ORIGINAL ARTICLE

Prevalence, Sociodemographic and Clinical Characteristics of G6PD Deficient Blood Donors in Terengganu and the Effects of Storage on Their Donated Blood

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ABSTRACT

Introduction: Glucose-6-phosphate dehydrogenase (G6PD) deficiency causes red blood cell destruction due to oxidative stress. G6PD is essential for NADPH conversion; which is critical for glutathione reductase to prevent damage to cellular structures. In Malaysia, blood donors are not routinely screened for G6PD deficiency. We hypothesise that G6PD-deficient red blood cells are more likely to haemolyse during storage due to increased oxidative molecules. The objectives of this study were to determine the prevalence of G6PD deficiency among blood donors, describe their characteristics and to evaluate the effects of storage on G6PD-deficient donated blood. **Methods:** This study was conducted at selected mobile donation centres in Terengganu. Consented blood donors were screened for G6PD status using fluorescent spot tests (FST). G6PD enzyme activities were measured for donors who were G6PD deficient. Effects of storage on haemolysis from G6PD-deficient donors were compared with non G6PD-deficient group. Sixty ml of blood was collected from blood unit to transfer pouch for estimation of haemoglobin (Hb), plasma Hb, percentage of haemolysis and plasma potassium. Serial sampling with a 7-day interval was done from Day 1 to Day 35. Statistical analysis was considered significant if $p \leq 0.05$. **Results:** A total of 440 blood donors were screened and 12 male donors were found to be G6PD deficient by FST. Enzymatic activities were measured in 11 donors as one donor sample failed to be sent to the centre due to logistic problem. Their enzymatic activities ranged from 1.66-2.93 U/g Hb whereby 6 have severe deficiency and the other 5 were categorised as partial deficiency. Donors were asymptomatic for haemolytic episode. Serial sampling showed there was no significant difference of haemolytic parameters in blood units of G6PD-deficient donors as compared to control ($p>0.05$). **Conclusion:** Prevalence of G6PD blood donors in Terengganu mobile centres was 2.7%. G6PD enzyme activities did not correlate with clinical symptoms. Haemolytic parameters were not affected in blood units which were G6PD-deficient.

Keywords: G6PD deficient donors, Enzyme activity, Haemolysis, Storage

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INTRODUCTION

The most common enzymopathy disease in the world is glucose-6-phosphate dehydrogenase (G6PD) deficiency. It is characterised by destruction of red blood cells (RBC) when exposed to oxidative stress caused by infection and ingestion of certain medications or food. The prevalence of G6PD deficiency worldwide is 4.9%. The geographical distribution of this disease is wide and has variable prevalence in different countries (1). In Malaysia, the overall prevalence of G6PD deficiency was 3.4 %; among which 5.3 % are males and 1.1% are females (2).

Oxidative stress on RBC may precipitate individuals with G6PD deficiency to present with neonatal jaundice and acute haemolytic anaemia. Causes of oxidative stress can be due to infection, some medications, or fava beans ingestion (3). Unfortunately, individuals who are G6PD-deficient usually are not aware of their status throughout their lives as most of them do not produce any symptoms; hence, the extent of the issue may be underrated (4).

The prevalence of G6PD deficiency among blood donors around the world has been reported and the frequency varies significantly. As the prevalence of G6PD deficiency depends on their racial or ethnic composition, this also affect the prevalence among

donors. In Malaysia, to the best of our knowledge, there is no published report on the prevalence of G6PD deficiency among blood donors.

There is dispute with regards to whether individuals with G6PD deficiency should be allowed to donate blood (5) and issues related to the value of donated blood during handling and storage (6). However, presence of G6PD deficiency is not an exclusion criterion in the selection of blood donors. According to the World Health Organization (WHO), they suggest that without history of haemolysis, blood donors with G6PD deficiency are eligible to donate their blood. However, the donated blood from those individuals cannot be used for intrauterine transfusion, exchange transfusion in neonates or for patients with G6PD deficiency. WHO also recommends that, those G6PD-deficient donors with a history of haemolysis are deferred from donating their blood (7). A similar approach has been adopted by the Malaysian National Blood Centre (8).

