Establishing the cut off value for glucose 6 phosphate dehydrogenase using the heel prick sample sent for newborn screening

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency remains the most prevalent enzyme deficiency in the world. It is estimated that nearly 330 million people may be affected by G6PD deficiency worldwide with a global prevalence of 4.9%. The overall incidence of G6PD deficiency in Malaysia were 5.3% among males and 1.05% among females.

G6PD is required for the generation of nicotinamide adenine dinucleotide phosphate (NADPH) which will maintain glutathione in its reduced form. This reduced glutathione is vital in defence against reactive oxygen species that can lead to destruction of the red blood cell. Therefore, G6PD deficiency may cause haemolytic anaemia and neonatal jaundice; which if severe enough can lead to permanent neurologic damage (kernicterus) and death.

In Malaysia, screening for G6PD deficiency using cord blood was commenced in 1980. The assays used in the screening programmes are based on the qualitative fluorescent spot test (FST) method which is simple, rapid and affordable. However, none of the screening tests can diagnose heterozygous females reliably, because X-chromosome mosaicism leads to partial deficiency. Heterozygous females with extremely skewed X inactivation have activity ranging from hemizygote to normal.

The objective of this study is to compare the cord blood FST with the quantitative enzyme assay using the heel prick sample and to establish the cut-off value for G6PD using the heel prick sample.

Methods and Materials

This study is retrospective in nature. Samples from newborn were collected after day 2 of life for newborn screening in our hospital. Guthrie cards were stored at -80^{o}C after the analysis of newborn screening. These samples were used for the study purpose.

A total of 602 (312 males and 290 females) neonates were included in this study. The 602 samples were analysed using neonatal G6PD assay (Labsystems Diagnostics Oy). It is based on an enzymatic method intended for the quantitative determination of G6PD activity from dried blood spots. The rate of NADPH formation is proportional to the G6PD activity, and is determined fluorometrically. G6PD activity was recorded as IU/gHb. The results were compared with the qualitative fluorescent assay using the cord blood.

Analysis of the frequency distribution of G6PD values were performed using Analyse-it software. The best cut off value for G6PD activity was obtained from the receiver operating characteristic (ROC) curve analysis, which will be able to discriminate G6PD deficient from normal.

Results

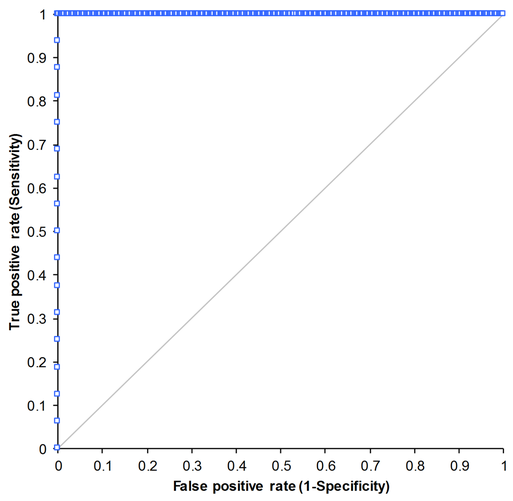
The descriptive statistics and histogram of G6PD levels of the dried blood spot samples showed the data was normally distributed (Gaussian distribution). Hence parametric method was used to determine the reference interval. The 2.5th percentile was used as the cut off value for the diagnosis of G6PD deficiency and it was noted to be <3.75 IU/gHb and <4.38 IU/gHb for males and females respectively (Table 1).

Analysis of G6PD activities from the cord blood samples using the fluorescence spot test showed that 21 newborns (16 males, 5 females) are G6PD deficient. Among the 5 females, 4 were found to have partial G6PD deficiency.

ROC curve analysis showed that the area under curve was 1.00 and at the cut off value of 3.75 IU/gHb for male newborn, the sensitivity and specificity were 100% (Figure 2A). At the cut off value of 4.38 IU/gHb for female newborn, the sensitivity and specificity were 80% and 97.2% respectively. Whereas at the cut off value of 4.55 IU/gHb the sensitivity improved to 100% and the specificity of the assay was 96.5% (Figure 2B).

