นิพนธ์ต้นฉบับ

Glucose-6-Phosphate dehydrogenase (G-6-PD) activity in Thai newborn and adults : Normal ranges and incidence.

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Background : *Glucose-6-Phosphate dehydrogenase (G6PD) is an essential enzyme involved in protecting red blood cell integrity against oxidative damage. Lack of this enzyme can cause cell lysis when it is exposed to external or internal oxidative agents. Quantitation of G6PD activity will help determine a person's susceptibility to hemolysis.*

Objective : *To study G6PD activity in Thai newborn and adults in regard cut point and normal range. The incidence of G6PD deficiency in males is also reported. The activity in obligate heterozygotes is included.*

Setting : *Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, during 1986-1991*

Research design : *Retrospective descriptive study.*

Patients : *The newborn subjected to G6PD screening as part of jaundice evaluation were selected in more or less equal numbers based on the results of G6PD screening showed deficiency or not deficiency (male = 490, female = 156). Some mothers with G6PD deficient newborn (N = 68), as well as healthy volunteers (male = 79, female = 50) were also included in the G6PD quantitative study.*

Methods : *Because the G6PD gene is transmitted via the x-chromosome, it is*

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fairly simple to distinguish between normal and G6PD deficient males. Therefore the distribution of G6PD activity in male was used to elucidated the cut off. G6PD activity in obligate heterozygotes (N = 68) were also studied. The incidence in male adults (N = 79) was established. The results in newborn and healthy volunteers above the cut point of the normal level were included in order to estimate normal ranges and these were represented as percentile between P_5 and P_{95}.

Results : *The cut point values for diagnosis of G6PD deficiency and normal G6PD activity were under 81 and over 150 IU/100 ml Rbc, respectively. Thirty percent of the obligate heterozygotes expressed G6PD activity over 150 IU/100 ml Rbc. The incidence in male adults was equal to the gene frequency, q = 0.101. The normal range in male newborn (N = 195) and female newborn (N = 84) amounted to 166-449 and 157-439 IU/100 ml Rbc, respectively, and to 160-337 and 158-341 IU/100 ml Rbc in male (N = 69) and female adults (N = 42), respectively. The activities were similar in both sexes and significantly higher in newborn than in adults.*

Conclusion : *The G6PD activities in newborn, healthy adults and obligate heterozygote females were determined. The incidence of G6PD deficiency in male adults was 10%. Some obligate heterozygote females could express normal G6PD activity. Therefore additional heterozygotes must have been among those with normal test results and who have an adequate reducing potential to protect red blood cells from hemolysis.*

Key words : *Glucose-6-Phosphate dehydrogenase (G6PD), Normal range, Incidence.*

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เหตุผลการทำวิจัย : *กลูโคส-6-ฟอสเฟต ดีไฮโดรจีเนส (G6PD) เป็นเอนไซม์ที่จำเป็นสำหรับการป้องกันตัวของเม็ดเลือดแดงต่อความเสียหายจากขบวนการออกซิเดทีฟ การขาดเอนไซม์นี้จะก่อให้เกิดการแตกของเซลล์ได้เมื่อสัมผัสกับสารออกซิเดทีฟ ทั้งจากภายนอกหรือจากภายในเซลล์เอง การวัดปริมาณเอนไซม์ G6PD จะช่วยตัดสินถึงความไวในการเกิดการแตก ของเม็ดเลือดแดง*

วัตถุประสงค์ : *เพื่อศึกษาระดับเอนไซม์ G6PD ในเด็กทารกแรกเกิดและผู้ใหญ่ชาวไทยในแง่ของจุดตัดและค่าปกติรวมทั้งอุบัติการของภาวะพร่องเอนไซม์ G6PD ในเพศชาย และระดับเอนไซม์ในผู้หญิงที่เป็นเฮเตโรไซโกตด้วย*

สถานที่ทำการศึกษา : *ภาควิชากุมารเวชศาสตร์ คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย, ระหว่างปี คศ. 1986-1991*

รูปแบบการวิจัย : *การศึกษาเชิงบรรยายแบบย้อนหลัง*

กลุ่มประชากรที่ศึกษา : *ศึกษาในทารกแรกเกิดที่ได้รับการเจาะเลือดเพื่อส่งตรวจกรอง G6PD โดยเลือกเด็กที่มีผลการตรวจกรอง G6PD เป็นปกติหรือมีสภาวะพร่องในจำนวนเท่าๆ กัน (เพศชาย = 490, เพศหญิง = 156) เพื่อศึกษาในเชิงปริมาณ รวมทั้งศึกษาในแม่ของเด็กทารกที่มีภาวะพร่องในเชิงปริมาณ (N = 68) และผู้ใหญ่ที่มีสุขภาพดี (เพศชาย = 79, เพศหญิง = 50)*