However, during the donation process, blood donors are not routinely screen for G6PD deficiency (5). Since most of individuals with G6PD deficiency are asymptomatic especially those with mild reduction in G6PD activity, blood donors are not aware that they may be a carrier of G6PD deficiency.

Current blood banking practice allows refrigerated storage of RBC for up to 42 days prior to transfusion. Standard processing and storage of RBC is accompanied by increased reactive oxygen species and accumulation of oxidative biomarkers with prolonged storage. As G6PD-deficient RBC lack the ability to cope with these oxidative stresses caused by refrigerated storage (9), our hypothesis is that G6PD-deficient RBC will accumulate more oxidative damage than G6PD-normal RBC during storage, thereby resulting in increased in haemolysis and potassium levels; thereby rendering the unit unsuitable for use and may cause detrimental effects to recipient if transfused. In previous research related to G6PD-donated blood, these blood units were subjected to methods which enhance RBC injuries (10, 11) and findings were conflicting. To our knowledge, there is no study which investigates the effect of conventional storage on G6PD donated blood units.

In light of these observations, the aim of this study is to determine the prevalence of G6PD-deficient blood donors, describe their clinical characteristics and determine the effects of storage on haemolytic parameters of G6PD-deficient blood.

MATERIALS AND METHODS

Ethical approval

This study was conducted at selected mobile donation centres in Terengganu and Department of Pathology, Hospital Sultanah Nurzahirah, Kuala Terengganu. Institutional ethical approval was granted by Universiti Putra Malaysia (FPSK(FR15)P018) and Ministry of Health Medical Research Ethical Committee (NMRR-14-1730-22998). Written informed consent was obtained from all donors prior to participation in the study.

Study population and sampling

Blood donors who attended the Mobile Blood Donation centres from August 2015 to February 2016 were invited to participate in the study. Using the prevalence of G6PD deficiency in Malaysia from a previous study (2), the sample size for this study was calculated to be 202. Generally, all voluntary and non-numerated blood donors had to answer a health questionnaire in the standard blood donation form and an interview before the donation procedure. A systematic sampling method was adopted. Every third donor was invited to participate in this study. Eligible blood donors were included in the study if they consented to participate after detailed explanation by the researcher. Donors with medical histories of red cell disorders e.g. red cell enzymopathy or membranopathy, thalassaemia or haemoglobinopathy or on oxidative or red cell haemolysing drugs were excluded from the study.

Blood sampling

Approximately 2.5 ml of venous blood samples were collected in ethylene diamine tetraacetic acid (EDTA) containers (BD Vacutainer®, USA) for G6PD screening from all respondents.

G6PD-deficient blood bags as well as the normal G6PD blood bags were identified. Only 50-60 ml of blood was taken from the principal bag and separated into the transfer bag, allowing the remaining blood in the principal bag to be used for future transfusion. Subsequent analysis of blood parameters were from the transfer bag with equivalent ratio of preservatives, stored separately from the principal bag. Approximately six ml of blood sample were collected from the transfer bag on days 1, 7, 14, 21, 28 and 35 for serial measurement of plasma and blood haemoglobin, haematocrit, and potassium level (Figure 1). Serial seven-day intervals measurement was modified from Kanias & Gladwin, 2012 (12). Samples for testing were collected by transferring the blood from the transfer bag into respective EDTA and plain tubes using the closed docking system.

G6PD screening and confirmation

The fluorescent spot test (FST) was used for G6PD screening to determine prevalence of G6PD deficiency among the blood donors.

