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ORIGINAL ARTICLE

Glucose -6- Phosphate Dehydrogenase Deficiency in the Dr. Cipto Mangunkusumo General Hospital

by

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Abstract

The incidence of G-6-P-D deficiency in Indonesia is not yet known. So far only Inyo Luan Eng (1964) reported the incidence in some parts of Indonesia. Other data are only from case reports.

This study which consisted of 3200 babies — born in the Dr. Cipto Mangunkusumo General Hospital from September 1975 through October 1976 proved that the incidence is high enough that is 2,66% (85 out of 3200).

The hemoglobin content of G-6-P-D deficient babies showed an average of 14,4 g%. This gives us an idea that there is slight anemia in these babies.

The incidence of jaundice was 35 out of 85 (41%). Factors inducing jaundice were infection, hypoxia and sub-aponeurotic bleeding.

Of 60 which could be examined quantitatively only 2 showed a G-6-P-D activity level of more than 120 mu/10^9 RBC, so that the screening method could be used for early detection of G-6-P-D deficiency.

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Introduction

G-6-P-D is an intra - erythrocyte enzyme which is needed for the stability and intergrity of the red blood cell. Erythrocyte stability depends on the presence of adequate reduced glutathione, which is necessary for maintaining sulfhydryl group within the red cell and in conjunction with glutathione peroxidase detoxify peroxide. When there is a deficiency of this enzyme, erythrocyte generates inadequate amounts of reduced nicotinamide - adenine dinucleotide - phosphate which is required as a cofactor for red cell glutathione reductase to maintain glutathione in a reduced state.

The deficiency of the activity of this enzyme was first detected by Carson in 1956 in a patient who developed hemolysis after ingestion of primaquine. Deficiency of this enzyme appears to be the most commonly identified inborn error of metabolism, more the 80 variant have been detected and it has been estimated that over a hundred million people throughout the world have this deficiency (Tarlov 1962, cited by Young et. al. 1974).

G-6-P-D deficiency is not evenly distributed throughout the world. In the Caucasian the incidence is very low. The highest incidence is among the Mediterranean people. It is mentioned that in the Kurdish Jews the incidence is almost 60%. In the Oriental people, Thailand has a high incidence. In the American Negro the incidence was estimated as 9 - 13%, while in some places in Africa the incidence can be as high as 25%. In Indonesia in 1964, Inyo Luan Eng reported an incidence of 1.1% with the highest occurence in West Irian (8%) and Borneo (6 - 30%) (Siswadi, 1972), and Fong (1977) reported an inciteince of 2.2 percent among Indonesians living in Sabah East Malaysia.

Problems of neonatal jaundice due to deficiency of this enzyme also differs. In American Negroes, G-6-P-D deficiency seldom causes severe hyperbilirubinemia during the neonatal period (Zinkham, 1963; O, Flynn and Hsia, 1963). Szienberg et al. (1963) concluded from a study of Jewish groups in Israel that G-6-P-D deficiency could not be regarded as an important factor in the etiology of hemolytic jaundice in Jewish neonates. However in Sardinia (Panizon, 1960), Malaysia (Smith and Vella, 1960, Weatherall, 1960), Nigeria (Capps et. al., 1963), Greece (Doxiadis et. al., 1964), Singapore (Brown et. al., 1968) and Italy (Sansone et. al., 1975), deficiency of G-6-P-D has been described as an important factor in the etiology of kernicterus or severe neonatal jaundice. Wong (1960) reported that 70% of hyperbilirubinemia in Singapore was caused by deficiency of this enzyme or immaturity of the liver or combination of both. In Jakarta Jayadiman Gatot (1974) performed 41 exchange tranfusion of which 10 were G-6-P-D deficient.

The purpose of this study is to find out the incidence of G-6-P-D deficiency and the problem of jaundice in the G-6-

P-D deficient babies, in the Dr. Cipto Mangunkusumo General Hospital, Jakarta.

Material and Method

Our material consisted of 3200 babies born in the Obstetric and Gynecologycal Department Dr. Cipto Mangunkusumo General Hospital, Jakarta between September 1975 and October 1976. During this period there were 3661 live births but only 3200 could be examined, consisting of 1774 boys, and 1486 girls. There were 55 from Chinese and 8 from Arab descendants.

On the second day of their lives they were screened for G-6-P-D deficiency using the method of Bernstein (1962). When on the first day jaundice was already present, screening was done on that day.

The procedure of the screening test is as follows :

1. Prepare a fresh mixture of

D.C.I.P.[1])	7.5 parts	
T.P.N.[2])	0.5 parts	
		by volume
P.M.S.[3])	2.5 parts	
G-6-P-D - acid	2.5 parts	

2. With a heparinized micro — pipet take 0.01 ml whole blood from a heel puncture.
3. This is added to 0.4 ml of water in a test tube and shake well.
4. Add 1 ml of mixture 1 to 3.
5. Cover with liquid paraffin.
6. Note time of complete color change. Normaly, the color change from bluish to reddish appears in less than 5 minutes.

When there is no color change after more than 5 minutes there is a deficiency of G-6-P-D.