วิธีการศึกษา : *เนื่องจากยีน G6PD ถ่ายทอดโดย เอ็กซ์โครโมโซม เพราะฉะนั้นในเพศชายสามารถแยกภาวะปกติและภาวะพร่องเอนไซม์ได้ชัดเจน จึงใช้การกระจายตัวของตัวเลขเชิงปริมาณในเพศชายนี้ เพื่อหาจุดตัดสำหรับวินิจฉัยภาวะพร่องเอนไซม์ G6PD โดยศึกษาในทารกเพศชาย 490 คน นำค่ามาจัดเรียงเป็นอันตรภาคชั้น และดูความถี่ในแต่ละชั้น แล้วจะแยกได้เป็น 2 กลุ่มใหญ่ คือ กลุ่มที่ปกติ และกลุ่มที่มีภาวะพร่อง และจะมีกลุ่มเล็กๆ ที่อยู่ระหว่าง 2 กลุ่มใหญ่นี้ ได้นำค่าจุดตัดนี้ไปวินิจฉัยภาวะเอนไซม์ G6PD ในแม่ที่เป็นเฮเตอโรไซโกต และหาอุบัติการในเพศชาย ค่าเอนไซม์ของทารกและผู้ใหญ่ทั้ง 2 เพศ ที่มากกว่าจุดตัดของ กลุ่มปกติจะถูกนำมาคำนวณเปอร์เซนไทล์ที่ 5 และ 95 เพื่อใช้ เป็นค่าปกติ*

ผลการศึกษา : *ค่าจุดตัดเพื่อวินิจฉัยภาวะเอนไซม์ G6PD คือต่ำกว่า 81 ยูนิตต่อ 100 มล. เม็ดเลือดแดงส่วนค่าที่มากกว่า 150 ยูนิตต่อ 100 มล. เม็ดเลือดแดงถือว่าปกติ จากการศึกษาในแม่ที่เป็นเฮเตอโรไซโกตพบว่ามี 30% ที่มีค่าเอนไซม์มากกว่า 150 ยูนิตต่อ 100 มล. เม็ดเลือดแดง อุบัติการของภาวะพร่องเอนไซม์ G6PD ในเพศชายเท่ากับ 0.101 ค่าปกติในเด็กทารกแรกเกิดเพศชาย (N = 195) และ เพศหญิง (N = 84) มีค่าเป็น 166-449 และ 157-439 ยูนิตต่อ 100 มล. เม็ดเลือดแดงตามลำดับ และมี ค่าเป็น 160-337 และ 158-341 ยูนิตต่อ 100 มล. เม็ดเลือดแดงในผู้ใหญ่เพศชาย (N = 69) และเพศหญิง (N = 42) ตามลำดับ พบว่าไม่มีความแตกต่างในทั้ง 2 เพศ แต่ในทารกจะสูงกว่าในผู้ใหญ่อย่างมีนัยสำคัญ*

สรุป : *บทความนี้รายงานผลการศึกษาเอนไซม์ G6PD ในเด็กทารกแรกเกิด ผู้ใหญ่ที่มีสุขภาพดี และแม่ที่เป็นเฮเตอโรไซโกต พบว่ามีค่าอุบัติการเป็น 10% การศึกษานี้ยืนยันว่าคนที่มีค่าเอนไซม์ปกติ ซึ่งหมายความว่ามีศักยภาพในการป้องกันการแตกของเม็ดเลือดแดงได้นั้นอาจมีภาวะเฮเตอโรไซโกตแฝงอยู่ได้*