For semi-quantitative FST, a drop of whole blood from each sample) was dried on Whatman's filter paper. The detailed procedure of the FST is found in Beutler,E. (1994) (13). Samples with normal or slightly depressed G6PD activity show strong fluorescence. No fluorescence after a 10-minute incubation period suggests complete

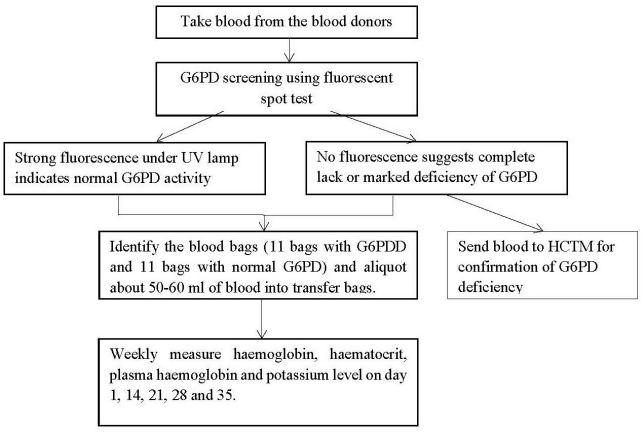

Figure 1: Flow chart of the methodology

lack or marked deficiency of G6PD. This test was run together with an internal positive and negative control. Quantitative G6PD test was performed if the FST showed deficient or intermediate results. This test confirms the reduced G6PD activity in G6PD-deficient blood donors. The test was performed in the Haematology Laboratory, Department of Diagnostic Laboratory Services, Hospital Canselor Tuanku Mukhriz (HCTM). The samples from the EDTA bottles were sent to HCTM and processed within 72 hours of collection to measure G6PD enzyme activity.

The OSMMR2000-D G6PD assay kit (R&D Diagnostics Holargos, Greece) with haemoglobin normalisation was used to quantitate G6PD enzyme activity using a spectrophotometer (Ultramicroplate Reader EL808, Bio Tek, Instruments). Principle of this kit involved an enzymatic colorimetric method for the quantitative determination of G6PD activity that employs the haemoglobin normalisation procedure. Detailed procedure of this quantitative G6PD test is found in Azma et al (2010) (14).

This assay kit was also governed by external QC (RCPA) & internal QC runs periodically as stated by the standard operating procedure.

Haemoglobin, plasma haemoglobin, haematocrit and percentage of haemolysis measurement

Effects of red blood cell storage upon red cell haemolysis were determined using the following parameters. All these parameters are measured using automated analyser, Sysmex XN-1000 series. This analyser has been evaluated and validated for use and is governed by external QC (RCPA) & internal QC runs periodically as stated by the standard operating procedure.

Measurement of haemoglobin and plasma haemoglobin were performed using Sysmex XN-1000. The measurement principle used is sodium lauryl sulphate (SLS)-haemoglobin method. This method uses SLS for measuring the haemoglobin concentration. SLS binds to the red blood cell membrane mainly by ionic bonding and partly by hydrophobic bonding. SLS- haemoglobin shows absorption curve with the maximum peak at wavelength 535 nm and a shoulder peak at 560 nm. The analyser irradiates light of 555 nm wavelength and measures the absorption.

Plasma haemoglobin is also measured using the similar principle whereby plasma is separated first from the red blood cells, and then haemoglobin in the plasma is measured.

Haematocrit (HCT) is a measurement of the ratio of the capacity occupied by the red blood cells to the volume of whole blood. Haematocrit is calculated by an automated analyzer by multiplying the red cell count by the mean cell volume (MCV).
As for percentage of red cell haemolysis, the following formula was used;

$$\text{Haemolysis} = \text{Plasma Hb} \times 1\text{-Hct} \times 100\text{B-hb}$$

where Hb- haemoglobin; Hct- haematocrit; B-hb- blood haemoglobin.

Potassium level measurement

The Beckman Coulter AU5800 biochemistry analyser was used to measure RBC potassium levels in stored donated blood. This analyser has been evaluated and validated and also took part in External Quality Control (EQC) by RCPA. Internal Quality Control (IQC) runs periodically as stated by the standard operating procedure.

The AU5800 analyser is a fully automated system for clinical chemistry analysis intended for the in vitro quantitative determination of analytes in body fluid. Potassium is measured using ion-selective electrodes.

Statistical Analysis

Data were entered and analysed using statistical package programme SPSS version 22.0 windows. Descriptive statistics for categorical variables were expressed as frequency and percentage whereas numerical variables were expressed as median and inter quantile range (IQR) for assumed not normally distributed data (as samples analysed less than 30). We used Mann Whitney U test to compare the median between the two independent variables. A p- value of < 0.05 was considered significant.

