USING THE FLUORESCENCE SPOT TEST FOR NEONATAL SCREENING OF G6PD DEFICIENCY

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Abstract. To establish the neonatal screening method of glucose-6 phosphate dehydrogenase (G6PD) deficiency, G6PD activity was measured using the fluorescence spot test (FST) using dried blood samples on filter paper. The G6PD/6PGD rate test of venous blood samples was further performed for confirmation. The positive G6PD deficiency rate was 4.2% and its detection rates were 3.7% for all neonates and 5.2% only for male newborns when FST was used for neonatal screening. Conformation rates by use of G6PD/6PGD ratio test for G6PD deficiency were 86.8% and 100% particularly in the severely deficient groups. Both sensitivity and specificity were very high in the severely deficient groups. FST can be used in neonatal screening of G6PD deficiency because of its high accuracy, applicability, and simplicity. Moreover, a high volume of dried blood samples on filter paper can be tested quickly. It is very favorable to diagnose and treat G6PD deficiency early in high incidence districts.

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an incomplete dominance sex-linked inherited disease. It easily presents symptoms of hemolytic jaundice, anemia, and neonatal hyperbilirubinemia. If severe, it may result in cerebral injury or even death. Therefore early neonatal screening and diagnosis of the disease using the fluorescence spot test (FST) will take an active role in preventing and treating G6PD deficiency and will be helpful in effectively protecting health.

MATERIALS AND METHODS

Materials. Dried blood specimens of 11,437 neonates for newborn screening were collected by heel prick on the neonates on the 3rd day of life. To ensure that the blood samples were fresh, samples were stored at 4°C and tested as soon as possible.

Quality control. The center participated in the external quality control program for the neonatal screening G6PD which is available at the Taiwan Yangming University.

Preparation of reagents. The following reagents were kept below 0°C and away from light: 0.01mol/l glucose-6 phosphate (G6P) solution, 0.007 5mol/l oxidized coenzyme II, oxidized glutathione (GSSG). At the same time, 0.027 mol/l digitalis and 0.75 mol/l Tris-HCl buffer solutions were preserved at 4°C. After each reagent was rewarmed at room temperature, it was mixed and used according to the needed quantity and proportion of 1 ml of 0.01 mol/l G6P, 1 ml of 0.0075 mol/l coenzyme II, 1 ml of 0.008 mol/l GSSG, 2 ml of 0.027 mol/l digitalis, 3 ml of 0.75 mol/l buffer solution and 3 ml of distilled water. The mixed solution could be reused for a week. When used subsequently, it was rewarmed for 15 minutes, protected from light at room temperature and used immediately.

Instrument. The ZF-I type tri-using ultraviolet analyzer was used which is an electrolight product of Shanghai Gucun .

Qualitative detection of G6PD activity by using FST. Dried blood samples on filter paper (for screening and for quality control) were punched and 3mm-diameter disk paper was put into each of the 96 wells in the micro plate. Then, 100 μl of the reaction solution was added. Before hatched, a drop of the reaction solution was placed on another filter paper for no-fluorescence comparison. These were all immediately put in an incubator at 37°C for 30 minutes. Using a pipette, 20μl of the solution was dropped onto the filter paper. The paper was dried

promptly with a blower. Irradiations with ultraviolet light were done and the samples were observed for fluorescence.

Brightness of the fluorescent spots on the paper or intensity of fluorescence (as ++) were measured. If the intensity of fluorescence appeared ± ~ -, it is considered positive for G6PD deficiency. If the fluorescent spot was dark and weak, it was considered as low-grade or moderate deficiency. The G6PD/6PGD rate test of venous blood samples was performed for further confirmation (Chuanshu, 1991).

RESULTS

Determination of FST

Of the 11,437 samples tested, 10,953 had normal fluorescent intensity and therefore had no G6PD. One hundred and sixteen had weak fluorescence thus were considered as low or moderately G6PD deficient and another 368 had no fluorescence at all, meaning severely G6PD deficient. The positive rate of G6PD deficiency was 4.2% (484/11,437). The reported time of the results was 4.5 ± 0.6 d after birth.

Of the 484 positive cases screened by FST, 420 positive G6PD deficiencies were confirmed by the G6PD/6PGD rate test. The conformation rate of FST with the G6PD/6PGD rate test for G6PD deficiency was 86.8% (420/484) and 100% particularly in the severe deficiency groups. This indicated that the sensitivity, specificity and accuracy were very high in the severe deficiency groups. As far as the low-grade or moderate G6PD deficiency samples were concerned, the confirmation rate was 44.8% (52/116). The confirmed detection rate was 3.7% (420/11,437). The confirmed detection rate of males was 5.2%.

The results of 120 quality control samples had a 100% feedback. Thirty-six (36) pieces of the samples were positive and the other 84 were negative. The accuracy rate was 100%, without false negative or false positive.

DISCUSSION

G6PD participates in the metabolizing course of G6P. In the catalysis of G6PD, G6P produces glucose-6-phosphate acid (6-PG) and reduces the coenzyme II (NADPH), which emit a gray-green fluorescence in the irradiation of ultraviolet wavelength. G6PD deficient groups weaken the fluorescence or produces none at all due to less production or shortage of NADPH. NADPH is also involved in the reaction wherein the erythrocyte oxidized glutathione (GSSG) is transformed into the reduced form (GSH). If GSSG is involved in the reaction solution, NADPH will be used up and will change into the oxidized form of coenzyme II (NADP). The detection rate of heterozygotes is thereby increased (Manyi and Lingjun, 1987). The test had a higher specificity than others when using G6P and NADP as the substrates of the enzymatic reaction (Yankang *et al*, 1980).

The positive G6PD deficiency rate was 4.2% and its detection rate was 3.7% in FST for neonatal screening. The confirmation rates of FST were 86.8% and 100% particularly in severe deficiency groups with G6PD/6PGD ratio test for G6PD deficiency. It showed high sensitivity, specificity and accuracy in FST while the test still had relatively low sensitivity for mild G6PD deficiency groups. On the whole, the results were consistent with the conclusions drawn from the evaluating tests of measuring G6PD activity such as FST, methylene chloride absorbing test, methemoglobin reduction test and improved blue tetrazolium test (Yankang *et al*, 1980). The superiority of FST lay in its high accuracy for both normal cases and cases found positive for G6PD deficiency. The detection rate was 100% especially for the severely G6PD deficient groups (male hemizygote and female homozygote). It is useful to prevent and treat hyperbilirubinemia and hemolysis (Jiang *et al*, 2000). Moreover, FST is simpler and spares time (only about 30 min). It is easy to duplicate and to grasp. A significant number of samples can be examined in one time. Expensive instruments are not needed and requires less quantity of reagents lessening the cost. FST is very favorable for the diagnoses and treatment of G6PD deficiency in high incidence districts.

Attention during the detection course showed that the samples are fresh enough (Chengyan *et al*, 1999). If the samples are preserved well in a 4°C refrigerator, G6PD activity will remain stable for 15d otherwise, fluorescence is easily reduced or is not produced at all and these will result in false positives. To avoid no effects, the reagents should not be refrigerated or repeatedly rewarmed. Only in a filter paper that is dried promptly can the fluorescence reaction be observed for the fluorenscence will be reduced in the state of wetting spots.

However, there are still disadvantages when using FST. For example, there is subjective deviation existing when using the naked eye to determine results and there are low detection rates in minor G6PD deficiency (heterozygote). There is need for further research on how to improve the method and technology to increase the

detection rate in female heterozygotes for G6PD deficiency.

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