ORIGINAL ARTICLE

The activity of glucose-6-phosphate dehydrogenase (G6PD) in stored blood

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ABSTRACT

Background: Blood transfusion is a critical component of supportive therapy. Red blood cell viability in stored blood determines successful transfusion**.** Glucose-6-phosphate dehydrogenase (G6PD) activity has been shown to maintain red blood cell membrane integrity. This study was, therefore, aimed at estimating the G6PD activity in stored blood bags at the blood bank of the University of Nigeria Teaching hospital (UNTH) Enugu.

Methodology: The activity of G6PD in 100 stored blood bags consisting of different ABO groups [A (*n=30*); B (*n=30*); O (*n=30*) and AB (*n=10*)], stored at the blood bank of the UNTH Enugu between April and August 2009, was determined using methhaemoglobin reduction and ultraviolet (UV) spectrophotometric quantitative methods. The data obtained were statistically analyzed using student's t-test and analysis of variance.

Results: There was statistically significant decrease in the G6PD activity from the third week of storage ($p<0.05$) at the blood bank, under optimum storage conditions. The different ABO blood groups did not show any significant variation ($p>0.05$) in G6PD activity.

Conclusion: Storage of whole blood for up to three weeks results in significant decrease in the G6PD activity and possibly, affects the red cell viability. Stored blood in the blood bank should be used up before the third week to ensure viability of red blood cells.

Keywords: *ABO groups, blood transfusion, blood donors, haemolytic anaemia, red blood cells*

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) is an important enzyme in red blood cell metabolism which catalyzes the initial step in the hexose monophosphate (HMP) or pentose phosphate shunt pathway, oxidizing glucose-6-phosphate to 6-phosphogluconolactone and reducing nicotinamide adenine dinucleotide phosphate (NADP) to NADPH.[1] This enzyme is, therefore, very important for red cell integrity.

The value of this enzyme was first recognized when it was noted that some African-American soldiers who took the anti-malarial drug primaquine developed acute haemolytic anaemia and had haemoglobinuria.[2] Further investigations revealed that the enzyme G6PD was deficient in the red cells of affected patients.[2,3]

Glucose-6-phosphate dehydrogenase deficiency is the most common enzyme deficiency in the world with about 400 million affected people.[3] All races are affected with the highest prevalence observed in Africans, Asians and the Mediterranean. In Nigerians, the prevalence of G6PD deficiency ranges from 4–26% with the male population having about 20–26%.[4,5] The ultimate effect of G6PD deficiency is acute haemolytic anaemia when affected red blood cells are exposed to oxidative damage. Predisposing factors to oxidative stress include infections, drugs or fava beans consumption.[5]

The use of anticoagulants has made it possible for red blood cells to be stored for varying lengths of time before transfusion depending on the anticoagulants used. The results obtained from different studies to determine the effect of the various anticoagulants on G6PD activity have not been consistent.[6] The most preferred anticoagulant in several Nigerian blood banks, including in UNTH Enugu, is the Citrate Phosphate Dextrose Adenine (CPDA).[5]

Most events of medical emergency require blood transfusion and this situation makes it imperative that stored blood remains a composite part of emergency healthcare delivery. The paucity of knowledge in G6PD activity of stored blood in our locality has necessitated this present study. The study was, thus, conducted to determine the G6PD activity and rate at which it is decreased or lost with time in stored blood, and also, to check the possible effect of ABO blood groups on G6PD activity in stored blood. The outcome of this study would reveal the effects of prolonged storage, and quality of donated blood before transfusion to recipients.

METHODOLOGY

Subjects

The study subjects included 100 screened male volunteer blood donors, attending the blood bank of the UNTH, Enugu between April and August 2009. The subjects were grouped according to their ABO blood groups into group A (*n=30*); group B (*n=30*); group O (*n=30*) and group AB (*n=10*). Informed consent was given by the subjects and ethical clearance was granted by the Ethics Committee of the institution, before the commencement of the study.

Sample Collection and Preparation

About 3 ml of blood was initially collected through a clean venepuncture from the ante-cubital vein into tri-potassium ethylene diamine tetracetic acid (K_3-EDTA) bottles for qualitative G6PD screening assay while the samples for quantitative assays were collected from the citrate phosphate dextrose adenine (CPDA) blood bags, immediately after blood donation by the donors. A total of 450ml of whole blood was donated by each donor into CPDA blood bags. The G6PD activity was estimated immediately after blood donation to obtain the baseline values before storage. The G6PD activity was, thereafter, repeated for the stored blood samples weekly for 4 weeks of storage at 4±2 ^oC.

Analytical Methods

Qualitative G6PD status screening was done using methhaemoglobin reduction method, whereas the quantitative G6PD activity was done by the ultra violet (UV) method.[6] The

data obtained were statistically analyzed with the Statistical Package for Social Sciences (SPSS) version 11, using student's t-test and analysis of variance at 95% confidence limit. The results were presented as mean ± standard deviation (±SD) and $p<0.05$ was considered significant.

RESULTS

The baseline values of G6PD activities in the blood samples in CPDA blood bags within one hour of collection are shown in Table 1, whereas Table 2 shows the comparison between the G6PD activities in the blood samples in CPDA blood bags after one week of storage at 4±2 ºC and baseline values.