RESULTS

Demographic data of blood donors

The sociodemographic characteristics of respondents are summarised in Table I. Most of the participants were male donors (70.2%) and female donors accounted for 29.8%. Most of them were Malay (93.9%), followed by Chinese (4.1%), Indian (0.9%) and others (1.1%). The mean (standard deviation) age of blood donors was 23.19 (7.16) years old. About 300 (68.2%) were first

Table I: Demographic data and donation status of blood donors (n=440)

Variable	Mean (SD)*
Age (years)	23.19 (7.16)
	Frequency n(%)
Gender	
Male	309 (70.2)
Female	131 (29.8)
Ethnic	
Malay	413(93.9)
Chinese	18(4.1)
Indian	4 (0.9)
Others	5(1.1)
Number of donation per year	
First time donor	300 (68.2)
2 times per year	90 (20.4)
More than 2 times per year	50 (11.4)

*SD- standard deviation

time donors and others had 2 times donation per year (20.4%) and regular donors who donate more than 2 times per year were 11.4%.

Prevalence of G6PD deficient blood donors and their severity

There were only 12 (2.7%) donors among the 440 samples collected found to have G6PD deficiency from the G6PD screening test. Among the G6PD deficient blood donors, we were able to measure G6PD enzyme activities in 11 out of 12 respondents as one sample failed to be sent to the referral lab within 72 hours due to logistic problem. The mean G6PD enzyme value was 2.24 (0.93) unit/g Hb (Table II).

G6PD deficiency was classified by severity based on its enzymatic activity whereby; severe deficiency is when enzyme activity is less than 20% of mean normal activity and partial deficiency is where its activity is 20-60% of mean normal activity. Out of 11 respondents, 5 (46%) of them had partial G6PD deficiency while another 6 (54%) of them had severe G6PD deficiency. Enzyme levels for G6PD-deficient partial and severe deficiency donors were 2.93 (0.91) [ref range: 2.36-7.27 Unit/g Hb] and 1.66 (0.40) Unit/g Hb [ref range < 2.36 Unit/g Hb], respectively. The range stated were 20% of normal mean equivalent to 2.36 U/gHb and 60% of mean normal activity equivalent to 7.27 U/gHb measured during the period of the study. Severe deficiency was diagnosed when the level was less than 20% equivalent to less than 2.36 U/gHb.

Table II: Severity of G6PD deficiency among G6PD deficient blood donors

	G6PD deficient blood donors n, (%)	G6PD enzyme activity Mean (SD) (Unit/g Hb)	Reference range (Unit/g Hb)
Partial deficiency	5 (46%)	2.97 (0.91)	2.36-7.27
Severe deficiency	6 (54%)	1.66 (0.40)	<2.36

Notes: Calculation of reference range for adult was based on WHO Working Group 1989 (Value of more than 60% of normal mean is considered to be the normal value of G6PD enzyme)

Demographic data of G6PD deficient blood donors by enzymatic activity

Out of 11 donors with G6PD deficiency, all of them (100%) were Malay male donors. The mean age was 21.6 (5.7) years old. Six (54.5%) of them were first-time donors followed by 4 (36.4%); who donated 2 times per year and 1 (9.1%) were those who donated more than 2 times per year. All G6PD deficient donors in this study were asymptomatic of haemolytic episodes. None of the 11 respondents were aware of being G6PD deficiency carriers, including the five who were periodic donors (45.5%).

Effect of storage on haemolytic parameters in G6PD-deficient donated blood compared to normal G6PD

Table III summarises the effect of storage on haemolysis parameters in blood from 11 G6PD-deficient donors as compared to 11 G6PD normal donors.

