40)
Group 2: G6PD carrier (n = 27)		27 (100)
Group 3: G6PD deficiency (n = 26)	23 (88)	3 (12)

Abbreviation: G6PD, Glucose-6-phosphate dehydrogenase.

* The subjects age range was 0.5 - 43 years old (mean age 17 years).

Table 2 Type and Frequency of *G6PD* mutations

Type and Frequency	No.	
	Group 2 (n = 27)	Group 3 (n = 26)
Heterozygous/Hemizygous		
Viangchan (871G > A)	15	13
Canton (1376G > T)	5	4
Kaiping (1388G > A)	3	3
Mahidol (487G > A)	2	1
Aures (143T > C)	2	1
Union (1360C > T)	-	1
Compound heterozygous		
Chinese4 (392G > T) / Canton (1376G > T)	-	1
Canton (1376G > T) / Kaiping (1388G > A)	-	1
Mahidol (487G > A) / Union (1360C > T)	-	1

Group 2 = G6PD carrier; Group 3 = G6PD deficiency.

Determination of G6PD Enzyme Level

To determine feasibility of the G6PD enzyme assay, test results upon various conditions were assessed. Ten repetitive assays performed within the same day using technical replicates resulted in 5.8% Coefficient of Variation (CV). To assess possible maximum pre-assay storage time in 4 °C, G6PD assay of a single wild-type subject was performed once daily for eight consecutive days. We found that results of G6PD enzyme level remained unchanged from day one to day seven (9.9 IU/gHb on day one and 9.3 IU/gHb on day seven) and decreased by 20.2% on day eight (7.9 IU/gHb).

Median G6PD enzyme level (interquartile range) of the subjects in Group 1 was 9.3 (8.0 to 10.3) IU/gHb. Median G6PD level of normal male was significantly higher than that of normal female, 9.8 (8.8-10.8) vs 8.0 (6.9 to 8.9) IU/gHb; $P = 0.003$, similar to a previous report.[4] Median G6PD level (interquartile range) of Group 3, 0.6 (0.3 to 1.5) IU/gHb, as expected, was not only significantly lower than that of Group 1 but also Group 2, 5.3 (4.6 to 6.7) IU/gHb, with P value of < 0.001. Additionally, median G6PD level of subjects in Group 2 was significantly lower than that of Group 1 ($P < 0.01$) as shown in Figure 1. There was no significant difference between G6PD activity of males and females in Group 3.

Identification of Cut-Off G6PD Enzyme Levels Based on *G6PD* Genotype

To identify cut-off values for classification of G6PD enzyme level into normal, intermediate and deficiency, we generated ROC curves. The enzyme level of $\leq$ 2.9 IU/gHb was found to be best for discrimination of subjects in Group 3 from those in Group 2 and Group 1, with area under the curve of 0.98 (95%CI, 0.95 to 1.0), 96% sensitivity and 97% specificity. The cut-off value of > 6.7 IU/gHb best differentiated subjects in Group 1 from those in Group 2 and Group 3, with area under the curve of 0.97 (95%CI, 0.94 to 1.0), 97% sensitivity and 87% specificity (Table 3). Enzyme level in between the two cut-offs were classified as intermediate deficiency. Figure 2 shows ROC curve analysis identifying genotype-based cut-off values for classification of G6PD enzyme level.

