SEMIQUANTITATIVE SCREENING TEST FOR G6PD DEFICIENCY DETECTS SEVERE DEFICIENCY BUT MISSES A SUBSTANTIAL PROPORTION OF PARTIALLY-DEFICIENT FEMALES

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Abstract. Neonatal screening for G6PD deficiency has long been established in many countries. The aim of the study was to determine whether the routine semiquantitative fluorescent spot test could detect all cases of G6PD deficiency, including those cases with partial deficiency (residual red cell G6PD activity between 20-60% of normal). We compared the results of G6PD screening by the semiquantitative fluorescent spot test and quantitative G6PD activity assay on a group of 976 neonates and 67 known female heterozygotes. The values for mean G6PD activity of G6PD-normal neonates and 293 healthy adult females were determined. There was no significant difference in the mean normal G6PD activity between the two racial groups in the neonates (669 Malays, 307 Chinese) and in the 293 healthy adult females (150 Malays, 143 Chinese) group. The values for the upper limits of total deficiency (20% of normal residual activity) for neonates and adult females were 2.92 U/gHb and 1.54 U/gHb, respectively. The upper limits of partial deficiency (60% of normal residual activity) were 8.7 U/gHb and 4.6 U/gHb respectively. The prevalence of G6PD deficiency among the male neonates was 5.1% (26) by both the fluorescent spot test and the enzyme assay method. The G6PD activity levels of all 26 cases of G6PD-deficient male neonates were <20% normal (severe enzyme deficiency). In the female neonate group, the frequency of G6PD deficiency was 1.3% (6 of 472) by the fluorescent spot test and 9.35% (44 of 472) by enzyme assay. The 6 cases diagnosed as deficient by the fluorescent spot test showed severe enzyme deficiency (<2.92 U/gHb). The remaining 38 female neonates had partial enzyme deficiency and all were misdiagnosed as normal by the fluorescent spot test. In the female heterozygote group, G6PD deficiency was diagnosed in 53% (35 of 67) by enzyme assay and in 7.5% (4 of 67) of cases by the fluorescent spot test. The 4 cases detected by fluorescent spot test had severe enzyme deficiency (<1.6 U/gHb). The remaining 31 (46.3%) cases, diagnosed as normal by fluorescent spot test, showed partial G6PD deficiency. In conclusion, we found that the semiquantitative fluorescent spot test could only diagnose cases of total G6PD deficiency and misclassified the partially-deficient cases as normal. In this study, the overall prevalence of G6PD deficiency was 3.28% by the semiquantitative fluorescent spot test and 7.17% by enzyme assay. This means that 3.9% of G6PD-deficient neonates were missed by the routine fluorescent spot test and they were found to be exclusively females. This study demonstrates a need to use a method that can correctly classify female heterozygotes with partial G6PD deficiency. The clinical implication is that these individuals may be at risk of the hemolytic complication of G6PD deficiency.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6 PD) is an important enzyme in the hexosemonophosphate shunt and plays a key role in the production of NADPH required for detoxification of toxic products of oxidative stress. G6PD deficiency is the commonest enzymopathy in humans, estimated to affect 400 million individuals worldwide. It has a wide geographical distribution, reaching high frequencies in certain parts of Africa, the Mediterranean and Asia. Although most individuals are asymptomatic, the clinical importance is that exposure of G6PD-deficient individu-

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als to oxidative stress may result in hemolytic anemia, and that G6PD-deficient neonates are at risk of severe hyperbilirubinemia with subsequent complications of kernicterus (Meloni *et al*, 1983; Fok and Lau, 1986).

The diagnosis of G6PD deficiency can be made easily by semiquantitative tests or by quantitation of red cell enzyme activity. The broad geographical distribution of this defect and the high prevalence in developing countries makes it important for many countries to adapt tests that are simple and inexpensive. The semiquantitative fluorescent spot test described by Beutler (1966a,b) and subsequently modified (Beutler and Mitchell, 1968) is widely used for diagnosis and population screening. Screening tests aim at classifying subjects as G6PD-normal or G6PD-deficient and therefore, reliably detect hemizygous males (and homozygous females). It is known that semiquantitative screening tests are not reliable in detecting heterozygous females whose overall residual red cell G6PD level may be normal or deficient. Many female heterozygotes may have mild to moderate reduction of red cell G6PD level, varying from 20% to 60% of normal (partial deficiency). Studies have shown that the semiquantitative test could only pick up those cases with a residual red cell G6PD activity level of less than 20% of normal, therefore missing the diagnoses of the partially deficient individuals (Reclos *et al*, 2000).