Table III: Effect of storage on haemolysis parameters in blood from G6PD normal donors compared G6PD deficient blood donors

Time	G6PD- Normal		G6PD-Deficient		*p* value (Mann Whitney U test)
Days	Median	IQR*	Median	IQR*	
Haemoglobin level (g/L)					
1	169.0	51.8	151.5	90.8	0.762
7	181.5	49.3	153.5	88.0	0.597
14	180.0	58.5	210.0	74.3	0.212
21	172.0	69.3	160.5	98.3	1.0
28	165.0	73.5	151.5	95.3	1.0
35	173.5	66.5	153.5	85.5	1.0
Plasma Haemoglobin level (g/L)					
1	0	0	0	0	0.146
7	0	0.3	0	1.0	0.3
14	0	1.0	0	1.0	0.445
21	0	1.0	0	1.0	0.923
28	0	1.0	0	1.5	1.0
35	0.5	2.0	0	2.5	0.854
Percentage of haemolysis (%)					
1	0	0	0	0	0.147
7	0	0.05	0	0.25	0.351
14	0	0.16	0	0.16	0.901
21	0	0.20	0	0.23	0.813
28	0	0.19	0	0.31	0.777
35	0.27	0.44	0	0.30	0.353
Potassium level (mmol/L)					
1	5.45	1.44	4.67	1.52	0.762
7	14.54	12.53	11.25	3.97	0.496
14	22.79	14.52	16.11	8.77	0.288
21	27.17	17.21	19.89	7.76	0.142
28	24.96	17.03	22.12	9.37	0.414
35	35.23	20.45	26.24	9.09	0.514

*IQR- interquartile range
p value > 0.05 – not significance.

Blood haemoglobin

Figure 2 compares haemoglobin levels between G6PD-deficient and normal G6PD blood based on storage time (days). Even though the value of blood haemoglobin in the G6PD-deficient group were lower than normal G6PD group in most measured days, statistical analysis using Mann Whitney U test showed there is no significance difference during storage of both G6PD-deficient and normal G6PD blood (p value > 0.05).

Free plasma haemoglobin & percentage of haemolysis

As for free plasma haemoglobin and percentage of haemolysis, free plasma haemoglobin and haemolysis were not detected in both groups of normal G6PD and deficient G6PD blood with time upon storage, hence there was no statistical significance noted in both parameters.

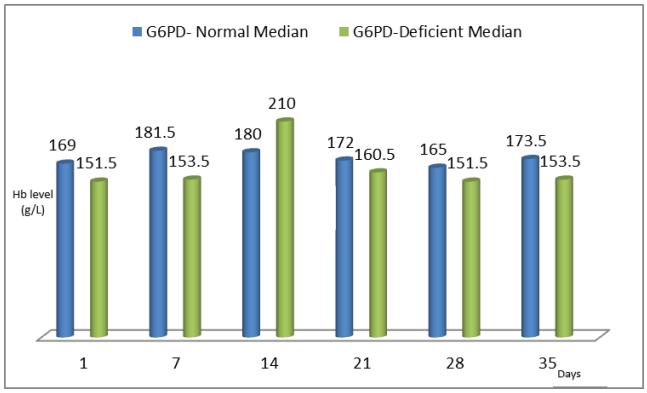

Figure 2: Effect of storage on blood haemoglobin level. Bar chart showing serial comparison of blood haemoglobin level of 7- day interval between blood donated by normal G6PD donors (blue) and deficient G6PD (green). There is no significant change in both groups with regards to blood haemoglobin level (p > 0.05)

Plasma potassium

Figure 3 shows an increasing trend of potassium level upon storage in both groups of normal G6PD and deficient G6PD blood. Potassium levels were consistently lower in the G6PD-deficient group as compared to normal G6PD. However, there was no significant differences (p >0.05) when values of both groups were compared.

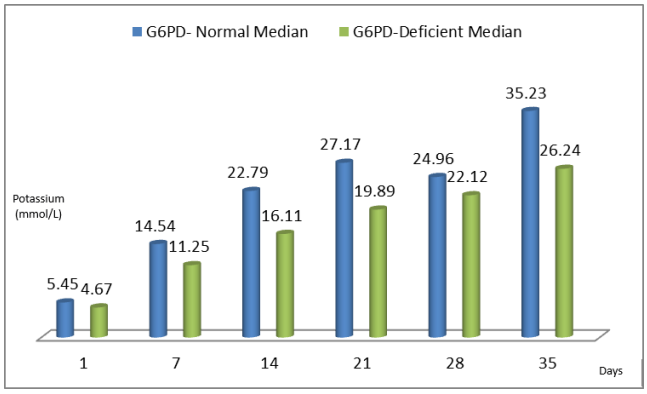

Figure 3: Effect of storage on potassium level. Bar chart showing serial measurement of potassium level of 7- day interval between blood donated by normal G6PD donors (blue) and deficient G6PD (green). There was increased in potassium level with increasing days of storage however, there is no significant change in both group (p> 0.05)