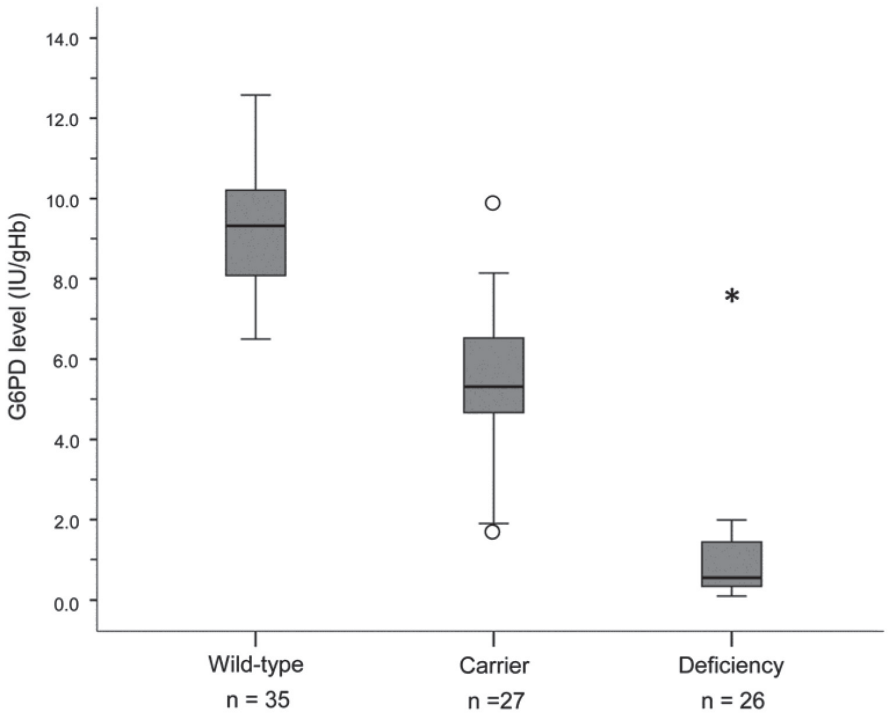

Figure 1 *G6PD Enzyme Level Based on Genetic Diagnosis*

The box plot shows G6PD enzyme activity level in each group. Bolded lines represent median G6PD level and the edges of the boxes represent the 1st and 3rd quartile values. Error bars show minimum and maximum G6PD level.

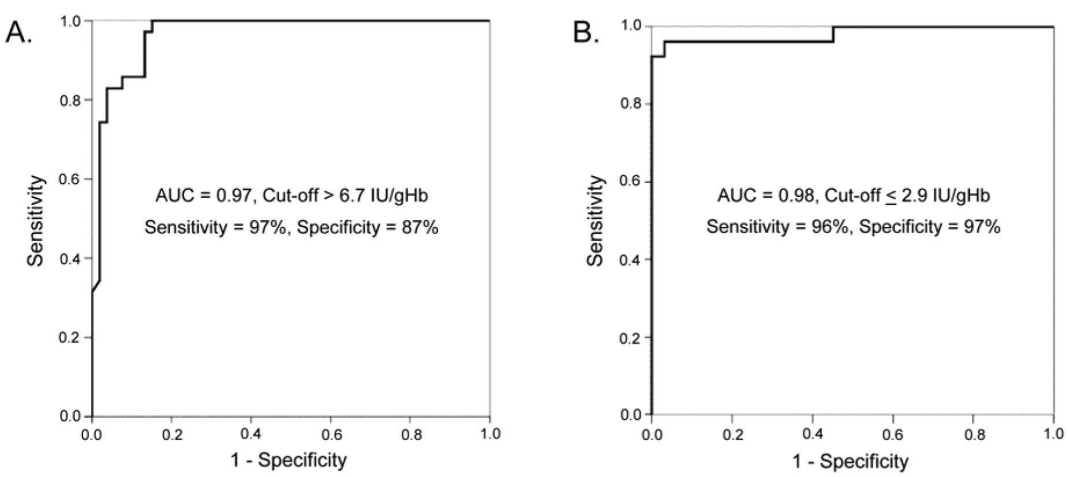

Figure 2 *Receiver Operating Characteristic (ROC) Curve Analysis for Cut-off Values of G6PD Enzyme Level.*

The curves demonstrates the most appropriate cut-off enzyme level for G6PD normal (A) and G6PD deficiency (B). Area under the curve (AUC), sensitivity and specificity for the cut-off values are shown in the middle area of each curve.