Table 1. The baseline mean ± SD of G6PD activities in the blood samples in CPDA Blood bags within 1hour of collection (baseline activity)

Blood Groups	n=100	Baseline Activity (U/g Hb)
A	n = 30	0.13 ± 0.01
B	n = 30	0.13 ± 0.01
AB	n = 10	0.13 ± 0.03
O	n = 30	0.14 ± 0.01

Table 2. The mean ± SD of G6PD Activities in the blood samples in CPDA blood bags after 1week of Storage at 4 ±2ºC and baseline

Blood Groups	n=100	1 week Activity (U/g Hb)	Baseline Activity (U/g Hb)	*p-Value*
A	n = 30	0.13 ± 0.01	0.13 ± 0.01	p > 0.05
B	n = 30	0.13 ± 0.01	0.13 ± 0.01	p > 0.05
AB	n = 10	0.13 ± 0.01	0.13 ± 0.03	p > 0.05
O	n = 30	0.13 ± 0.01	0.14 ± 0.01	p > 0.05

The results revealed no significant difference ($p>0.05$) after 1 week of storage. Tables 3, 4 and 5, respectively, show the comparisons between the G6PD activities in the stored blood bags after 2, 3 and 4 weeks of storage, at 4 ± 2 ºC vis-à-vis the baseline values. The results also revealed no significant difference ($p>0.05$) in the G6PD activity of the stored blood after 2weeks, whereas significant decrease ($p<0.05$) in activity was recorded after 3 and 4 weeks of storage, respectively, compared to the baseline values. Different ABO blood groups however, did not show any significant variation ($p>0.05$) in G6PD activity throughout storage.

Table 3. The mean ± SD of G6PD activities in the blood samples in CPDA blood bags after 2weeks of storage at 4 ± 2 ºC and baseline

Blood Groups	n=100	2 weeks Activity (U/g Hb)	Baseline Activity (U/g Hb)	*p-Value*
A	n = 30	0.13 ± 0.01	0.13 ± 0.01	p > 0.05
B	n = 30	0.13 ± 0.01	0.13 ± 0.01	p > 0.05
AB	n = 10	0.13 ± 0.01	0.13 ± 0.03	p > 0.05
O	n = 30	0.13 ± 0.01	0.14 ± 0.01	p > 0.05

Table 4. The mean ± SD of G6PD activities in the blood samples in CPDA blood bags after 3weeks of storage at 4 ± 2 ºC and baseline

Blood Groups	n=100	3 weeks Activity (U/g Hb)	Baseline Activity (U/g Hb)	*p-Value*
A	n = 30	0.11 ± 0.01	0.13 ± 0.01	p < 0.05
B	n = 30	0.11 ± 0.01	0.13 ± 0.01	p < 0.05
AB	n= 10	0.11 ± 0.02	0.13 ± 0.03	p < 0.05
O	n= 30	0.11 ± 0.01	0.14 ± 0.01	p < 0.05

Table 5. The mean ± SD of G6PD activities in the blood samples in CPDA blood bags after 4weeks of storage at 4 ± 2 ºC and baseline

Blood Grps	n=100	4 weeks Activity (U/g Hb)	Baseline Activity (U/g Hb)	*p-Value*
A	n = 30	0.11 ± 0.01	0.13 ± 0.01	p < 0.05
B	n = 30	0.11 ± 0.01	0.13 ± 0.01	p < 0.05
AB	n = 10	0.11 ± 0.02	0.13 ± 0.03	p < 0.05
O	n = 30	0.11 ± 0.02	0.14 ± 0.01	p < 0.05

DISCUSSION

The present study estimated the G6PD activity in the stored blood of different ABO groups and results of the study revealed no significant difference in the G6PD activity of the stored blood after 2weeks, whereas significant decreases in activity were recorded after 3 and 4weeks of storage, respectively, compared to the baseline values. Different ABO blood groups did not show any significant variation in G6PD activity.

This result pattern shows that G6PD activity decreases with prolonged storage of donated blood from the 3rd week, irrespective of the expiry date. This might probably be due to the type of anticoagulant (CPDA) in the blood bags. However, the G6PD activity was stable in the blood bags when the test was carried out within one hour (baseline value) as well as when the blood was stored in CPDA blood bags at 4 ± 2°C for up to 2weeks. But, beyond 2weeks, there was significant loss of activity in the CPDA stored blood.

Deficiency of G6PD results in the inability of red cells to regenerate reduced nicotine adenine dinucleotide phosphate (NADPH). Only male subjects were selected as G6PD deficiency has been shown to mostly affect males, because the G6PD gene is x-linked.[7] Haemolytic anaemia and neonatal jaundice are the two major conditions associated with G6PD deficiency, which may result in neurological complications and death.[7] Screening and detection of G6PD deficiency helps in reducing such episodes, through appropriate selection of treatment, patient counselling and abstinence from precipitating drugs such as anti-malarial and other agents.[7]

Previous studies have documented that G6PD deficiency decreases cholesterol synthesis, superoxide production, and reductive stress, whereas, more recent studies show that G6PD deficiency leads to moderate susceptibility to ventricular dilation in response to myocardial infarction or pressure overload-induced heart failure.[8]

Blood donors in our locality (especially males) need to know their G6PD status so as to ensure that those found to be deficient will avoid the predisposing factors to acute haemolytic attack, such as indiscriminate ingestion of drugs.[9]

Also, transfusion of blood stored for more than 3weeks (with decreased G6PD activity) or G6PD deficient blood should be discouraged. Transfusion with G6PD-deficient blood has been reported to carry a potential risk of haemolytic complications, especially if it is used for exchange blood transfusion in neonates.[10] The shelf-life for blood stored in blood bags containing CPDA anticoagulant is about 35days in our environment and our study has further shown that G6PD activity starts to decrease after 2weeks of storage.

CONCLUSION

This study revealed that G6PD activity is significantly reduced in blood stored beyond 2 weeks. Considering the negative effects on red cell membrane integrity and viability concerted efforts must be made to improve the outcomes of blood transfusion services in this environment. Screening for G6PD deficiency should, therefore, be included among the screening tests for blood donors before blood donation.

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