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Formazan ring method: a simple test for screening of glucoce-6-phosphate dehydrogenase (G-6-PD) deficiency in the neonates

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ABSTRAK

Purnomo Suryantoro – *Metode cincin formazan : suatu uji saring sederhana defisiensi glukosa-6-fosfat dehidrogenase pada neonati*

Kekurangan glucose-6 phosphate dehydrogenase merupakan suatu penyakit keturunan yang bersifat x-linked yang berupa anemia hemolitika yang muncul secara periodik. Insidensinya bisa dihubungkan dengan terjadinya epidemi malaria pada suatu daerah, misalnya di Mediterania, Afrika dan Asia (termasuk Asia Tenggara)

Metode formazan ring yang dikembangkan oleh Nishiyama merupakan metode yang mudah, murah, tidak menyakitkan dan bisa dipakai untuk skrining. Dari 145 bayi baru lahir yang diteliti ternyata sebanyak 9 orang (6,2 %) memperlihatkan aktivitas enzim yang rendah, bahkan 2 orang (1,4 %) kekurangan glucose-6 phosphate dehydrogenase.

ABSTRACT

Glucose-6-phosphate dehydrogenase deficiency, is an x-linked inherited disease which is responsible for episodic hemolytic anemia. The incidence correlates with the malarial epidemic in many area like Mediterranean, Africa and Asia including South East Asia.

This paper reports a simple, cheap, traumatic, and reliable method screening test called formazan ring developed by Nishiyama. Among 145 new-born infants in this report the low enzyme activity was as high as 9(6.2%) including 2(1.4%) G-6-PD deficiency.

***Key words*: glucose-6-phosphate dehydrogenase deficiency – x-linked inherited disease – formazan ring test – neonatal screening**

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Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is an X-linked inherited anomaly which is responsible for episodic hemolytic anemia induced by infection or certain drug and spontaneous nonspherocytic hemolytic anemia[1]. The role of this enzyme is to catalyse the conversion of glucose-6 phosphate to 6-phospho gluconate, maintaining a high intracellular level of NADPH which is important to defend against oxidative stress by detoxification of peroxide[2]. Without any nucleus contained DNA so far, G-6-PD synthesis in the erythrocytes is not possible and shortened life time will result if deficiency exists. In the neonate it may be one of many causes of hyperbilirubinemia.WHO report showed these disease subjects were not less than 100 millions people[3] in the world especially around Mediterranean sea and Asia[4] correlated with some mechanisms of protection against malarial infection[5]. It is estimated that in Indonesia the frequency G-6-PD deficiency 3 - 6%, but this figure has not been clarified yet.

A number of screening procedures have been introduced and found effectively to detect

G-6-PD deficient persons. These include the spot test of Fairbanks and Beutler[5], the dye decoloration test of Motulsky and Cambell-Kraut[6] and methylen blue reduction test of Oki and Growney[7]. All micro screening tests are performed with a few drops of capillary blood the methemoglobin reduction test of Brewer et al[8]. The ascorbic acid-cyanate test of Yacob and Jandl[9] and the THNH fluorescence test of Beutler[10] require venous blood which is more difficult. These techniques are psychologically and physically more traumatic for the individuals during the screening programs.

Recently, Knudsen and Brewer[11] introduced a micro-modification of the methemoglobin reduction test, based on the inability of G-6-PD deficient erythrocytes to generate NADPH (TPNH). Through pentose phosphate shunt stimulation by glucose and methylene blue, the NADPH generation reduces sodium nitrite-induced methemo-globinemia in normal red cells, whereas methemoglobinemia persists in G-6-PD deficient erythrocytes.

The purpose of this study was to determine the potential usefulness of the formazan ring method for the screening test of G-6- PD deficiency in the neonates. This technique will be briefly explained in the material and method mentioned below.

MATERIALS AND METHODS

After informed consent was obtained from their parents, 145 blood spots were collected from the newborn babies not less than 3 days of age delivered in Panti Rapih Hospital Yogyakarta. These blood spots on filter papers were dried up in the room air and then kept in the 4°C refrigerator. This sample was then round cutted 4 mm in diameter and applied on the formazan gel before incubated for 5 hours on 37°C. The blue ring would appear around the papers.