Table 3 Genotype-Based Cut-off Values for Classification of G6PD Enzyme Activity

Classification	G6PD Enzymatic Activity, IU/gHb
Normal	> 6.7
Intermediate deficiency	> 2.9 - 6.7
Deficiency	≤ 2.9

Abbreviation: G6PD, Glucose-6-phosphate dehydrogenase.

Overall, the established cut-off values for G6PD level resulted in 87% sensitivity and 97% specificity for correct classification of enzyme level according to the genetic diagnosis. G6PD level of all but one subjects in Group 3 were classified into "deficiency", whereas the enzyme activity of as many as 21 subjects in Group 2 (21/27, 78%) were classified as "intermediate deficiency" as expected. The remaining subjects in this group, however, were miss-classified as "normal". G6PD level of one individual in Group 1 was miss-classified as "intermediate deficiency" (6.5 IU/gHb) and that of one individual in Group 3, *G6PD* Viangchan (871G > A), was miss-classified as "normal" (7.6 IU/gHb). Sanger sequencing of *G6PD* gene was performed for these two cases and results showed no additional mutation. Therefore, the G6PD level from these two individuals were most likely due to technical errors and were excluded from subsequent analysis.

WHO Classification of G6PD Enzyme Activity and Clinical Manifestation

WHO has divided G6PD-deficient individuals into five classes according to their residual enzyme activity and clinical presentation of chronic hemolytic anemia.[2] Employing median level of G6PD enzyme of subjects in Group 1 (9.3 IU/gHb) as a normal level, 68% (17/25) of subjects in Group 3 were defined as Class II; severely deficient. The remaining subjects in this group were defined as Class III; moderately deficient (Table 4). The majority (59%, 16/27) of subjects in Group 2 were categorized as Class III, whereas all subjects in Group 1 were categorized as Class IV; normal level.

The two most common *G6PD* mutations found in this study were *G6PD* Viangchan and Canton. Sixty-seven percent (8/12) of *G6PD* Viangchan hemizygous males and 67% (10/15) of those heterozygous females were categorized

Table 4 WHO Classification of G6PD Deficiency According to *G6PD* Genotype

Class	No.		
	Group 1	Group 2	Group 3
I (CNSHA)	-	-	-
II (severely deficient: < 10% residual activity)	-	-	17
III (moderately deficient: 10 - 60% residual activity)	-	16	8
IV (normal activity: 60 - 150%)	34	11	-
V (increased activity)	-	-	-
Total	34	27	25

Abbreviation: CNSHA, Chronic non-spherocytic hemolytic anemia; G6PD, Glucose-6-phosphate dehydrogenase.

Group 1 = *G6PD* wild-type; Group 2 = *G6PD* carrier; Group 3 = *G6PD* deficiency.

as Class II. All of *G6PD* Canton hemizygous males (4/4) and all but one of those heterozygous females (4/5) were categorized as Class II.

Discussion

G6PD deficiency is a common condition found in tropical countries, such as in Thailand. A previous study of 350 cord blood samples demonstrated that G6PD deficiency was found in 11.1% of Thai male and 5.8% of female.[4] Although the vast majority of individuals with G6PD deficiency are asymptomatic in a steady-state condition, precise diagnosis of the condition is important for genetic counseling. This is because individuals with G6PD deficiency are at risk of having severe hemolysis after exposure to certain biochemical agents leading to massive hemoglobinuria and renal dysfunction. Fava beans, anti-malarial drug, such as primaquine, sulfonamide and aspirin being the most common among those agents causing hemolysis. Not only G6PD-severely-deficient individuals, but also *G6PD* mutation carriers whose RBCs exhibit -intermediately-deficient G6PD enzyme can be severely affected. Therefore physicians should provide a precaution food and drug list to both individuals with G6PD deficiency and the carriers.

