GLUCOSE 6 PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY IN BAD PREGNANCY HISTORY IN ST. VINCENTIUS A PAULO CATHOLIC HOSPITAL, SURABAYA

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ABSTRACT

G6PD is an enzyme that has been proved to have cytoprotective effect against oxidative stress. It has been widely known that exposure of oxidants could trigger hemolytic in G6PD deficient patients. Researches have found that there is a correlation between G6PD deficiency and miscarriage or spontaneous abortion in pregnant women. G6PD is very important for normal embryonal development, during this time the G6PD activity is increased because of increasing oxidative stress, especially after the maternal fetal blood circulation begins to function. Researchers have proved the increase of intrauterine fetal death rate, stillbirth and fetal abnormality in G6PD deficient rats. This study analyzed the G6PD enzyme in women who experienced abortion, either once or recurrent. Using descriptive study samples are women, aged 21 years or more who showed no abnormality reproduction organs, no TORCH infection, diabetes mellitus, thyroid abnormalities and heart disease. The results showed that 10 (43.5%) of samples are G6PD deficiency. Hemoglobin levels, erythrocyte and reticulocyte count of the samples that could influence G6PD activity levels were normal.

Keywords: *abortion, G6PD deficient, oxidative stress*

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INTRODUCTION

Glucose 6 phosphate dehydrogenase (G6PD) is an enzyme that acts in the early part of pentose phosphate pathway, an important glucose oxidation pathway for the formation of reduced Nicotinamide Adenin Dinucleotide Phosphate (NADPH) and pentose (ribose-5-phosphate). NADPH has a function to attenuate oxygen radicals that continuously emerge during the physiological process. The G6PD gene consists of 13 exons and 12 introns. The G6PD presents in cytoplasm, and spread around the cells in different concentration. G6PD deficiency is an enzymatic abnormality due to the mutation of the G6PD gene and thus decreased the enzyme activity. In studies on G6PD deficiency at molecular level, there are 130 G6PD mutants that have been found. G6PD gene mutant that induced G6PD deficiency may account for clinical symptoms ranging from the mild ones to the emergence of Kern-icterus, and severe neurological disorder that may lead to fatality (Beuler 1994; Glader & Lukens 1999; Lukens 1993; Luzzatto 1995; Piomelli 1987).

It is estimated that 400 million human beings are G6PD deficient. The highest frequency is in the eastern hemisphere. In Indonesia, frequencies of G6PD deficiency are as follows: Western Papua 8%, Sasak 18.4%, Bima 12%, and Flores 4% (Sofro 1984), Central Java 14% (Soemantri et al. 1995), in the islands of Southeast Maluku ranging between 1.6 % and 6.7% (Suhartati 2000), Buru and Halmahera islands about 6 % (Iwai et al. 2001). Damanik et al., (2001), using Formazan screening test to 263 neonates in Dr Soetomo Hospital, Surabaya, found that 3.0% of them G6PD deficient.

It is recently proved that critical period in the organogenesis of embryonal tissue involves several antioxidant enzymes, including superoxide dimustase (SOD), glutathione peroxidase (GSHPX), and catalase whose function is to attenuate oxidant compounds. To carry out their functions, glutathione peroxidase (GSHPX) and catalase requires NADPH, a product of G6PD enzyme. The increase in G6PD activity during embryonal development is along with the increase in cell proliferation and DNA synthesis (Newburgh et al. 1962; Papaconstantinou 1967). Jauniaux (2000) found that by the beginning of blood circulation between mother and the fetus the aerobic metabolism is started. In aerobic metabolism the reactive oxygen species

(ROS) is formed through the process of respiratory chain single electron transfer in mitochondria, and through the cytochrome P450 in cytoplasm that produces superoxide (O_2*-). The superoxide (O_2*-) will be altered into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD), and hydrogen peroxide (H_2O_2) will be altered into water (H_2O) and oxygen (O_2).