The formazan gel composition for 20 ml was as follows:

- G-6-PD, Na2 (Nacalai Co) 25mg
- B NaDP (Oriental yeast Co) 5mg
- Agar (Dosindo Co) 150mg
- MTT (Nacalai Co) 5mg
- MS (Wakko Chem) 5mg
- 0.1 TrisHCl-0.01M MgCl2 (pH 6.5)

As shown in FIGURE 1, two blood spot as control were used. The first was part of an episodic hemolytic anemia case induced by infection (Parotitic Epidemica), the diameter of blue were 7x7 mm and had been proved to have 77 μU G-6-PD/10^{10} erythrocytes. The second was normal in diameter (8x8 mm) containing 115 μU G-6-PD/10^{10} erythrocytes, the measurement of the G-6-PD levels was done using laboratory (Prodia). According to this laboratory, the normal range is 110-130 μU/1010 erythrocytes.

RESULTS

One hundred forty five blood spots from newborn infants were collected consisting of 71 males and 74 females. The blue color diameter of the males were (8.2 ± 0.7) mm and of the females were (8.6 ± 0.7) mm.

As seen in Table I. there were 9 (12.6%) of the males that had low G-6-PD levels possibly of G-6-PD levels below 77 U/10^{10} erythrocytes, this include 2 cases of G-6-PD deficient. Among the females neither low nor deficient G-6-PD was detected in this screening test.

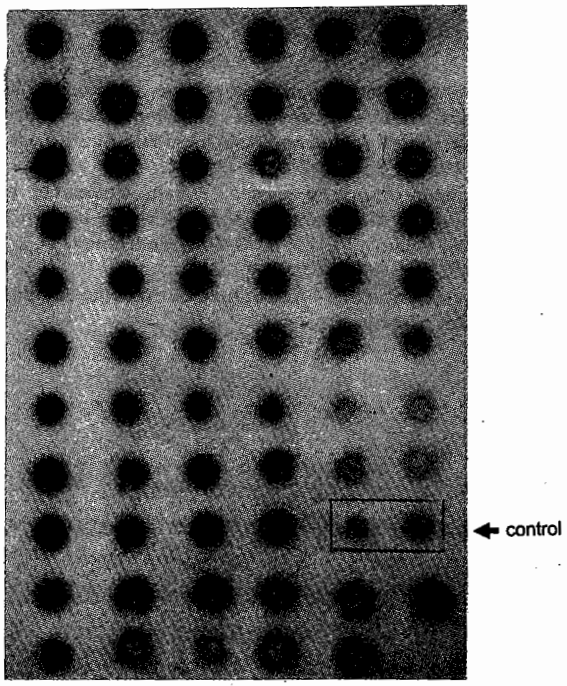

FIGURE 1. – The result of formazan ring test of dried blood spot after 5 hours incubation at 37°C.

Small diameter (7mm) contains G-6-PD activity: 77 μU/1010 erythrocytes and normal diameter (8mm) similar 115 μU/1010 erythrocytes. Normal range activity were 110-130 μU/10101 erythrocytes.

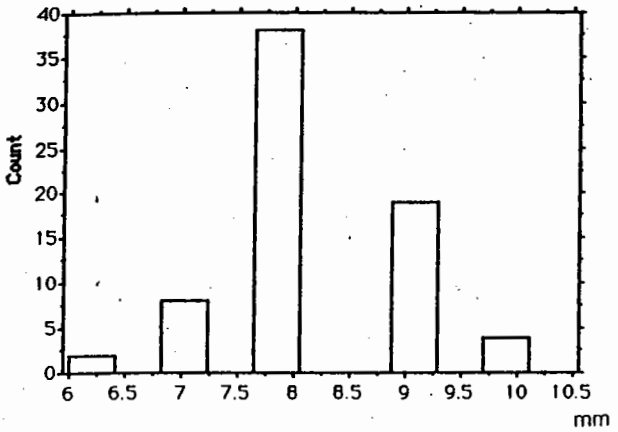

FIGURE 2. – The distribution of blue ring diameter as results of the formazan ring test in the male neonates. Seven cases of low (7 mm) and 2 deficient (6 mm) were detected.

