Studies On G6PD Stability In Blood Stored With Different Anticoagulants

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Abstract

Background: G - 6PD screening is not carried out on overnight K_2EDTA anticoagulated blood samples in our hospital. This was due to belief that the
G6PD enzyme would have lost its activity. Literature datas on stability of G6PD in blood stored with different anticoagulants varied from one author to another.

Objectives: To investigate the viability of G6PD in blood stored with different anticoagulants.

Setting: The work was undertaken in the special Investigation unit of the department of Haematology Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Nigeria.

Methodology: G6PD screening was performed using methaemogobin reduction method on 100 apparently health Nigerians. Their blood samples were collected into dispotassium ethlenediamine tetraacetic acid (K_2EDTA); acid citrate dextrose, (ACD); citrate phosphate dextrose (CPD); sodium citrate, lithium heparin, ammonium oxalate and Fluoride oxalate. The tests were performed within an hour of blood collection, the blood samples were them kept at 4°c and the tests repeated at 12, 24, 48 and 72 hours of storage.

Result and conclusion: the result of G6PD screening within an hour of blood collection and at 12, 24, 48 and 72 hours after storage at 4°c in K_2EDTA, ACD, CPD, sodium citrate and lithium heparin were the same. The results of G6PD screening within an hour of blood collection and 12 hours after storage at 4°c in ammonium oxalate at 24, 48 and 72 hours in the oxalates. G6Pd was stable for up to 72 at 4°c in K_2EDTA, ACD, CPD, sodium citrate and lithium heparin and just for 12 hours in the oxalates,

INTRODUCTION

Glucose- 6- phosphate is a cytosolic enzyme that generates reduced nicotinamide adenine dinuclueotide phosphate (NADPH) in the oxidative branch of the pentose phosphate pathway (PPP) (Salvemini and France, 1997) The clinical significance of G6PD is the deficiency state which is an inheritable sex-linked recessive disorder whose primary effect is the oxidation of double bonds in the red cell membrane to epoxide by hydrogen peroxide which is a byproduct of the formation of oxyhaemoglobin in the lungs. The cell membrane becomes incompetent and sodium and water enters with resultant haemolysis. G6PD deficient red cells are more susceptible than normal red cells to oxidative damage. The reason for this is that NADPH, produced by G6PD, is required for regeneration of reduced glutathione (GSH) Dxidative damage can be caused by fava beans, infection and drugs Luzzato and Gardonsmith 1999).The ultimate effect of the disease is to produce anaemia either acute haemolytic or a chronic spherocytic type (Rebecca, 2001) The G6PD deficient state is frequently associated with neonatal hyperkernicterus and death (Beutler, 1994; Valees, 1994 and slusher et al, 1995). It is also the most important aetiological factor associated with neonatal jaundice in full term normal weight infants in Nigeria (Effong and Lehtan, 1995) and other parts of Africa (Nkrumah, 1978)

G6PD deficiency in the most common enzyme deficiency status in the world with about 400 million people living with it, all races are affected and the highest prevalence is found among Africans, Asians and Meditarraneans (Carter and Gross 2002). The prevalence and pattern of G6PD

deficiency in different haemoglobin types in Ile-Ife, Nigeria has been determined (Oduola and Olayink er al 2004) also reported demonstration of Heiz bodies for G6PD screening.

The literature datas on stability of G6PD in whole blood stored with different anticoagulants tend to be contradictory; some authors advocate the use of ACD and glucose-EDTA while others said that ACD and glucose-EDTA do not sufficiently stabilize G6PD in whole blood. The same argument goes for other anticoagulants such as K_2EDTA, citrate, oxalate, heparin, fluoride and CPD [12]. In many Nigerian hospital laboratories, including our own, G6PD screening is not carried out on overnight EDTA blood samples based on the fact that G6PD would have lost its activity. Since most of these anticoagulants are easily available, and for scientific documention, therefore, the present study was conducted in order to evaluate the rate at which the loss of G6PD activity is taking place in different anticoagulants in vitro, and determine the maximum post collection time that would give reliable results.