G6PD deficiency is common in Malaysia, with an overall incidence of 3.1% among males, and is a very important cause of severe neonatal hyperbilirubinemia (Tan, 1981; Singh, 1986; Hon *et al*, 1989). In Malaysia, a neonatal screening program using the fluorescent spot test has been in place in all public hospitals since 1980. We carried out a study to determine whether the fluorescent spot test could detect all cases of G6PD deficiency, where we defined G6PD deficiency as a residual red cell G6PD activity level of < 60% normal (WHO Working Group, 1989). We compared the results of the fluorescent spot test and the G6PD activity assay on a group of neonates whose cord blood samples were sent for routine screening and on a group of known female heterozygotes (mothers of G6PD-deficient neonates). The study on known female heterozygotes would provide us with information on the range of G6PD activity, frequency of phenotypically-deficient female heterozygotes, and determine whether they can be diagnosed by the semiquantitative test. We first established normal ranges of G6PD activity in both neonates and adults for the two major ethnic groups (Malays and Chinese) and used the mean activity to establish the 20% and 60% cut-off points for each group.

MATERIALS AND METHODS

Neonatal population

A total of 669 Malay (335 males; 334 females) and 307 Chinese (163 males; 144 females) neonates born in Hospital UKM (universiti Kebangsaan Malaysia) between July 1999 and December 1999, were studied. The fluorescent spot test and the G6PD activity assay were performed on EDTA cord blood samples from these subjects sent to the laboratory for routine screening for G6PD deficiency. Values of mean normal G6PD activity and the 2 standard deviations were established for those neonates with normal fluorescent spot test results.

Normal female population

G6PD activity assays were performed on EDTA peripheral blood samples from 293 (150 Malays and 143 Chinese) normal healthy adult female blood donors and perinatal women (Malays: 75 /150, Chinese: 73/143) in order to determine the mean normal activity. None of the females had any diagnosed health problem at the time of sampling.

Female heterozygotes

To support the theory that G6PD activity in female heterozygotes is variable, *ie* that it can range from total to partial deficiency and normal, and to determine whether the semiquantitative test can diagnose partial G6PD deficiency, we studied 67 female heterozygotes (40 Malays, 27 Chinese) who were mothers of known G6PD-deficient babies born in Hospital UKM between September 2000 and December 2000. G6PD-deficient males were identified during routine neonatal screening and their mothers were either

traced back to the wards or recalled for G6PD evaluation. For all cases, EDTA blood samples were collected. The fluorescent spot test, full blood pictures and reticulocyte counts were performed on the same day, and the G6PD assay was performed within 2 days of collection.

Blood samples received from all neonate and adult cases were subjected to semiquantitative fluorescent spot test, full blood picture and reticulocyte count, performed on the same day of collection. For the semiquantitative fluorescent spot test, blood specimens from all samples were spotted and dried on Whatman's filter paper. The test was carried out according to the method described by Beutler and Mitchell (1968). This technique is based on the visual evaluation of fluoresced reduced pyridine nucleotide (NADPH) when activated by UV light. Samples that do not show fluorescence are classified as G6PD-deficient and those showing fluorescence are classified as normal. For the quantitative evaluation of G6PD activity, the G6PD kit from Randox Laboratories Ltd was used. The kit utilizes the chemical reaction described by Beutler (1968), and the NADPH produced is measured at 340 nm in a kinetic mode. The method involved an elution stage for the lysis of red cells and an assay stage that involved incubation with reagents containing substrate and the cofactor NADP, followed by photometric measurement of the kinetic reaction at 340 nm. The assay was performed according to the manufacturer's instructions with modification for normalization of hemoglobin concentration. 0.2 µl of whole blood was washed with 2 ml aliquot of NaCl solution, centrifuged for 10 minutes at 3,000 rpm; the process was repeated three times. The washed erythrocytes were then suspended in 0.5 ml of digitonin solution and allowed to stand for 15 minutes at 4ºC, followed by centrifugation. The supernatant was then used for the enzyme assay in a Hitachi 717 (Boehreinger Manheim, Germany) autoanalyser within 2 hours of preparation where the G6PD activity was expressed in G6PDH mU/erythrocytes per ml x 100. In this study, G6PD activity was finally expressed in U/gHb using the following calculation:

$$\text{G6PDH U/gHb} = \frac{\text{G6PDH mU/erythocytes per ml x 1,000}}{\text{Hb g/dl}}$$

The hemoglobin concentration (g/dl) for each sample was measured on a Coulter Stac S cell counter. 100 = a factor to convert G6PD activity to 100 ml.