When on examination G-6-P-D deficiency was found, the following things were done.

1. Blood was taken for quantitative examinations for G-6-P-D activity using the U.V. method by Kornberg. The normal activity is between 131 $\pm$ 13 mu/10^9 R.B.C.
2. Hemoglobin content was determined by the colori meter.
3. Follow up.
   When on follow up jaundice appears.
4. The date of onset of jaundice was noted.
5. The level of bilirubin was determined by photo — electric method for at least 4 days.
6. The jaundice was treated by phototherapy or exchange transfusion according to the local guidelines.

Results

Three thousand and two hundred babies of which 55 were of Chinese and 8 Arab descendants were examined by the qualitative Bernstein method. Eighty five

1) DCIP = Dichloro Indophenol
2) TPN = Tri Phosphopyridine Nucleotide 0.005M
3) PMS = Phenazine Metho Sulphate

were found to be G-6-P-D deficient, consisting of 57 boys and 28 girls. The average Hemoglobin content of those with G-6-P-D deficiency was 14.4 gram %, ranging from 8.2 gram % to 19 gram %. From the 85 babies 35 became jaundiced. In 77 percent the jaundice was noted after the third day. In 80 percent the bilirubin content was below 15 mg %. Those with bilirubin content of more than 10 mg% had also other morbidity which by it self could cause jaundice. Enchange transfusion was only necessary in 3 cases. From 60 babies who could be examined quantitatively, only two showed an activity of more than 120 mu/10^9 R.B.C. and from 20 controls all showed an activity of more than 140 mu/10^9 R.B.C.

The results could be seen on Table 1 to 7.

Discussion

Incidence :

We found a total incidence of 2.66 percent (85 out of 3200). That is 3.3. percent in males and 1.9 percent in females. If we compare it to areas near Indonesia this is between the incidence found in Singapore 1.14 percent (Brown, et. al. 1968) and Thailand 11.98 percent (Tuchinda et. al. 1968 cited by Panich and Na — Na korn (1973) and almost the same as the incidence found by Vella (1958) in Chinese males in Singapore (2.63 percent). If we look at it from the racial point of view we see that the incidence is higer among the Chinese and the Arabs, but the sample is too small to take conclusions from.

Sex Distribution :

We know that G-6-P-D deficiency is sex linked inherited. Using this screening method also the female heterozygous could be detected. We found 28 deficient girls compared to 57 deficient boys. If we calculate it according by the Hardy — Weinberg formula, the incidence of female heterozygote should be about twice that of the males. Here we found the incidence is 1.9 percent in females. This proves that the female heterozygote x'x undergoes Lyonisation of one of 2 x - sex chromosome. Thus the expression of an x - linked abnormality would depend on the ratio of cells carrying the abnormal gene in an active x - chromosome against those carrying the gene in an inactive x. So theoreti cally the G-6-P-D activity in heterozygous females may vary from zero to normal.

Hemoglobin Level :

In our study we found an average Hemoglobin content of 14.4 gram percent in G-6-P-D deficient babies, with a range between 8.2 to 19 gram percent, (Table 2). Unfortunately we could not compare it to the normal babies as we did not examine the Hemoglobin level of our normal babies. However we consider this a rather low level and concluded that in our deficient babies there was slight anemia. This is in accordance to the experience of Brown et. al. (1968) in Singapore who found that the Hemoglobin level and the Hematocrit were lower and

the Reticulocyte count was higer in the G-6-P-D deficient babies than in the normal.

Jaundice :

I. Incidence :

Thirty five out of 85 babies developed jaundice, an incidence of 41 percent. At almost the same time at the same hospital Monintja et al. found that the overall incidence of jaundice was 32.1%. Only in 7 babies (20 percent) are the highest bilirubin level more than 15 mg%. This is lower than what Yue et al. (1965) reported in Hongkong where he found that 27 percent of the G-6-P-D deficient babies had serum bilirubin levels of over 15 mg%, and what Brown et al. (1968) reported in Singapore that 19 percent of G-6-P-D deficient babies had plasma bilirubin levels exceeding 19 mg%.

II. Onset of jaundice :

Capps (1963) stated that jaundice due to G-6-P-D deficiency appears late and does not stay long. Yue (1965) found that in 73 percent of his cases jaundice appeared on the 3rd or 4th day. This is also what we found in our series. We found that in 77 percent the onset of jaundice were after the third day.

III. Factors responsible for jaundice:

Zinkham and Child (1957; cited by Capps, 1963) proved that G-6-P-D deficient babies were prone to hemolysis when given vit K. analogues. However Capps proved that in his series 8 mg manaphtone given at birth did not enhance the incidence of jaundice. We used vit K_1 1 mg given once at birth only in premature babies and those whose birth were not spontaneously. And in the 35 babies with jaundice in our serie only some babies got vit K_1 at birth, so we can not take conclusions out of this.