In G6PD deficient pregnant women, the G6PD activity is reduced, and this increases hydrogen peroxide (H_2O_2) level and thus induces the formation of hydroxyl radical (*OH). The latter's highly reactivity may damage important components that maintain cell integrity and survival. In the placenta particularly the syncytiotrophoblast, the concentration of antioxidant enzyme (G6PD) is lower than that in other trophoblast cells. The syncytiotrophoblast cells synthesized human chorionic gonadothropin (hCG) required for the development of placenta. In addition, syncytiotropho blast is located at the surface of the villi, vulnerable of being attacked by reactive oxygen (ROS) specific compound (Burton 2004; Kliman 2000). In gestational age of 10-12 weeks, oxygen pressure in the placenta increases three times, which may result in oxidative stress in trophoblast. If the capacity of oxidant is more than that of antioxidant, such as in G6PD deficiency, syncytiotrophoblast or trophoblast degeneration may occur more extensively and result in miscarriage (Jauniaux 2000; Kliman 1994).

G6PD deficiency generally does not result in remarkable clinical symptoms. However, exposure to oxidants can be a trigger of clinical symptoms. Currently, the observation of clinical symptoms resulting from G6PD deficiency is only directed towards hemolytic anemia. Experiment on animals with G6PD deficiency reveals intra- or extrauterine fetal death and abnormal fetal delivery (Nicol 2000). This finding was confirmed by Longo (2002), who proved that G6PD could distort placental development and result in fetal death. According to Jalan Anil (2004) The G6PD gene located in chromosome X region q 28 can be attributed to the occurrence of repeated abortion. The G6PD gene is located in telomer region of the chromosome X long chain (Xq28). Result of the study showed that 50-70% of spontaneous abortion is that of unknown cause (Jalan Anil 2004). Can the G6PD deficiency be connected to abortion with unknown cause? It is still unclear whether G6PD does serve as the cause of women with bad pregnancy history?

This study analyzed G6PD deficiencies in women with bad pregnancy history. Such question remains a health problem until today, since the clinical symptoms of women with bad pregnancy history due to G6PD deficiency are still unknown. This study might be used as a base for determining G6PD deficiency in pregnancy to prevent miscarriages or bad pregnancy.

MATERIALS AND METHODS

This is an observational laboratory explorative study. Blood samples were taken from St. Vincentius A Paulo Catholic Hospital (RKZ) patients who will the inclusion and exclusion criteria. Patients visiting this hospital comprised various ethnicities in Surabaya, such as Javanese, Chinese, and Madurese. The inclusion criteria were as follows: age 21 years old or more; gestational age of or more than 8 weeks; ever experienced miscarriage, bleeding, intra- or extrauterine fetal death, or gave birth to abnormal neonate; normal ultrasonographic (USG) and vaginal toucher (VT); with no TORCH (toxoplasma, rubella, cytomegalovirus or herpes) infection; no hormonal abnormalities (diabetes mellitus or thyroid) and no heart diseases; and not alcoholic or cigarette smokers. The exclusion criteria were the unwillingness to join the study and experienced miscarriage in gestational age less than 8 weeks.

Blood samples were taken purposively as much as 2 ml from cubital veins from those who met the criteria and agreed to participate in this study by signing informed consent. Blood with Na2EDTA anticoagulant was divided into 2 parts: a) 1 ml for hemoglobin level, erythrocyte count, and reticulocyte count, b) 1 ml for G6PD activity test. Blood could be kept in refrigerator to wait for examination time. Assays of hemoglobin level (Hb), reticulocyte and erythrocyte count were assayed using Coulter Jt (quantitative, automated hematology analyzers). Blood was automatically absorbed and analyzed by the instrument Coulter Jt, and the result will be recorded automatically. G6PD assays were carried out using Randox Kid (Catalog No. PD 410 and DG 2693). UV method was done using Photometer 5010 reader. The principle was that the enzyme activity was measured from NADPH formed in the reaction of ($G6P + NADP^+ \rightarrow 6\ PG + NADPH + H^+$), read in 340 nm wavelength.