Diagnosis of G6PD deficiency by enzyme activity includes total deficiency (activity<20% of mean normal activity) and partial deficiency (20-60% of mean normal activity).

RESULTS

Neonatal population

The results for mean normal G6PD activity, and the cut-off points for partial G6PD deficiency for 976 neonates (669 Malay and 307 Chinese) are shown in Table 1. There were no significant differences in the mean G6PD activity between sexes within and between both racial groups. The lower and upper cut-off points for partial deficiency were 2.92 U/gHb (20% normal mean) and 8.7 U/gHb (60% normal mean), respectively.

Results of screening for G6PD deficiency, by the fluorescent spot test and enzyme assay for Malays and Chinese, are shown in Tables 2 and 3, respectively. In the male Malay neonate group, G6PD deficiency was diagnosed in 5.1% of cases by both the semiquantitative fluorescent spot test and enzyme assay. In the female Malay neonates, G6PD deficiency was diagnosed in 1.2% of cases by fluorescent spot test and 9.0% of cases by G6PD activity assay. In the male Chinese neonate group, the percentages of G6PD deficiency were the same, by both the fluorescent spot test and the G6PD assay, *ie* 5.5%, whereas in the female group the percentages of G6PD deficiency differed markedly between the two methods, *ie* 1.4% and 9.7%, respectively. All the neonates (males and females) who were diagnosed as deficient by fluorescent spot test showed G6PD activity levels of < 2.92 U/gHb (<20% normal activity), with a range of 0-1.7 U/gHb; the mean G6PD activity was 0.65 U/gHb and 1.05 U/ gHb for the Malay and the Chinese neonates, respectively (Table 4). 7.8% of female Malay and 8.3% of female Chinese neonates had partial enzyme deficiency with their G6PD activity levels ranging from 2.98-8.5 U/gHb. None of these cases was picked up as deficient by the fluorescent spot

Table 1
G6PD activity levels for normal Malay and Chinese neonates.

	Mean G6PD activity U/gHb	Upper limit of total deficiency (20% of mean value U/gHb)	Upper limit of partial deficiency (60% of mean value) U/gHb
Malay (n=669)	14.8	2.96	8.8
Chinese (n=307)	14.3	2.86	8.5
Total (976)	14.55	2.92	8.65

There was no significant difference in mean G6PD activity between male and female neonates in each racial group ($p < 0.05$).

Table 2
Diagnosis of G6PD deficiency in Malay neonates by semiquantitative and quantitative methods.

	Method	No. of cases with total deficiency (G6PD activity <20% normal)	No. of cases with partial deficiency (G6PD activity 20-60% normal)	No. of cases with normal G6PD activity	G6PD-deficient individuals (%)
Male (n=335)	Semiquantitative FST	17	0	318	5.1
	Quantitative	17	0	318	5.1
Female (n=334)	Semiquantitative	4	0	330	1.2
	Quantitative	4	26	304	9.0

G6PD activity ranged from 0 -1.48 U/gHb for neonates with total G6PD deficiency, and 3.2-8.6 U/gHb for neonates with partial G6PD deficiency.

test. The overall frequencies of G6PD deficiency were 3.28% by fluorescent spot test, and 7.17% by enzyme assay (Table 4).