Tarlov (1962, cited by Young et. al. 1974), proved that several drugs which caused severe hemolysis in the Caucasian G-6-P-D deficient, did not or only cause slight hemolysis in the Negro G-6-P-D deficient. This gives additional weight to the opinion of Fessas et. al. (1962), based on a family analysis, that for the development of severe neonatal jaundice in the G-6-P-D deficient newborns a second factor (probably genetically determined) is necessary. The postulation of a second genetically determined factor may also explain the absence of severe neonatal jaundice, due to G-6-P-D deficiency in some races known to have a high insidence of this enzyme defect. (Szeinberg et. al 1963, Zinkham 1963). In Singapore mothballs (naphthalene) and certain herbs were the factors responsible for jaundice. In our neonatal wards fortunately mothballs are not being used.

In our cases hypoxia, infection and subaponeurotic bleeding, are conditions which could be considered as factors inducing jaundice. We agree with Brown et. al. (1968) that factors responsible for hyperbilirubinemia in G-6-P-D deficient infants may bee at least three fold :

1. Ethnic group predisposition towards hyperbilirubinemia.

2. Slightly excessive hemolysis in G-6-P-D deficient infants.
3. Possibly, exposure to exogenous hemolytic agents.

IV. Kernicterus :

Exchange transfusion was only performed in three cases, two with blood incompatibility and one with sepsis, none which was due to G-6-P-D alone. One developed kernicterus despite of exchange transfusion. In this baby the Coombs test was positive. Free incomplete antibodies with anti - D specification was found in the serum of the baby with a titer of 1/16. On the first day the bilirubin level was already 11 mg percent and the Hb level only 8.2 gram percent.

The quantitative determination of G-6-P-D activity could be performed in 80 babies, sixty with G-6-P-D deficiency and 20 with no deficiency which serve as controls. Those 20 with no G-6-P-D deficiency by the qualitative method all have G-6-P-D activity of more than 140 mu/10^3 R.B.C. From table 7 we can see that only in two cases with G-6-P-D deficiency the activity were more than 120 mu/10^9 R.B.C. that is 124,963 mu/10^9 R.B.C. in one and 123,125 mu/10^9 R.B.C. in another. From this examination we can take the conclusion that the qualitative method is realible for screening G-6-P-D deficiency.

Conclusions

As a conclusion we can mention here that the incidence of G-6-P-D deficiency among newborns in dr. Cipto Mangunkusumo General Hospital is high enough. Though according to Yoshida (1973), the Indonesian variant is not associated with chronic hemolytic anemia, neonatal jaundice could be a problem as factors inducing jaundice are frequently present. Screening is necessary in our country because our population has many ethnic background and anti malarial prophylaxis are being given in areas where malaria is still prevalent. Further studies in this field are necessary.

TABLE 1: *Incidence and sex distribution of G-6-P-D deficiency*

Ethnic	Number of infants			G-6-P-D deficient			
	boys	girls	total	boys	girls	total	%
Indonesian	1679	1458	3137	53	26	79	2.51
Chinese	30	25	55	3	2	5	9.09
Arabs	5	3	8	1		1	12.5
	1714	1486	3200	57	28	85	2.66

TABLE 2 : *Hb content of G-6-P-D deficient babies*

Hb	N u m b e r
< 10 gram %	1
10 — 14 gram %	36
> 14 gram %	48
T o t a l	85

Average 14.4 gram%

Range 8.2 gram % — 19 gram %

TABLE 3 : *Day of onset of jaundice in G-6-P-D deficient babies*

Day of onset	N u m b e r	%
day 1	1	2.85 %
day 2	1	2.85 %
day 3	6	17.15 %
day 4	13	37.15 %
day 5	10	28.55 %
day 6	4	11.45 %
T o t a l	35	100 %

TABLE 4 : *Serum bilirubin levels in jaundiced G-6-P-D deficient babies*

Serum bilirubin levels	Number	%
— 10 mg %	6	17.15%
10 — 15 mg %	22	62.85%
15 — 20 mg %	6	17.15%
> 20 mg %	1	2.85%
T o t a l	39	100%

TABLE 5 : *Morbidity of 35 jaundiced babies with G-6-P-D deficiency*

Blood incompatibility	2 cases
Hypoxia	8 cases
Infections	15 cases
Subaponeurotic bleeding	5 cases
Without morbidity	5 cases
T o t a l	35 cases

TABLE 6 : *Serum bilirubin associated with morbidity*

Highest bilirubin level	Blood incompatability	hypoxia	Infection	SB	Without morbidity	Total	%
— 10 mg%			1		5	6	17.15
10.1 — 15 mg%	1	6	10	5		22	62.85
15.1 — 20 mg%		2	4			6	17.15
> 20 mg%	1					1	2.85
T o t a l	2	8	15	5	5	35	100

* Subaponeurotic bleeding

TABLE 7 : *Quantitative determination of G-6-P-D activity of 60 qualitatively deficient babies*

G-6-P-D activity	boys	girls	total
0 — 40 mu/10^9 RBC	5	2	7
41 — 80 mu/10^9 RBC	11	9	20
80 — 120 mu/10^9 RBC	29	2	31
> 120 mu/10^9 RBC	2		2
	47	13	60

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