DISCUSSION

Sociodemographic characteristics of blood donors

In this present study there were 309 (70.2%) male donors whilst 131 (29.8%) were female donors. This is similar to the worldwide trend of more male blood donors than female blood donors. Malaysia has multiracial population with Malays, Chinese and Indians being the major ethnics in Peninsular Malaysia. In Terengganu, we found that Malay blood donors were the highest (93.9%) followed by Chinese (4.1%), Indian (0.9%) and others (1.1%). Our result was consistent with a study done by Rosline et al which reported 91.3% of blood donors were among Malays (15) and report from population census whereby 97% of Terengganu population were of Malay ethnicity (16). The mean age (SD) of blood donors was 23.19 (7.16) years old. This showed that majority of the blood donors were among the younger age group.

Prevalence of G6PD deficient blood donors and severity of G6PD deficiency

In this study, we found that prevalence of G6PD deficiency in blood donors is 2.7%. The reported proportions of G6PD deficiency among blood donors worldwide varies significantly; Nigeria (19.5%) (18), Canada (0.17%) (19), Brazil (3.2%) (20), Iran (16.3%) (21), India (0.8%) (22), Riyadh, Saudi Arabia (0.78%) (6), Italy (1.1%) (23) and United States (0.3%) (5). This shows diversity in study population and background affects prevalence of G6PD deficiency among population as well as its prevalence among blood donors. G6PD deficiency is highly diverse, with at least 217 genetic variants expressing a spectrum of enzyme phenotypes. At the global scale, distinct regional patterns of G6PD deficiency epidemiology emerge based on unique combination of alleles originating from different regions (24).

WHO Working Group in 1989 defined G6PD deficiency as a residual red cell G6PD activity level of less than 60% normal (25). Individuals with activity of < 20% of mean normal activity is called severe deficiency and those with 20-60% of mean normal activity; they are classified as partial deficiency (26).

In this study, all G6PD deficient blood donors were male. Out of 11 G6PD deficient donors assessed; 54% (6 of 11) had severe enzyme deficiency and 46% (5 of 11) had moderate enzyme deficiency with the values for G6PD activities of 1.66 (0.40) and 2.93 (0.91) U/gHb, respectively. Differences in the level of G6PD enzyme among G6PD deficient males' donor are most probably due to different G6PD variant they have. The most common G6PD variant causing G6PD deficiency belongs to the class II variant in which the enzyme is severely deficient. Previous local study reported among Malay and Chinese neonates with G6PD enzyme deficiency, majority of them were males with severe enzyme deficiency (27, 28). Similarly in this study, more

than half of the G6PD deficient were males with severe enzyme deficiency.

In another local study, they found the prevalence of partial enzyme deficiency in female children and female neonates were 15.7% and 3.42% respectively (29). However, no study was done among adult population to compare with our study. Therefore, there is possible underestimation in our prevalence of G6PD deficiency especially among the partial deficient female donors as we did not use the quantitative enzyme assay as the screening method.

The risk of haemolytic episode is not critically dependent on the true level of the G6PD enzyme alone. In affected males, as they can only be hemi zygotes of G6PD deficiency, they have always been associated with high risk of haemolysis. Nevertheless, even in partially deficient individuals, there is increased risk of oxidative haemolysis when exposed to certain drugs, food, chemicals, infection and not to mention cultural practices (30). The clinical severity of G6PD deficiency also depends on the different type of G6PD variants. Specific G6PD allele mutations give rise to variable sensitivity against oxidative damage. In some cases with extremely low enzyme level, chronic haemolysis may occur (31). However, effects of haemolysis on blood storage between these partial and severe deficiency has not been determined yet in the literature.