Adult female population

The results of the G6PD activity assay in 293 adult normal females (150 Malays; 143 Chinese) are shown in Table 5. The mean values for G6PD activity, for adult Malay and Chinese females were 7.45 U/gHb and 7.50 U/gHb, respectively. There was no significant difference in the mean values between these two racial groups ($p = 0.54$). The mean value for G6PD activity for women in their perinatal period was slightly higher compared to normal adult females but the difference was not statistically significant ($p=0.08$). Since all samples from female heterozygotes were taken during the perinatal period, we used the mean G6PD activity values for perinatal women (Malay: 7.67 U/gHb; Chinese: 7.75 U/gHb) to determine the upper and lower limits of partial G6PD deficiency (Table 5). The values for the upper limit of partial deficiency were 4.6 U/gHb (Malays) and 4.65 U/gHb (Chinese); the lower limits were 1.53 U/gHb (Malays) and 1.55 U/gHb (Chinese).

Female heterozygotes

Using the above established upper and lower limits for G6PD deficiency, 53% (23 of 40 Malays, 12 of 27 Chinese) were classified as G6PD-deficient by enzyme assay *ie* G6PD activity < 60% normal residual activity (<4.65 U/gHb) (Table 8). Of these 35 G6PD-deficient females, 31 cases (19 of 40 Malays, 12 of 27 Chinese) had enzyme levels between 20-60% normal (1.53 U/gHb-4.65 U/gHb) *ie* partial enzyme deficiency and 4 (all Malay) cases had severe deficiency, *ie*

Table 3
Diagnosis of G6PD deficiency in Chinese neonates by semiquantitative and quantitative methods.

	Method	No. of cases with total deficiency (G6PD activity <20% normal)	No. of cases with partial deficiency (G6PD activity 20-60% normal)	No. of cases with normal G6PD activity	G6PD-deficient individuals (%)
Male (n=153)	Semiquantitative FST	9	0	154	5.5
	Quantitative	9	0	154	5.5
Female (n=144)	Semiquantitative	2	0	142	1.4
	Quantitative	2	12	142	9.7

G6PD activity ranges from 0-1.7 U/gHb for neonates with total G6PD deficiency, and 2.98-8.17 U/gHb for neonates with partial G6PD deficiency.

Table 4
G6PD activity in G6PD deficient neonates and overall frequency of G6PD-deficiency according to ethnic group, by semiquantitative and quantitative methods.

G6PD deficiency	Ethnic Group (no. of neonates)	Range of G6PD activity U/gHb	Mean of G6PD activity U/gHb	No. (Frequency) diagnosed by semiquantitative method	No. (Frequency) diagnosed by quantitative method
Total	Malays (n=669)	0-1.48	0.65	21 (3.1%)	21 (3.1%)
Deficiency	Chinese (n=307)	0-1.7	1.05	11 (3.6%)	11 (3.6%)
Partial	Malays (n=669)	3.2-8.6	7.01	0	26 (3.9%)
Deficiency	Chinese (n=307)	2.89-8.17	6.0	0	12 (3.9%)
Total no of G6PD-deficient cases				32 (3.28%)	70 (7.7%)

Total number of neonates studied: 976

Table 5
Mean G6PD activity for normal perinatal females and G6PD activity value used as cut-off points for partial G6PD deficiency.

	Mean G6PD activity U/gHb	Lower limit for partial G6PD deficiency (20% of normal mean G6PD activity) U/gHb	Upper limit for partial deficiency (60% of normal mean G6PD activity) U/gHb
Malays (n=75)	7.67	1.53	4.60
Chinese (n=73)	7.75	1.55	4.65

G6PD activity assay was performed on a total of 293 normal females including 148 perinatal women. The overall mean G6PD activity was 7.47 U/gHb, SD 1.34. There was no significant difference in the mean G6PD activity between perinatal women and female blood donors (p=0.08). The G6PD activities for perinatal women was used for the normal range in this study because all the test female heterozygote samples were taken during the perinatal period G6PD activity ranges.

Table 6
Diagnosis of G6PD deficiency in Malay female heterozygotes by semiquantitative and quantitative methods.

Method	Cases with total G6PD deficiency (No.)	Cases with partial deficiency (No.)	Cases with normal G6PD activity (No.)	Total no. of G6PD deficiency detected (percentage)
Semiquantitative (n=40)	4	0	36	4 (10%)
Quantitative (n=40)	4	19	17	23 (59%)

Total no. of G6PD-deficient cases includes total and partial deficiency.
Range of G6PD activity for heterozygotes with total deficiency: 0.79-1.47 U/gHb and partial deficiency: 2.10-4.42 U/gHb.

Table 7
Diagnosis of G6PD deficiency in Chinese female heterozygotes by semiquantitative and quantitative methods.