G6PD deficiency was found in up to one fifth of Thai neonates who experience neonatal hyperbilirubinemia.[4, 17] Severe neonatal hyperbilirubinemia associated with G6PD deficiency has long been a well-known cause of kernicterus leading to fatality or permanent neurological damage.[18] In the era of comprehensive prenatal diagnosis and ever-improved post-natal care, mothers should be screened for G6PD carrier state and at risk neonates should be carefully monitored for hyperbilirubinemia. Methods for detection of G6PD deficiency, therefore, should be feasible and precise for diagnosis of residual G6PD enzyme activity.

The most commonly used method for diagnosis of G6PD deficiency in routine clinical practice is FST, which is either a qualitative or semi-quantitative screening method.[19] This method, however, shows low accuracy (approximately 30%) and reliability especially for detection of individuals with intermediate deficiency.[13, 14] This study provided an easy-to-follow spectrophotometric method for an improvement in the diagnosis of G6PD deficiency and its reference cut-off values.

G6PD enzyme assay performed in this study was adapted from WHO protocol.[15] However, we replaced an assay temperature of 25°C with 37 °C and followed the difference in optical density up to 10 minutes. These adaptations were aimed to make an *in vitro* reaction more likely to mimic that occurs *in vivo* and to ensure any residual enzymatic activity was detected. To avoid inaccuracy of the enzyme activity level measured in individuals with anemia or hypochromic red cells, adjustment of red cell concentration in each sample to yield a certain Hb level was performed just before red cells being hemolyzed. We found that the adjusted Hb level of 13 to 15 g/dL was optimal for subsequent procedures. Unlike a previously reported method performed in Thailand involving rather complicate procedure and equipment[20], our G6PD assay is simple, requiring only basic chemical agents and a standard temperature-controlled spectrophotometer. This makes it appropriate to be applied in general hospitals to serve their patients. Additionally, a number of blood samples can be stored in 4 °C and pooled for an assay day up to once a week, resulting in a practical and less-time consuming procedures.

Although diagnosis of G6PD deficiency using spectrophotometric method ultimately requires laboratory-specific reference values, initial provided reference values can be extremely helpful for in-house procedural set up. Unlike previous studies using cross-reference values between qualitative and quantitative tests[21-23], we used genetic analysis to correlated *G6PD* genotype with the enzyme activity level and generated ROC curve to identify cut-off values for determination of patients' G6PD status. Nevertheless, an important point to be aware of is that

reference values determined in this study were based on *G6PD* mutations commonly found in the South East Asian subcontinent.[4, 24, 25] Therefore, they may not be appropriate to be employed in regions with different mutations, such as Africa and Mediterranean.[23]

The established cut-off values showed a high sensitivity and specificity to determine relevant enzyme levels according to their *G6PD* genotype in the vast majority of the subjects with G6PD normal and deficiency. Additionally, these reference cut-offs resulted in a better identification of individuals with intermediate deficiency of G6PD as compared to FST.[13] This is because as many as 78% of *G6PD* mutation carriers were precisely identified as having intermediate level of residual G6PD enzyme. The remaining 22% of the carriers, however, were miss-interpreted as having G6PD normal if they were screened using G6PD activity assay alone. This gap suggested that additional novel assays focusing on isolation of individuals with intermediate deficiency from those with normal or severe deficiency remains in need.

The *G6PD* gene, consisting of 13 exons and 12 introns, encodes 515 amino acids protein and a GC-rich promoter region.[10] More than 200 mutations in *G6PD* gene have been reported to date (reviewed in reference 25). In keeping with previous reports of common *G6PD* mutations found in Thai population[4, 6, 10], *G6PD* Viangchan (871G >A) was the most prevalent among enrolled subjects, accounting for 57% of hemizygous males and 56% of heterozygous females. This study also demonstrated that mutations found in Thai population most likely result in Class II or Class III deficiency as classified by WHO. None of the subjects showed the phenotype of CNSHA.

Conclusions

This study provided an easy-to-follow methods for G6PD enzyme assay and reference cut-off values for result interpretation. This will be of most benefit for initial set up of the procedure in order to replace or to use in conjunction with available qualitative tests for improvement in the diagnosis of G6PD deficiency.