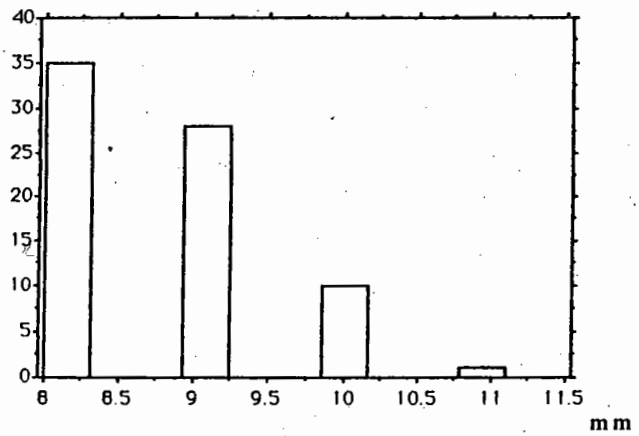

FIGURE 3. – The distribution of the blue ring diameter as results of the formazan ring test in the neonates. Low and deficient G-6-PD activity were not found.

DISCUSSION

The formazan ring method as a screening test appears to be reliable, inexpensive and simple. A further virtue is the relatively less traumatic procedure of obtaining the blood sample. The test requires one or two drops of blood obtained by heel or finger prick, avoiding the need of venipuncture, and the cost is approximately Rp. 200,-.

Some difficulties with the test were encountered: storage in the room temperature for a long period caused decrease this enzyme activity, small blue ring will result. It is also possible that the ingredients do not dissolve homogeneously in the gel, therefore the diameter of the blue ring is not perfectly accurate.

Eventhough, this technique is suitable for screening test in the childhood.

The incidence of G-6-PD deficiency in Indonesia as reported by Injo Luang Eng (1964) was 1.1 % with the highest in West Irian (8%) and Kalimantan (6-30%), while Notopuro and Donoseputro found out 2 cases of lowered G-6-PD activity among 105 cases studied[12].

This report showed that the incidence of lowered G-6-PD levels was as high as 6%. This finding is similar to the number estimated previously. Deficiency occurred in 2 (1.4%) cases, close to those reported by Injo Luang Eng.

Since the G-6-PD deficiency is X-linked, low or deficient case may exist depending on a double or single X-chromosome affected in females. This report fails to show this phenomenon. In males only single X chromosome is available, therefore males should be homozygote. Manifestation of deficiency will exist especially if the deficient indivual is exposed to infection or drugs. This is the case we use as small-size sample control. Among neonates the risk of male (12%) is twice compare to those of all over (6%), eventhough we cannot find any correlation with jaundice occurred during the first week of life.

G-6-PD molecule consists of 514 amino acids accounted for 400 variants which are differ in severity, clinical expression and biochemical properties. Variation of the enzyme activity is still unexplained yet, for instance among class 3 type such as A-type which is common in Africa. The activity of the enzyme is ranging from at 10 to 60%. At the molecular levels 58 different muta- tions have been identified accounting for 97 variants. The mutations are almost exclusively missense mutation causing single amino acid substitution. They are spread throughout the coding region of the gene, appeared to be a cluster of mutation that causes a more severe clinical phenotype towards the 3'end of the gene[13]

SUMMARY

The formazan Ring method is simple, less traumatic and reliable method for the screening of

G-6-PD deficiency in the neonates. The frequency of lower G-6-PD activity is 6.2% and deficiency is 1.4%. The risk of male neonate is twice (12.4%) than all over the case (6.2%). This figures closed to those estimated previously (3.6%) and or reported by Injo Luang Eng in Surabaya (1.1%). Further study are needed to detect the enzyme abnormality of activity at the molecular levels.

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