Method	Cases with total G6PD deficiency (No.)	Cases with partial deficiency (No.)	Cases with normal G6PD activity (No.)	Total no. of G6PD deficiency detected[a] (percentage)
Semiquantitative (n=27)	0	1	26	1 (3.6%)
Quantitative (n=27)	0	12	15	12 (44%)

[a]Total no. of G6PD deficient cases includes total and partial deficiency.
Range of G6PD activity for partial deficiency: 1.82-4.14 U/gHb.

G6PD activity < 20% normal (<1.55 U/gHb). All the partially-deficient cases, except for one Chinese female (G6PD activity level : 1.82 U/gHb), were misclassified as normal by fluorescent spot test. All the 4 cases with severe enzyme deficiency were picked up as deficient by the fluorescent spot test. Tables 6 and 7 show the frequency of G6PD deficiency, by both methods, in Malay and Chinese female heterozygotes. The overall range of G6PD activity in female heterozygotes with total G6PD deficiency was 0.79 U/gHb-1.47 U/gHb and the range for partial deficiency was 1.82 U/gHb-4.42 U/gHb (Table 8). The highest G6PD activity value detected as deficient by fluorescent spot test in this group of adult females was 1.82 U/gHb (23.5% normal residual activity) and the lowest value detected as normal by fluorescent spot test was 2.1 U/gHb (23.3% normal residual activity).

DISCUSSION

G6PD deficiency, although X-linked, is not a recessive disorder. In female heterozygotes, red-cell mosaicism arising from random X chromosome inactivation results in two populations of G6PD-deficient and G6PD-normal cells (Beutler *et al*, 1963; Davidson *et al*, 1963). The proportions of these two cell types can vary enormously, ranging from completely normal activity to complete deficiency. Many of the female heterozygotes may have overall G6PD activity that ranges from 20-60% normal residual activity, classified as partial deficiency.

Hemolysis has always been thought to be a problem of the hemizygous male, who is almost always phenotypically deficient. However, the actual G6PD content of the cell is not the only critical determinant of a hemolytic episode. Oxi-

Table 8
Overall frequency of G6PD deficiency by semiquantitative and quantitative methods and G6PD activity in female heterozygotes.

	Range of G6PD activity for cases with total G6PD deficiency	G6PD U/gHb	Activity	Frequency of G6PD deficiency diagnosed by semiquantitative method	Frequency of G6PD deficiency diagnosed by quantitative method
	Lowest value	Highest value	Mean		
Malays (n=40)	0.79	9.95	4.50	10 (10%)	23 (59%)
Chinese (n=27)	1.82	9.8	5.06	1 (3.7%)	12 (44%)
Overall (n=67)	0.79	9.95	4.71	5 (7.5%)	35 (53%)

Overall range of G6PD activity for cases with total G6PD deficiency is 0.79-1.47 U/gHb and partial deficiency 1.82-4.42 U/gHb.

dative stress induced by factors such as infections, administration of drugs, ingestion of certain kinds of food, contact with mothballs and certain cultural practices have been reported to induce hemolysis even in partially-deficient individuals (Luzzatto and Mehta, 1995). Individuals with class III G6PD variants (G6PD activity 10-60% normal) do have problems of intermittent hemolysis. A common Class III variant found in this part of the world is the G6PD Mahidol variant (Panich *et al,* 1972). Partial enzyme deficiency is expected to be seen more commonly among female heterozygotes as a result of X inactivation. The majorities of G6PD variants that cause G6PD deficiency seen in most populations belong to the class II variants (severe enzyme deficiency). Previous studies of Malay and Chinese neonates with G6PD deficiency in Malaysia showed that the majority of the male G6PD-deficient neonates had severe enzyme deficiency (Boo *et al,* 1995; Ainoon *et al,* 1999). Therefore, partial deficiency remains a problem of heterozygous females who may be at risk of hemolysis. Heterozygous female neonates have been shown to be at risk of neonatal hyperbilirubinemia (Davidson *et al,* 1963; Kaplan *et al,* 1999). Meloni *et al* (1983) found that 15.5% of female heterozygotes developed neonatal jaundice. Reclos *et al* (2000) reported a case of a 16-year-old female with partial deficiency (G6PD activity 5.5 U/gHb) who developed severe hemolysis. It was recently shown that G6PD plays an embryoprotective role in the development of oxidative stress and chemical teratogenesis in mice. Litters of G6PD-deficient animals showed increased incidence of perinatal and postnatal death and that the developmental risk was potentially substantial even for heterozygous G6PD-deficient animals (Nicol *et al,* 2000).