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Original Article/นิพนธ์ต้นฉบับ

การพัฒนาการวินิจฉัยภาวะพร่องเอนไซม์จีซิกพีดีโดยการวัดระดับเอนไซม์ด้วยวิธีสเปคโตรโฟโตเมทริกเรทดีเทอมิเนชั่น

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บทคัดย่อ

บทนำ: ภาวะพร่องเอนไซม์จีซิกพีดีเป็นภาวะพร่องเอนไซม์ในเม็ดเลือดแดงที่พบบ่อยที่สุดในประชากรไทย การวินิจฉัยที่แม่นยำจะนำไปสู่การให้คำปรึกษาที่ถูกต้องแก่ผู้ป่วย

วัตถุประสงค์: เพื่อพัฒนาการตรวจระดับเอนไซม์จีซิกพีดี และกำหนดค่าอ้างอิงในการวินิจฉัยภาวะพร่องเอนไซม์จีซิกพีดี

วิธีการศึกษา: รวบรวมประชากรไทยที่เคยได้รับการวินิจฉัยว่ามีภาวะพร่องเอนไซม์จีซิกพีดี และผู้ที่มีสุขภาพแข็งแรงเข้าสู่งานวิจัย ตรวจระดับเอนไซม์และการกลายพันธุ์ของยีนในผู้เข้าร่วมงานวิจัยทุกราย กำหนดค่าอ้างอิงในการวินิจฉัยภาวะพร่องเอนไซม์โดยใช้ ROC

ผลการศึกษา: ผู้เข้าร่วมงานวิจัยจำนวน 88 คน แบ่งเป็น 3 กลุ่มตามลักษณะการกลายพันธุ์ของยีน กลุ่มที่ 1 ปกติ (35 คน), กลุ่มที่ 2 พาหะ (27 คน) และกลุ่มที่ 3 พร่องเอนไซม์จีซิกพีดี (26 ราย) ค่ามัธยฐานของระดับเอนไซม์จีซิกพีดีในกลุ่ม 3 น้อยกว่ากลุ่ม 2 และกลุ่ม 1 อย่างมีนัยสำคัญ (0.6 (0.3 - 1.5) vs 5.3 (4.6 - 6.7) vs 9.3 (8 - 10.3) IU/gHb, $P < 0.01$) ค่าระดับเอนไซม์ที่เหมาะสมในการจัดแบ่งผู้เข้าร่วมวิจัยเป็น ผู้มีภาวะพร่องเอนไซม์ พร่องเอนไซม์ระดับปานกลาง และผู้มีระดับเอนไซม์ปกติ คือระดับ < 2.9, > 2.9 - 6.7 และ > 6.7 IU/gHb ตามลำดับ โดยให้ความไวร้อยละ 87 และความจำเพาะร้อยละ 97 ในการแปลผลระดับเอนไซม์จีซิกพีดีสอดคล้องกับลักษณะการกลายพันธุ์ของยีน ค่าอ้างอิงนี้สามารถวินิจฉัยผู้เข้าร่วมวิจัยกลุ่ม 2 ว่ามีภาวะพร่องเอนไซม์ระดับปานกลางได้ถูกต้องถึงร้อยละ 78 ชนิดกลายพันธุ์ของยีนจีซิกพีดีที่พบมากคือ Viangchan (871G > A) และ Canton (1376G > T)

สรุป: วิธีวัดระดับเอนไซม์จีซิกพีดีและค่าอ้างอิงที่พัฒนาขึ้นในงานวิจัยนี้ ให้ความไวและความจำเพาะสูงในการวินิจฉัยผู้มีภาวะพร่องเอนไซม์ พร่องเอนไซม์ระดับปานกลาง และระดับเอนไซม์ปกติ

คำสำคัญ: วิธีวัดระดับเอนไซม์จีซิกพีดี ค่าอ้างอิงการวินิจฉัยภาวะพร่องจีซิกพีดี ภาวะพร่องเอนไซม์จีซิกพีดี

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