Glucose-6-Phosphate dehydrogenase (G6PD) is an essential enzyme in the pentose phosphate pathway. Under steady-state conditions 95 per cent of glucose is metabolized to lactate generating adenosine triphosphate (ATP). The other 5 per cent of the glucose enters the pentose phosphate shunt which serves as the source of reduced nicotinamide adenine dinucleotide phosphate (NADPH). The reducing potential of NADPH is important for the integrity of the red blood cell versus intrinsic and drug-induced oxidative damage. G6PD-deficiency is an inherited condition in which the activity of red blood cell G6PD is markedly decreased. The gene for G6PD is located on the x-chromosome where it spans 18 kilobases. Hence, enzyme deficiency finds full expression in males carrying a variant gene (hemizygote). When the enzyme activity is low or decreases below a critical level, external or internal oxidative agents cause a loss of NADPH and reduced glutathione (GSH) with concomitant precipitation of denatured hemoglobin and Heinz body formation; hemoglobin breakdown products may bind to the red blood cell membrane and cause cell lysis.[1]

In steady-state conditions, individuals with G6PD deficiency are usually healthy but are susceptible to acute hemolysis initiated by infection, some kinds of drugs and food, etc. Therefore precise determination of G6PD activity is important. Spectrophotometric assay of G6PD enzyme activity represents one of the more accurate methods. Hathirat et al reported that the values of 30.29 $\pm$ 39.2 U/100 ml was censidered to be deficient.[2] The normal activity not significantly different between both sexes. This is due to the phenomenon of X-chromosome inactivation, which leaves only one functional X-chromosome in females. Individuals with normal G6PD activity will have an adequate level of reduced glutathione (GSH) which, due to its antioxidant activity, is to a great extent responsible for the stability of the red blood cell. However, the females with normal G6PD activity group may be heterozygous and therefore may transfer the defective gene to their offspring, because the unique phenomenon of X-chromo-some inactivation occurs randomly in each cell of the female embryo with the respective X-chromosome remaining inactive throughout subsequent cell divisions for the duration of life (Lyon hypothesis). The result is a mosaic of X-chromosome activity so that the total G6PD activity of blood from heterozygous females varies markedly, ranging from normal red blood cell G6PD activity to levels as low as in hemizygous males.[3] The value of 146-376 U/10^{12} Rbc, obtained from 90 clinically healthy males and females was reported in Sigma Diagnostics (Procedure No. 345-UV). The aim of this report was to study the normal range of red blood cell G6PD activity in Thai newborn and adults and also the incidence of G6PD deficiency in males. G6PD activity was also studied in heterozygotes.

Materials and Methods

Blood collection

Samples were obtained from newborn, delivered at King Chulalongkorn Memorial Hospital, whose blood specimens were subjected to a methemoglobin reduction test, a screening test for G6PD, as part of jaundice evaluation at the Hematology Unit, Department of Pediatrics. Among these blood specimens more or less equal numbers of "deficiency" and "not deficiency" result were selected for quantitative assay. (male = 490, female = 156). Blood obtained from mothers of some newborns were, sometimes, sent to our unit for G6PD screening test. Among these mothers, there were 63 and 5 mothers having G6PD quantitatively deficient male and female newborn, respectively. Blood obtained from these 68 mothers were included in this study. Blood from healthy volunteers (male = 79, female = 50) were studied in 1988 for the quantitative assay. These volunteers were blood donors of the National Blood Center, nurses and staff of the Hospital. However, their G6PD status were not know before. One ml of blood was collected in 0.25 ml acid citrate dextrose (ACD) solution containing 1.3% trisodium citrate, 0.5% citric acid and 1.5% dextrose. Blood samples can be kept at 4°C for about 3 days before testing.

Determination of G6PD level[4,5]

Enzyme activity was quantitated by adding 0.05 ml of hemolysate to an 0.95 ml assay mixture containing buffer (0.1 M tris HCl PH8.0, 0.01M $MgCl_2$) , G-6-P (0.6mM) and NADP (0.2 mM). The rate of NADPH generation was measured at 340 nm at 30°C from 0-10 min. The average OD/min was calculated to determine the activity. The details of preparation of the hemolysate, test reagent and assay procedure have been described elsewhere.[4]

Calculation The G6PD activity is expressed in IU/100 ml Rbc. G6PD-Activity = $\frac{1 \times OD/min}{6.22} \times$ dilution $\times \frac{100}{Hct} \times \frac{100}{0.05}$ IU/100 ml Rbc

100 = Hct as %

100 = expressed as IU/100 ml Rbc

0.05 = amount of hemolysate used

1 = amount of assay mixture

6.22 = Absorbance of one μmol/ml of reduced NADP in a light path of one centimeter.

dilution = 0.1 ml washed Rbc sample: 1.9 ml DW = 1: 20

Hct = Hct of washed Rbe sample.