Socio demographic and clinical characteristics of G6PD deficient blood donors

Sex distribution in this study showed that 100% of the deficient donors were male. This is because the gene that expresses G6PD enzyme is located at chromosome X, hence, the male preponderance of the deficiency. An abnormality at specific alleles of X chromosome may give rise to G6PD deficiency. A female heterozygote has 50% chances of passing the abnormal X chromosome to her son. Therefore, the son who receives the abnormal gene will be G6PD deficient. On the other hand, her daughter may be normal or a heterozygote carrier (15).
As stated earlier, males are hemi zygotes of G6PD deficiency thus the enzyme activity is always low. Females on the other hand, may be heterozygous or less commonly homozygous for the G6PD gene mutation. In homozygous females, the enzyme activity can be as low as male hemizygotes. However, in heterozygous females, a lyonization phenomenon may occur. This phenomenon causes random inactivation of the normal X chromosome giving rise to genetic mosaicism in the red cells G6PD activities. The overall effect of the G6PD deficient severity depends on the proportion between deficient and normal residual red cells enzyme's activity (23).

Another factor contributing to the absence of G6PD deficiency in female donors was due to the insensitive method used in this study. Female heterozygotes may have partial deficiency in which the enzyme level ranges between 20-60% (26). Previous study had shown that semi quantitative method like FST is not reliable to detect heterozygotes female as it is only sensitive when the enzyme activity is less than 20% (32). In this study, we used FST as first line to screen all donors followed by confirmatory test of red cell enzymatic activity quantitation. Semi quantitative method is reliable in detecting severe G6PD deficient but may missed substantial number of partial deficient donors. This reason may explain the possibility of missing partially G6PD deficient female blood donors in this study.

Based on racial distribution, from this study, we found that all G6PD deficient male donors are Malays. This is consistent with previous study which stated G6PD deficiency is more prevalent among Malays and Chinese, and less commonly occurred among Indian. The prevalence of G6PD deficiency among males was 5.3%; with racial distribution of 7.2%, 4.6% and 2.7% among Chinese, Malays and Indians males respectively (2).

In this study, none of the G6PD deficient donors was aware of their G6PD status even though half of them were periodic donors. They were also asymptomatic and admitted that there was no history of favism in the past. Similarly, few studies reported that most of the G6PD deficient blood donors are symptomless with no history of previous haemolytic reaction or jaundice due to febrile illnesses or drugs (33, 17, 18).

Effects of storage on levels of blood haemoglobin, plasma haemoglobin, percentage of haemolysis and potassium between G6PD deficient and normal G6PD blood

Markers of storage lesions assessed in this study include blood haemoglobin, plasma haemoglobin and potassium level. Haemolysis is defined as the breakdown or the disruption of red blood cells' membrane integrity with subsequent release of haemoglobin to the plasma. Findings of the present study shows there were no significant differences in the effect of storage on haemolysis of G6PD deficient blood as compared to normal G6PD blood in any of the time interval assessed (p value > 0.05).

These findings are almost similar with findings of past study by Agarwal et al. whereby G6PD deficient red cells (cases) were irradiated by gamma rays and compared with red cells with normal G6PD (controls). Markers for storage lesion, such as, free plasma haemoglobin and potassium; as well as lactate dehydrogenase (LDH), were not elevated significantly following irradiation. Hence, it was concluded that, there was no significant extra damage to RBC membrane due to G6PD deficiency when subjected to gamma irradiation as compared to controls (10). Although they used gamma irradiated blood to compare between these two groups, the final

findings were similar to our study; there was no different in the effects of storage on haemolysis of blood belonged to G6PD deficient individuals.

In contrast to another study by Westerman et al, ionizing radiation had adverse effect on G6PD-deficient red cells and RBCs survival times were shortened when compared with normal red cells (11). The differences in results could occur due to variation in methodology such as differences in cut off level of G6PD activity in the RBCs (e.g., G6PD-deficient RBC with 10% versus 10%-60% residual enzymatic activity). Apart from that, variation in radiation dose and different methodologies used to assess G6PD deficiency (i.e., cesium-137 versus x-rays) also had to be considered. Materials used for blood storage also varies (blood bags vs plastic containers), as well as different variants of G6PD activity (because the type and degree of haemolysis differs according to variant) (10, 11).

