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Screening for glucose-6-phosphate dehydrogenase (G-6-PD) deficiency by a simple method

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Summary

The methaemoglobin reduction test (MHRT) was used at room temperature (27–32°C) to screen for G-6-PD deficiency. Blood (0.5 ml) was incubated for 4 h. The results obtained were in good agreement with those obtained using the fluorescent screening method (FSM) ($P < 0.01$). In the males, there was agreement in 95% of the cases. Most of the differences occurred in the females. It is concluded that since MHRT can be used at room temperature, and since the materials needed are cheap and easy to get and electricity is not essential, most hospitals should be able to use the MHRT method to screen for G-6-PD deficiency.

Résumé

Le test de réduction de la méthémoglobine (MHRT) a été employé en température ambiante pour détecter une déficience de la G-6-PD. Le sang (0.5 ml) a été mis en incubation pour 4 heures. Les résultats obtenus ont été en bonne concordance avec ceux obtenu par la méthode de fluorescence ($P < 0.01$). Chez les hommes il y'avait un accord dans 95% des cas. La plupart des disparités se sont manifestées chez les femmes. Il est conclu que puisque le MHRT est utilisé en température ambiante, et les matériels ne coûtent pas cher, et ils sont faciles à obtenir, et puisqu'on n'a pas besoin d'électricité, la plupart des hôpitaux pourraient utiliser MHRT comme méthode de dépistage pour la déficience de la G-6-PD.

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Introduction

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is the most prevalent clinically significant enzyme deficiency of man [1], and the single most important aetiological factor associated with severe neonatal jaundice (NNJ) in full-term normal weight infants in Nigeria [2–4] and other parts of Africa [5]. It is, therefore, a sufficiently common public health problem to require routine screening of new-born infants in Nigeria and many other parts of Africa.

Many simple and efficient screening procedures for G-6-PD deficiency are available [6]. Some of them require stringent conditions that may be difficult to meet. The desire for a cheap, simple and sensitive method, for which materials are readily available, cannot be over emphasized. The methaemoglobin reduction test (MHRT) [7] satisfies the above requirements. A micro-method of MHRT has also been described [8]. In the latter, incubation is done at room temperature and much smaller volumes of blood are needed. The modification used in the present study is based on both the original method [7], in which tubes are used, and the micro-method [8], which was done at room temperature using much smaller volumes of blood. This makes the procedure easy to carry out even in the rural centres.

The fluorescent screening method (FSM) is simple and requires a very small amount of blood, but the reagents are not easy to get and are more expensive. The long-wave UV lamp needed for interpretation requires electricity, which is not always available even in major centres.

Patients and methods

The study was done in two stages. Firstly, the

MHRT was done by one of us (J.A.O.), exactly as described by a WHO scientific group [9] at room temperature (27–32°C) on ten samples, divided into five tubes each. The test was read at 30-min intervals starting after 3-h incubation (i.e. at 3, 3.5, 4, 4.5 and 5 h). The normal and the deficient samples were clearly differentiated throughout the period, but when read after overnight (12 h) incubation the deficient samples appeared normal. An attempt was then made to reduce the amount of blood required, proportionately to 0.25 ml, which did not affect the sensitivity of the procedure.

The materials for the second part were made up of five adults and 57 neonates. The adults were healthy individuals from whom blood was collected for control in the MHRT. The neonates (21 females, 36 males) were mostly babies with neonatal jaundice.

In the second part, 0.5 ml of blood was used for the MHRT, with incubation carried out at room temperature. The methylene blue solution and sodium nitrate/glucose solution, prepared as previously described [9], were mixed in equal proportion just before each batch of tests. A portion (0.05 ml) of the mixture was added into each test tube and 0.5 ml of blood was then added. For the normal control, no reagent was added to the blood sample but for the positive control, 2 ml of blood were added to 0.1 ml sodium nitrite/glucose solution. Each batch (sometimes as many as ten) was incubated for approximately 4 h at room temperature. After collection, the samples were immediately kept in a refrigerator or wet ice before analysis, which was done the same day (usually within 8 h) by one of us (J.A.O.). The remaining samples used for the FSM were kept cold before transporting them to Ibadan in wet ice (approximately 1-h journey). The FSM test was performed by one of us (V.O.O.), immediately, at the Department of Paediatrics Laboratory at the University College Hospital, Ibadan. The FSM was as modified by Beutler and Mitchell [10]. The results obtained by one author were not known to the other until the end of the study when results were compared.

Results

A total of 62 samples from 22 females (21 neonates, one adult), and 40 males (36 neonates, four adults) were screened by the two methods. Thus there were 57 neonatal and five adult samples. Table 1 shows that 26 patients were G-6-PD deficient by the MHRT while 22 were G-6-PD deficient by the FSM. There was no significant differences in the results obtained from the two methods. For the whole group $\chi^2 = 29$, and for the males $\chi^2 = 29$, $P < 0.01$ in each case (Tables 1 and 2). There was disagreement in 10 female samples: seven had normal G-6-PD status by the FSM but were deficient by the MHRT method, and three experienced the reverse. Three of the seven females reported deficient by the MHRT method were suspected to be heterozygous for G-6-PD status. One of them was a mother of a male patient reported as deficient by the two methods and two others

Table 1. Comparisons of the results obtained from the fluorescent screening method (FSM) and the methaemoglobin reduction test (MHRT) in 62 patients

	FSM normal	FSM deficient	FSM total
MHRT normal	33	3	36
MHRT deficient	7	19	26
MHRT total	40	22	62

$\chi^2 = 29$, $P < 0.01$.

Table 2. Comparisons of the results obtained from the fluorescent screening method (FSM) and the methaemoglobin reduction test (MHRT) in males

	FSM normal	FSM deficient	FSM total
MHRT normal	20	1	21
MHRT deficient	1	18	19
MHRT total	21	19	40

$\chi^2 = 29$, $P < 0.01$.

were reported as intermediate (both were neonates) by the MHRT. In the males, one recorded as normal by MHRT was recorded as deficient by FSM and one recorded as normal by FSM was deficient by MHRT. However, in 38 (95%) of males both tests agreed.

Discussion

The MHRT is based on the principle that methaemoglobin (brown) is formed through the action of nitrate on the red cells. In the presence of methylene blue, methaemoglobin is reduced to haemoglobin (red) [9].

The MHRT, as described above, should be very useful in the rural centres because it is simple, sensitive, and the reagents are cheap and easily available. The cost of the reagents per test is under 2 cents (US). Conversely, the FSM kit costs over 60 cents per test. Moreover, these reagents (kits) are not always available. There is also the added advantage of incubating at room temperature with the MHRT, meaning that electricity is not essential and all that is required to interpret the test is bright daylight. The actual time spent per test is less than 5 min when done in batches.

The results obtained by the two methods were in agreement (95% in males), which is good enough for routine screening procedures. The disparity in the two males is not easy to explain. The MHRT may have false positive results in rare conditions such as methaemoglobin reductase deficiency (L. Luzzatto, personal communication) or NADPH diaphorase deficiency [11]. No screening method for detection of the heterozygotes for G-6-PD deficiency is entirely satisfactory [12]. The micro-method of MHRT for G-6-PD deficiency as first described [8] has not had extensive field testing [13]. The present report is not an attempt at proving that one method is superior to the other, rather it shows that the MHRT done at room temperature with reduced blood volume can be as reliable as most other screening methods, especially in males. The MHRT as described here needed just 0.5 ml blood for a test that is done only once. This amount of blood is small and can easily be obtained from most neonates. Moreover, the amount of blood required can still be reduced further.

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