Hence G6PD Activity = $\frac{OD/min \times 6.44 \times 10^5}{Hct}$ IU/100 ml Rbc

Because the G6PD gene is transmitted via the x-chromosome, it is fairly simple to distinguish between deficiency and normal activity in males. Therefore the distribution of G6PD activity in male can show the cutpoint of G6PD deficiency.

Result

ACD blood from 490 male newborns who had jaundice were examined for G6PD activity. The data were arranged in class intervals. The distribution of these data is shown in Table 1 and Figure 1. According to the frequency in each class interval, the data could be divided into three groups, which were two large groups with G6PD deficiency and normal activity and a smaller one with G6PD activity in between the other two. The groups were called, Group I: Deficiency, Group II: Suspected and Group III: Normal Activity. The G6PD activity of each group was below 81, 81-150 and above 150 IU/100 ml with the cumulative frequencies of 274, 21 and 195, respectively

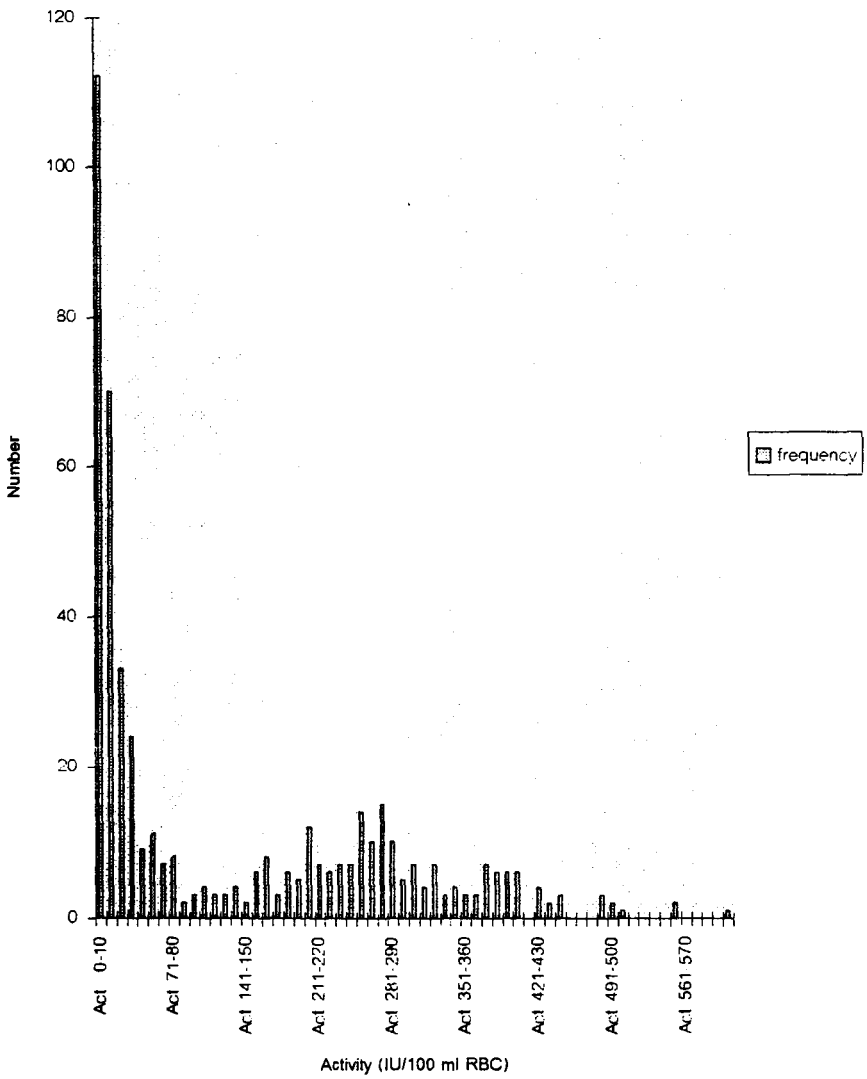

Figure 1. G-6-PD levels of selected male newborn (n=490)

Table 1. G6PD activity in 490 male newborn.