RESULTS

Table 1. Samples with bad pregnancy history

No	Age (years)	Number of pregnancy	Number of miscarriage (weeks)	Number of intrauterine death (weeks)	Number of stillbirths	Number of living birth
1	38	1	1 (12 wks)	0	0	0
2	30	3	1 (12 wks)	1 (28 wks)	0	1
3	34	1	1 (8 wks)	0	0	0
4	29	2	2 (24 wks)(28 wks)	0	0	0
5	36	2	2 (12wks)(16 wks)	0	0	0
6	32	4	4 (12 wks)(10wks) (12 wks)(15 wks)	0	0	0
7	37	2	2 (12 wks)(28 wks)	0	0	0
8	27	2	2 (12 wks)(10 wks)	0	0	0
9	35	1	1 (11 wks)	0	0	0
10	34	3	1 (8 wks)	0	1 (anencephal)	1
11	33	3	2 (8 wks)(12 wks)	0	0	1
12	2	2	Bleeding: 16 wks and 8 wks	0	0	2
13	35	2	2 (12 wks)(16 wks)	0	0	0
14	36	2	2 (12 wks)(16 wks)	0	0	0
15	35	2	2 (12 wks)(9 wks)	0	0	0
16	31	1	0	1 (28 wks) anenchepal	0	0
17	40	1	1 (8 wks)	0	0	0
18	25	3	3 (12 wks)(12 ks) (12 wks)	0	0	0
19	29	1	1 (12 wks)	0	0	0
20	34	9	8 (8-12 wks)	0	0	1
21	26	3	1 (12 wks)	1 (24 wks) anenchepal	1	1
22	28	1	0	1 (28 wks)	0	0
23	29	1	1 (16 wks)	0		0

Table 1 shows women with bad pregnancy history. Four (4) of the 23 samples showed intra uterin death, 20 samples had experienced spontaneous miscarriage (except no. 16, 22), 1 sample had bleeding without miscarriage (samples no. 12), 4 samples had intra uterine death (no.2,16,21 and 22) and 2 samples had anencephaly (no. 16 and 21). Of 20 samples who ever experienced miscarriage, 6 had living child or children. Of the samples 6 have living children Of the 23 samples G6PD deficiency were detected on 10 samples no.1, 4, 5, 9, 10, 12, 13, 17, 18 and 20.

Results of laboratory tests were as follows: G6PD activity of the 23 samples showed that in 10 (43.5%) samples G6PD activity was 60% of normal value. Hemoglobin measurement from 23 samples showed mean level of 13.16 g/l, while the normal level was 11.6 -17.0 g/l, only one sample (no. 5) had hemoglobin level of 10.00 g/l. Mean erythrocyte count in 23 samples were 4.62 (10^{12}/L), while the normal value was 3.80-5.50 (10^{12}/L). Mean reticulocyte from 23 samples were 0.92%, with normal value of 0.5%-2 %.

DISCUSSION

The objective of this study was to analyze the G6PD deficiency in mothers who had bad pregnancy history. Results of hemoglobin level, erythrocyte and reticulocyte count tests showed that the total mean of 23 samples had normal value. Hemoglobin and erythrocytye counts influenced the G6PD activities assays. Reticulocyte contains a nucleus that is able to synthesize G6PD, increase in reticulocyte count will increase G6PD activities indicates resulting in a false interpretation of G6PD status of the patients.

Table 2. Laboratory results. G6PD activities, hemoglobin level, erythrocyte and reticulocyte count

No	G6PD ($MU/10^9E$) or activity in %	Hemoglobin (g/l)	Erythrocyte ($10^{12}/L$)	Reticulocyte %
1	51 / 39.2%	11.1	5.3	0.5
2	91 / 70.0%	13.1	4.05	1.4
3	83 / 63.8%	12.1	5.36	0.9
4	60 / 46%	15.3	4.92	0.6
5	67 / 51.5%	10.0	4.55	0.3
6	101 / 77.7%	13.2	4.23	1.3
7	109 / 83.9%	13.0	4.22	0.8
8	128 / 98.5%	14.0	4.48	0.9
9	69 / 53.1%	13.9	4.71	1.09
10	69 / 53.1%	13.6	4.61	0.5
11	79 / 60.8%	12.4	4.10	1.08
12	74 / 59.9%	13.2	4.40	0.8
13	73 / 56.2%	15.2	5.38	1.2
14	95 / 7/3.1%	13.6	4.21	0.8
15	91 / 70.0%	11,7	4.16	0.6
16	106 / 81.3%	14.0	5.27	1.0
17	66 / 50.8%	14.3	5.01	1.0
18	98 / 52.3%	13.2	4.53	0.87
19	90 / 69.2%	12.3	3.91	0.51
20	66 / 50.8%	14.8	5.03	1.2
21	85 / 65.4%	12.5	4.60	2.1
22	90 / 69.2%	14.2	5.48	0.8
23	90 / 69.2%	12.1	3.91	0.7
	X:83.96 SD:17.64	X:13.16 SD:1.26	X: 4.62 SD: 0.49	X: 0.92 SD: 0.37