However, in our study, the blood under storage was not subjected to any method which may enhance the RBC injury, like previous studies. They used gamma irradiation on G6PD deficient red cells to induced oxidative injuries while in this study, the blood were stored in conventional condition without any addition of oxidative stress.

The main characteristic of G6PD deficiency is RBC destruction in response to oxidative stress. Following blood donation, conventional blood storage system has factors which contribute to oxidative stress. These factors include presence of reactive oxygen, free radicals and other oxidative biomarkers that accumulates over time. An example of this is malondialdehyde and other products of oxidation from the human serum albumin, lipid as well as spectrin, which is on RBC membrane protein (34). Changes that occur during storage will contribute to haemolysis in vitro and in vivo. Oxidative damages do occur on normal RBCs during conservative blood banking storage. Main variations usually occur between 7 to 14 days of storage (12). These consequences would most probably be increased with red cells which are G6PD deficient (13). However, other than G6PD deficient blood donor, storage injuries appear to be reliant on donor age, sex, smoking habit and may also be subjected by the other genetic background of the donor for example thalassemia traits (34) which are not specifically assessed in this study.

Haematological parameters which include blood haemoglobin, plasma haemoglobin and percentage of haemolysis can be used as quality indicators for stored blood. One of the most used quality indicator for stored red cell units is the extent of haemolysis. There are many factors that caused abnormal haemolysis in RBC units. These include inappropriate handling during processing and storage of blood, RBC membrane defects, complement lysis due to bacterial haemolysin antibodies or an abnormality in the blood donor (35). The extent of haemolysis is described as the percentage of free haemoglobin in relation to the total haemoglobin with appropriate correction for the haematocrit. Council of Europe and US FDA guidelines have stated that the allowable degree of haemolysis should not more than the permissible value which is 0.8% and 1% respectively, even on the day of 42nd of storage (36). There was no sample in this study which showed percentage haemolysis of more than 0.8%, which is the maximum acceptable limit of haemolysis as per Council of Europe Guideline.

There was some discrepancy noted in the result of blood haemoglobin level at day 14 analysis. This was probably due to pre analytical error occurred during blood sampling for example improper mixing of blood. This will affect haemoglobin and haematocrit level .Another reason why these haematological parameters showed not much different between these two groups most probably because the blood donors with G6PD deficiency have normal haematological parameters thus, they are well, asymptomatic and unaware of their genetic defect especially those with mild to moderate level of G6PD enzyme. Even with severe G6PD deficiency due to class II molecular variant, they are often asymptomatic and haemolytic attacks are triggered only by an oxidative damage (3). Since the blood donors are asymptomatic and most of them have normal haematological parameters, this probably explained the storage lesions in both groups were almost similar throughout the storage time.

In a study to evaluate effect of blood storage on complete biochemistry, there were significant increase in serum phosphorus, AST, serum protein, calcium and potassium level and decrease in pH, serum chloride, sodium and bicarbonate levels throughout the storage time (37). Similarly, in our study, we noted the median value of potassium level increased during storage period in both groups. However, comparing G6PD deficient and normal G6PD blood, the difference was not statistically significant (p value > 0.05) for plasma potassium level. Accumulation of potassium in the supernatant due to alteration of Na+/K+ fluxes in the older RBCs can cause adverse clinical consequences especially in paediatric patients (34).

CONCLUSION

From this study, as there was no difference in the effect of storage on haemolysis of G6PD deficient blood, screening for G6PD deficient donors appear to be unnecessary. There is a need to perform similar study with larger sample size, G6PD variant molecular characterization and more numbers of parameters taken into account for study of oxidative damages. Apart from that, there is a need to study about the effects of receiving G6PD deficient blood in the recipient. Although the

risks for most adult recipients are minimal, specific cases as in premature infant, neonates and in patients who may require multiple transfusions, screening for G6PD deficiency in the donors may be essential (4).

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