G6PD activity (IU/100 ml Rbc)	No. of cases	G6PD activity (IU/100 ml Rbc)	No. of cases
0 - 10	112	311 - 320	4
11 - 20	70	321 - 330	7
21 - 30	33	331 - 340	3
31 - 40	24	341 - 350	4
41 - 50	9	351 - 360	3
51 - 60	11	361 - 370	3
61 - 70	7	371 - 380	7
71 - 80	8	381 - 390	6
81 - 90	2	391 - 400	6
91 - 100	3	401 - 410	6
101 - 110	4	411 - 420	0
111 - 120	3	421 - 430	4
121 - 130	3	431 - 440	2
131 - 140	4	441 - 450	3
141 - 150	2	451 - 460	0
151 - 160	6	461 - 470	0
161 - 170	8	471 - 480	0
171 - 180	3	481 - 490	3
181 - 190	6	491 - 500	2
191 - 200	5	501 - 510	1
201 - 210	12	511 - 520	0
211 - 220	7	521 - 530	0
221 - 230	6	531 - 540	0
231 - 240	7	541 - 550	0
241 - 250	7	551 - 560	2
251 - 260	14	561 - 570	0
261 - 270	10	571 - 580	0
271 - 280	15	581 - 590	0
281 - 290	10	591 - 600	0
291 - 300	5	601 - 610	1
301 - 310	7		

Table 2. G6PD Activity in 490 male newborn

Group	Activity*	No.of Cases	P_{50}*
Gr.I deficiency	≤80	274	15
Gr.II Suspected	81-150	21	116
Gr.III Normal	>150	195	275

* Activity in IU/100 ml Rbc.

(Table 2).

The median values (Percentile at P_{50}) were 15, 116 and 275 IU/100 ml Rbc, respectively. The 274 male newborn were diagnosed hemizygous for G6PD-deficiency. The other group of 195 cases were diagnosed as normal However, these figures were not statistically significant due to sample selection based on the screening test. Only a few samples were found in the suspected group. This group may be the result of the clinical symptoms of an acute hemolytic episode and of the high G6PD activity in young red blood cells which circulate earlier in the blood stream in order to compensate for the anemia (self-limited hemolysis). These newborn should be followed up and the activity should be confirmed. If the activities remain at the same level, they may be hemizygous for other G6PD variants.

From this study, the cut point values of G6PD activity for diagnosis of "deficiency" and "normal activity" were found to be below 81 and above 150 IU/100 ml Rbc. These cut point values would be used for diagnosis of other blood specimens.

G6PD activity of the 156 female newborns were grouped into three groups according to the G6PD-activity of the male newborn. The median values (Percentile at P_{50}) (IU/100 m Rbc) and the number of cases of each group were 33, 116, 247 IU/100 ml Rbc, and 36, 36 and 84 cases respectively.

In accordance with genetic transmission in a sex-linked fashion, the mothers of male G6PD-deficient newborn may either be homo-zygotes or obligate heterozygotes for G6PD deficiency. The G6PD-activity was studied in 63 mothers of male G6PD-deficient newborn and is shown in Table 3.

The results showed two mothers (3.2%) whose G6PD activity was zero. The remaining 61 cases were grouped into the three groups according to the cut point values. Because of a small number in each group, the actual activities assayed (IU/100ml Rbc) were shown instead of P_{50}. The activities and number of cases (%) of each group were 21-77, 86-139, 152-372 IU/100ml Rbc and

Table 3. G6PD-Activity in 63 mothers of male G6PD-deficient newborn

Group	Range of activity*	Actual assayed activity*	No. of cases	% cases
	0	0	2	3.2
I	1-80	21-77	26	41.3
II	81-150	86-139	16	25.4
III	>150	152-372	19	30.1

*(IU/100 ml Rbc)

41.3, 25.4 and 30.1%, respectively. Mothers in group.I were diagnosed either homozygous or heterozygous because, as said before, some heterozygotes can have low activity as in hemizygotes. The mothers in group II and III were diagnosed heterozygous. There were about 30% obligate heterozygotes with normal levels of red blood cell G6PD activity. There were very few data on mothers with G6PD-deficient female newborn (N = 5). The actual activities assayed (IU/100 ml Rbc) and number of cases of each group were 29, 99-125, 169 and 1, 3, 1, respectively. However, without examining the husbands of these mothers, their G6PD status could not be determined.