X: mean

To determine that the low G6PD activities are not false positive correction factors such as hemoglobin level, erythrocyte and reticulocyte count, are needed. In this study, G6PD activities were assayed using Randox Kid that had been completed with correction factors, both hemoglobin level and erythrocyte count. In 10 samples with G6PD activity of less than 60% the hemoglobin value, erythrocyte count and reticulocyte count were normal, except sample no. 5 with Hb level of 10 mg/dl, but it has normal erythrocyte count and reticulocyte count. It can be concluded that the G6PD activities value in these samples are not false positive results. The World Health Organization (WHO) has futher classified the different G6PD variants on the magnitude of the enzyme deficiency and also the severity of hemolysis (Beutler, 1993).

1. Class I variants have very severe enzyme deficiency (less than 10% of normal) and have chronic hemolytic anemia.
2. Class II variants also have severe enzyme deficiency, but there is usually only intermittent hemolysis.
3. Class III variants have moderate enzyme deficiency (10% to 60% of normal), with intermittent hemolysis usually associated with infection or drugs.
4. Class IV variants have no enzyme deficiency or hemolysis.

5. Class V variants are those in which enzyme activity is increased.

Of the 23 samples, 10 had G6PD activity less than normal. According to WHO, a patient with G6PD activity of less than 60% is regarded as having G6PD deficiency.

According to Burton in gestational age of 8 and 10 weeks, blood circulation from mother to fetus and the change between anaerobic metabolism and aerobic metabolism start to occur. Aerobic metabolism produces free radicals, particularly through the transfer of single electron to oxygen molecules in respiratory chain. This results in the formation of superoxide (O_2*-). Superoxide dismutase (SOD) in mitochondrial matrix and cytoplasm may change superoxide (O_2*-) to become hydrogen peroxide (H_2O_2). Hydrogen peroxide (H_2O_2) will be attenuated by glutathion peroxidase (GSHPX) and catalase will alter it into water (H_2O) and oxygen (O_2). In pregnant women with G6PD deficiency, NADPH is reduced and resulting in accumulation of hydrogen peroxide (H_2O_2) and this induces oxidative stress. Hydrogen peroxide (H_2O_2) accumulation may change into hydroxyl radical (*OH) through Haber-Weiss and Fenton reaction. Hydroxyl radical (*OH) is the most reactive radical cluster that may damage three types of compound, i.e., DNA, protein, and fatty acid needed to maintain cell integrity (Burton et al. 1999; Kliman 2000).

The 10 (43.5%) samples that showed G6PD deficiency had bad pregnancy history. This confirmed the result of the study from Longo. The living birth found may be because during pregnancy the patient was not exposed to oxidants or infection and because the provision of sufficient antioxidants. The antioxidant in the body, besides obtained from endogenous factors, such as catalase and glutathion peroxidase (GSHPX), is also obtained from exogenous factors from diet, such as vitamin E, vitamin C, beta karoten, lipoic acid, riboflavin, selenium and zinc. Thus, in G6PD deficiency, if the diet is sufficient with antioxidants, the oxidants can be attenuated, so that miscarriage can be prevented (Chan 1999; Longo et al. 2002; Nicol et al. 2000).

CONCLUSIONS

The low values G6PD activity in 10 (43.5%) samples classified in class III are not due to, since almost all affecting factors (hemoglobin level, erythrocyte and reticulocyte count) are normal. Further studies using molecular analysis should be carried out to patients with G6PD deficiency who had miscarriage. Since the number of variants of G6PD gene mutation found so far have reached more than 100, while certain variants may induce severe clinical symptoms, the identification of miscarriage-resulting G6PD gene variation patterns can be expected to raise alertness and improve the life quality of pregnant G6PD deficiency patients.

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