Routine neonatal screening for G6PD deficiency by fluorescent spot test is carried out in all hospitals in Malaysia as part of a strategy for early detection and prevention of severe hyperbilirubinemia and kernicterus. In this study, we performed both the fluorescent spot test and the G6PD assay on two groups of individuals at risk of complications of G6PD deficiency, to determine whether our screening test could diagnose all cases of G6PD deficiency (total and partial). In establishing the normal values of G6PD activity, there was no significant difference in the mean G6PD activity between males and females between the two racial groups, and within each racial group, for both the neonates and adults. The means of G6PD activity for the Malay and Chinese neonates were 14.8 U/gHb and 14.3 U/gHb, respectively. However, the adult mean G6PD activity values was found to be significantly lower than the neonates *ie* 7.67 U/gHb and 7.75 U/gHb, for the Malay and Chinese groups, respectively. These findings are comparable to previous findings where G6PD activity has been shown to vary according to age group (Normah *et al,* 1991; Boo

et al, 1995).

The results of the fluorescent spot tests and the G6PD assays on 472 male neonates showed no differences in the frequencies of G6PD deficiency, *ie* both tests diagnosed G6PD deficiency in 5.3% (26) of cases. All the 26 male G6PD-deficient neonate cases had severe enzyme deficiency, *ie* their residual red cell G6PD activity was lower than 20% of normal (< 2.92U/gHb), ranging from 0-1.7 U/gHb, with a mean of 0.85 U/gHb. We did not find any male neonate case with partial enzyme deficiency, which means that all of the G6PD-deficient male neonates in this group had severe enzyme deficiency, a finding comparable to our previous studies (Boo *et al*, 1995; Ainoon *et al*, 1999) and that there was no problem in using the screening fluorescent spot test to diagnose male G6PD-deficiency.

However, in the group of 446 female neonates, we found a marked difference between the results of fluorescent spot test screening and the G6PD activity assay. The fluorescent spot test diagnosed G6PD deficiency in only 1.3% (6) of cases and the quantitative G6PD assay diagnosed 9.8% (44) of cases as G6PD-deficient (both total and partial). All the 6 female neonate cases diagnosed as G6PD-deficient by fluorescent spot test had total deficiency (residual red cell G6PD activity lower than 20% normal). Therefore, 8.5% (38) of the G6PD-deficient female neonates were misdiagnosed as normal by the routine semiquantitative screening test and their residual G6PD activity ranged from 19.8-58% of normal residual activity (2.98 U/gHb-8.5 U/gHb) with a mean G6PD activity value of 6.5 U/gHb. These cases were neonates with partial G6PD deficiency.

Examining the overall (both male and female) prevalence of G6PD deficiency in this study, we found that by using the semiquantitative screening test, the prevalence of G6PD deficiency was 3.28%, but using the quantitative enzyme assay the prevalence was 7.17%. Thus the semiquantitative screening test failed to diagnose 3.9% of neonates with G6PD deficiency and these neonates were found to be exclusively females. Similar findings were made by Reclos *et al* (2000), where they screened 2,000 neonates and found that the fluorescent spot test missed the diagnosis of 2.4% of neonates with partial G6PD deficiency, and they were all females. It appears that the semiquantitative fluorescent spot test used for population screening is not able to diagnose a substantial proportion of neonates with G6PD deficiency who were exclusively heterozygous female neonates.