G6PD activity was also studied in healthy adult male (N = 79) and female (N = 50) volunteers. The data derived was also grouped into three groups. The number of cases in each group is shown in Table 4. The data obtained with male and female volunteers were 10.13, 2.53, 87.33 and 6.0, 10.0, 84.0%, respectively. On average, about 85.5% of the healthy volunteers had normal G6PD activity. In the male groups, 8 of the 79 men tested were found to be G6PD-deficient. As mentioned above, G6PD is transmitted via the x-chromosome and therefore, the incidence in males is equal to the gene frequency[6] q (= 0.101). The expected frequency of homozygotes is q^2 = 0.0102 and that of heterozygotes 2q (1-q) = 0.182. The frequencies of homozygotes and heterozygotes found in this study were 0.06 and 0.100, respectively.

The normal range of G6PD activity in newborn and in adults has previously been reported.

Table 4. G6PD activity in male (N = 79) and female (N = 50) normal volunteers.

Group	Activity*	Actual assayed activity		No of cases		% cases	
		male	female	male	female	male	female
1.	0-80	10-37	12-72	8	3	10.13	6.0
2.	81-150	100-126	81-146	2	5	2.53	10.0
3.	>150	151-425	151-399	69	42	87.33	84.0

* Activity in IU/100 ml Rbc

Male and female newborn, as well as healthy volunteers, whose G6PD activities were above 150 IU/100 ml Rbc were included in this study. The G6PD activity in each group was arranged in class intervals. The percentile at P_5, P_{50} and P_{95} was calculated. The values between P_5 and P_{95} represent the normal range and the P_{50} values are the median values. (Table 5).

Table 5. Normal range and median value of G6PD activity in newborns and adults.

		P_5-P_{95}*	P_{50}*
Newborns	Male (N = 195)	166-449	275
	Female (N = 84)	157-439	247
Adult	Male (N = 69)	160-337	229
	Female (N = 42)	158-341	219

*Activity in IU/100 ml Rbc.

The normal ranges in male and female newborn and in male and female adults amounted to 166-449, 157-439, 160-337 and 158-341 IU/100 ml Rbc, respectively. The median values are 275, 247, 229 and 219 IU/100 ml Rbc, respectively.

In comparison with normal adults, the G6PD activity in newborn was found to be significan-tly higher (Mann-Whitney U test, P = 0.000 and 0.004 in males and in females respectively).

The values were more or less identical in males and females (Mann-Whitney U test, P = 0.990 and 0.791 in newborn and in adults respectively).

Discussion

Quantitative assays of G6PD activity can be based on the reference of one g Hb or ml of red blood cells. But the latter may be more meaningful when red blood cells are hypochromic.[4] Based on quantitative studies in male newborn, G6PD activity under 81 IU/100 ml Rbc is considered G6PD deficiency. This value is close to the value of 70 IU/100 ml Rbc reported by Hathirat P et al.[2] The maximum activity of the deficient group in male adults amounted to 37 IU/100 ml Rbc which represents about half of 80 IU/100 ml Rbc. This may be due to the small number of cases. G6PD activity over 150 IU/100 ml Rbc which is close to the value of 146 reported in Sigma Diagnostics (Precedure No. 345-UV), is considered normal. Due to the irregular distribution of the data, the mean value and normal range were represented as percentile at P_{50} and between P_5 and P_{95}, respectively. The normal range was similar in both sexes. About 30% of the obligate heterozygotes had normal results, therefore, additional heterozygotes must have been among those with normal test results. The incidence of G6PD deficiency was studied. Eight of the 79 males were found to be G6PD deficient. The incidence in males is equal to the gene frequency (q = 0.101). This figure was comparable with the figure of 0.12 reported by Panich V.[7] The expected frequency of heterozygotes plus homozygotes in females could be calculated according to the following formula: $2q(1-q) + q^2$[6] which yields a figure of 0.192. From this study, and the study of obligate

heterozygotes the frequency found for homozygotes was higher than expected. This apparent excess of G6PD-deficient females is likely to be due to imbalanced x-inactivation in heterozygotes.[3] However, the total frequency of homozygotes and heterozygotes found in this study was 0.160 which is comparable with the expected value of 0.192.

The quantitative assay of G6PD activity for diagnosis of G6PD deficiency is important because G6PD deficiency can cause susceptibitity to neonatal jaundice and consequently fever-or drug-induced hemolytic anemia. Individuals with normal levels of G6PD activity will have an adequate reducing potential of NADPH to protect red blood cells from oxidative damage. However, for the detection of heterozygotes, determining the extent of enzyme activity cannot be relied upon to that end. The most accurate method consists of detecting the mutation in genomic DNA. There are many G6PD variants in Thailand[7-9] and some variants have been characterized at the DNA level.[10]

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