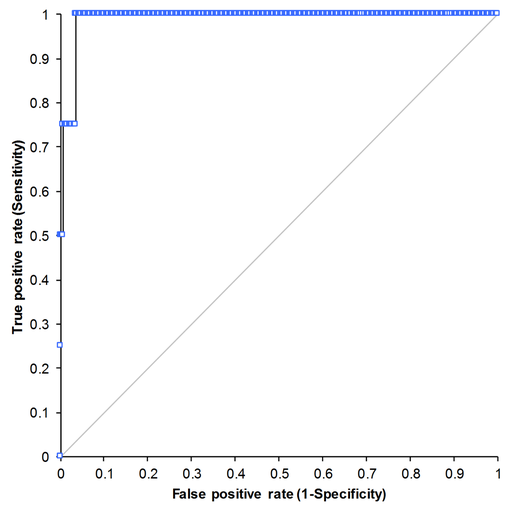
	Lower 2.5th percentile	Confidence interval
Male	3.75	3.3 to 4.2
Female	4.38	3.99 to 4.78

Table 1 : Cut off value for G6PD in male and female



G6PD (Positive test < cut off)	TP rate (sensitivity)	95% CI	TN rate (specificity)	95% CI
3.703500	1.000	0.794 to 1.000	1.000	0.988 to 1.000

Figure 2A: Receiver operating characteristic (ROC) curve of G6PD level in male newborns



Test	Area	95% CI	SE	Z	p
G6PD	0.99	0.98 to 1.00	0.007	66.69	< 0.0001

G6PD (Positive test < cut off)	TP rate (sensitivity)	95% CI	TN rate (specificity)	95% CI
4.37020	0.800	0.284 to 0.995	0.972	0.945 to 0.988
4.40650	0.800	0.284 to 0.995	0.968	0.941 to 0.985
4.42030	0.800	0.284 to 0.995	0.965	0.936 to 0.983
4.55310	1.000	0.478 to 1.000	0.965	0.936 to 0.983

Figure 2B: Receiver operating characteristic (ROC) curve of G6PD level in female newborns

In this study, at cut off value of 3.75 IU/gHb for male newborn, all newborn was identified as G6PD deficient. Based on the cut off value of 4.55 IU/gHb, 15 females newborn should have been classified as G6PD deficient/intermediate type. However, by FST only five females newborn have been identified as G6PD deficient/intermediate. Ten females newborn have been identified as normal by FST.

Studies have shown that FST will be able to detect G6PD deficient males but may erroneously classify partial deficiency G6PD females as normal. A work by Ainoon et al. showed that in Malay population of Malaysia, FST, the chosen approach for screening program, could detect only 7.5% of heterozygote cases, while quantitative enzyme assay detected 53% of heterozygote females. This could be the probable cause of difference between newborn screening by quantitative method and FST.

There is no reliable biochemical assay to detect G6PD heterozygotes, and DNA mutation analysis may provide alternative.

The limitation of the study is that we did not compare our results with more reliable enzymatic and molecular analysis to identify the heterozygous state of the female newborn. This is a preliminary study to look at the feasibility of performing G6PD screening using heel prick samples that is sent to our hospital.

Discussion

World Health Organization (WHO) recommends screening for G6PD deficiency in populations with a prevalence of 3 to 5 percent or more in males. Our data showed that the overall prevalence of G6PD deficiency was 3.48%, with 5.13% in males and 1.72% in females; thus, justify the necessary for a screening programme.

Conclusions

Screening for G6PD deficiency by qualitative fluorescent spot test using the cord blood has its own disadvantage. Our hospital has introduced extended NBS for amino acid and organic acid disorders, and hence screening for G6PD deficiency using quantitative method can be incorporated as part of the NBS programme.

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