The results of our study on mothers of known G6PD-deficient male babies supported the notion that the semiquantitative screening test fails to diagnose partial deficiency in female heterozygotes. The study of 67 known female heterozygotes revealed a similar discrepancy in the diagnosis of G6PD deficiency between the fluorescent spot test and the quantitative enzyme assay. Firstly, we found that the range of residual red cell G6PD activity in the female heterozygotes was wide, ranging from 8-128% of normal activity, in concordance with Lyon's theory. Fifty-three percent (35 of 67) of cases were diagnosed G6PD-deficient (G6PD activity level of < 60% normal) by enzyme assay and only 7.5% (5 of 67) were found deficient by the fluorescent spot test. Out of the 35 cases diagnosed as deficient by enzyme assay, 31 (46.3%) had partial G6PD deficiency; their G6PD activity values ranged from 23.3-57.6% of normal (2.10 U/gHb-4.42 U/gHb) and all of these partially-deficient cases, except one, were misclassified as normal by the fluorescent spot test. The remaining 4 cases had severe enzyme deficiency (0.79-1.47 U/gHb) and all were picked up by the fluorescent spot test. The one case of partial deficiency diagnosed as deficient by the semiquantitative screening test was a Chinese female heterozygote whose enzyme activity level was 1.82 U/gHb (23.5% of normal). Therefore, in this group of heterozygous females, G6PD deficiency was diagnosed in 7.5% (5 of 67) of cases by the fluorescent spot test, whereas the G6PD assay diagnosed 53% (44 of 67) of the cases. This means that 46.3% of female heterozygotes had partial G6PD deficiency and in almost all of these cases, the diagnosis was missed by the fluorescent spot test. Reclos *et al* (2000) demonstrated a similar discrepancy in the results of the two tests. The quantitative enzyme assay detected 28 cases of G6PD deficiency in a group of relatives of G6PD-deficient individuals and the semiquantitative only detected 9 (32%) of these 28 cases to be deficient.

Residual red cell G6PD activity values as low as 2.98 U/gHb (20.6% of normal activity for neonates) in a female neonate and 2.1U/gHb (23.3% of normal activity for female adults) in an adult female heterozygote, were classified as normal by the fluorescent spot test. A residual red cell activity of 1.82 U/gHb (23.5% of normal activity for female adults) in a Chinese female was picked up as deficient by the fluorescent spot test. It appears that the fluorescent spot test we employed, could only detect cases with a residual red cell enzyme activities lower activities lower than a cut-off point that is lies between 2.1 U/gHb and 1.82 U/gHb. A G6PD activity level of 2.1 U/gHb has been shown by other workers to be the cut-off limit for the fluorescent spot test to detect G6PD deficiency. Reclos *et al* (2000) found that the 2.1 U/gHb level was a value 20% of their normal mean. In their study of 2,000 neonates, using 20% of normal activity as the upper limit of G6PD deficiency, they found that the results of the two methods would be identical *ie* both methods would give the same frequencies. However, if the cut-off point for G6PD deficiency were set at 60% of normal mean activity, as is generally accepted, the frequency of G6PD deficiency by enzyme assay was higher than the frequency detected by fluorescent spot test, and there were more G6PD-deficient females than G6PD-deficient males. The findings in our study are similar to those of Reclos *et al* (2000) where, when 20% normal activity was set as the cut-off point for the diagnosis of G6PD deficiency, there was little difference in the frequencies between the two tests, both in the neonate and the adult female heterozygote groups. Although the actual value for the cut-off G6PD activity level for the fluorescent spot test to detect deficiency in our study appears to be between 2.1U/gHb and 1.82 U/gHb, a level that is slightly more than 20% normal activity for adults and slightly lower than 20% normal activity for neonates, we found that the fluorescent spot test still missed almost all cases of partial deficiency and detected all cases of total deficiency. Among the severely deficient neonates and female heterozygotes the highest G6PD activity values detected by the fluorescent spot test were less than 20% normal activity *ie* 1.7 U/gHb (12% of normal activity for neonates) and 1.47 U/gHb (19.2% of normal activity for adult females), and among the partially-deficient neonates and female heterozygotes the lowest G6PD activity values missed by the fluorescent spot test were more than 20% normal residual activity *ie* 2.98 U/gHb (20.6% of normal neonates) and 2.1 U/gHb (23.3% of normal adult females).

When we set the cut-off point for G6PD deficiency at 60% normal residual activity, by using the enzyme assay, there appeared to be more G6PD-deficient female neonates than male neonates (9.8% *vs* 5.3%) compared to frequencies based on the fluorescent spot test (1.3% *vs* 5.3%). The frequency of G6PD deficiency is usually expressed as the proportion of males who are found to be hemizygous and this figure is usually considered to be equal to the gene frequencies and the prevalence of G6PD-deficient females and the heterozygotes are usually derived by calculation.

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