Materials And Methods

Thirty five millitres of blood was collected from each of 100 healthy subjects and dispensed into the anticoagulants as follows: 5ml each into K_2EDTA, lithium heparin, and fluoride oxalate 5ml each into 0.5ml of the following 3.8% sodium citrate, ACD and CPD bottles. And ammonium oxalate bottles.

Methaemoglobin reduction method (Beutler, 19971) was employed for G6PD screening. The test was carried out in all the anticoagulants within an hour of blood collection, the samples were then stored at 4°C and the test repeated at 12, 24, 48 and 72 hours.

RESULTS

One hundred blood samples from apparently healthy subjects were screened for G6PD status using methaemoglobin reduction method in seven different anticoagulants, namely: K_2EDTA, lithium heparin, ACD, CPD, sodium citrate, ammonium oxalate and fluoride oxalate. 89(89%) and 11 (11%) blood samples had normal and deficient G6PD status respectively in EDTA, ACD, CPD, sodium citrate and lithium heparin within an hour of blood collection, then at 12, 24, 48 and 72 hours after storage at 4°C. 89 (89%) and 11 (11%) had normal and deficient G6PD status respectively within an hour of blood collection, then at 12 hours after storage at 4°C in ammonium oxalate and fluoride oxalate. After storage for 24hours at 4°C in both oxalates, none (0%) had normal G6PD status, 89 (89%) had intermediate and 11 (11%) had G6PD deficient status. After storage at 48 and 72 hours at 4°C, all the samples were deficient in G6PD in the two oxalates.

Figure 1

Table 1: Effect of storage an anticoagulants on G6PD status.

	Within 1hr				12hr				24hr				48hr				72hr			
	T	N	IM	D	T	N	IM	D	T	N	IM	D	T	N	IM	D	T	N	IM	D
EDTA	100	89	-	11	100	89	-	11	100	89	-	11	100	89	-	11	100	89	-	11
ACD	100	89	-	11	100	89	-	11	100	89	-	11	100	89	-	11	100	89	-	11
CPD	100	89	-	11	100	89	-	11	100	89	-	11	100	89	-	11	100	89	-	11
Sodium Citrate	100	89	-	11	100	89	-	11	100	89	-	11	100	89	-	11	100	89	-	11
Lithium heparin	100	89	-	11	100	89	-	11	100	89	-	11	100	89	-	11	100	89	-	11
Fluoride oxalate	100	89	-	11	100	89	-	11	100	-	89	11	100	-	-	100	100	-	-	100
Ammonium oxalate	100	89	-	11	100	89	-	11	100	-	89	11	100	-	-	100	100	-	-	100

T = Total no screened
N = Normal
IM = Intermediate
D = Deficient

DISCUSSION

The result of this study showed that G6PD activity was normal in blood samples collected in EDTA, ACD, CPD, 3.8% sodium citrate and lithium heparin when the test was carried out within an hour and when blood was stored at 4°C for up to 72 hours. G6PD activity was normal within an hour of blood collection and at 12 hours of storage at 4°C in blood collected in ammonium oxalate and fluoride oxalate however, G6PD started losing its activity (intermediate) at 24 and the activity completely lost at 48 and 72 hours of storage at 4°C.

In many hospitals, G6PD screening was not always carried out on blood samples that had been kept overnight at 4°C just on the basis that G6PD activity would have been lost. Fresh samples were always taken from the affected patients. But this became a problem when patient had been transfused as G6PD screening cannot be done on patients that were just transfused. The patients had to wait for about three months before the test could be repeated. This study has solved that problem since it has been shown that G6PD activity is normal at 24,48 and 72 hours of storage at 4°C if the blood samples were collected in K_2EDTA, ACD, CPD, sodium citrate and lithium heparin. However, if the blood samples were collected in ammonium oxalate or fluoride oxalate, G6PD screening must be done with 12hours of blood collection if kept at 4° C.

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