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The Occurrence of Glucose 6 Phosphate Dehydrogenase Deficiency amongst Blood Donors at the Regional Blood Transfusion Centre-Mombasa, Kenya

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Abstract

Glucose-6-phosphate dehydrogenase deficiency (G6PDd) is an X-linked hereditary genetic defect that is estimated to affect 400 million people worldwide. This deficiency is associated with hemolytic disorders that may manifest depending on the molecular variant present, exposure to hemolytic triggers such as consumption of foods including fava beans and exposure to drugs including dapsone and primaquine. This disorder has been found to be more prevalent in malaria endemic zones of Asia, Africa and South America. This study determined the occurrence of G6PD deficiency among the donors at the Regional Blood Transfusion Centre-Mombasa, and whether any correlation existed between the occurrence of G6PD deficiency and either ABO blood type or haemoglobin concentration. Methaemoglobin reduction test was used to check for the presence of G6PDd among the blood donors, anti A and anti B sera were used to determine the blood types. Haemoglobin concentration was estimated using haematology analyzer. Multivariate analysis was done to establish the point prevalence of G6PDd in the donor population, the relationship between G6PD and ABO blood types and the correlation between G6PDd and haemoglobin concentration. Out of the 676 donors 9.6% were deficient of G6PD activity while 13.17% had red cells exhibiting partial activity. The point prevalence for all forms of G6PDd was found to be 22.79%. Blood group AB donors were least likely to exhibit G6PD deficiency compared to the rest of the ABO blood types. The association between G6PDd and haemoglobin concentration was inconclusive. The current findings indicate that G6PD deficiency exists among healthy donors without manifestation of clinical symptoms. G6PDd screening as part of donor blood testing regime would therefore allow for the discriminate use of G6PDd blood in transfusion dependent patients and neonates. It would also aid in establishing risk in the use of drugs associated with triggering clinical manifestations.

Key Words: *Transfusion, Glucose-6-Phosphate dehydrogenase, deficiency, blood donors.*

Introduction

Red blood cells are an important part of the body's metabolic processes. With a lifespan of 120 days, the non-nucleated red cells perform their metabolic functions and are also able to perform their specialized core function of oxygen transport and delivery to the body tissues [1]. The transfer of oxygen across cell membranes and its utilization are dependent on the cytochrome P24 group of molecules, the Embden Meyerhof glycolysis pathway and the hexose monophosphate shunt. The free oxygen radicals that are produced during these processes are harmful to cells and tissues [2]. To mitigate the effects of these free radicals, various enzymes and metabolic intermediates are involved in these glycolytic pathways. These include the 2-3 diphosphoglycerate, pyruvate kinase and glucose 6 phosphate dehydrogenase (G6PD) enzymes [3]. Glucose-6-phosphate dehydrogenase plays an important role in all cells particularly the red blood cells (RBC) [4]. It protects the cells from potential damage by reactive oxygen species (ROS) [5]. G6PD reduces nicotinamide adenosine dinucleotide phosphate (NADP) to nicotinamide adenoside dinucleotide phosphate hydrogenase (NADPH). The NADPH then reduces oxidised glutathione (GSSG) to reduced glutathione (GSH). The reduced glutathione is then able to bind to the ROS and protect the cells from oxidative stress [6]. The lack of mitochondria in the RBCs make the action of G6PD vital in the protection against oxidants [5]. A Glucose 6 Phosphate Dehydrogenase deficiency (G6PDd) would therefore be potentially hazardous to Red Blood Cells (RBCs) in particular due to their role in oxygen

transport. The G6PDd was first reported by the Greek philosopher Pythagoras who noticed that the consumption of fava beans resulted in an ailment to some of his subjects, which he named favism [4].

The G6PD gene has been shown to have several mutant alleles that have decreased enzyme activity thus expressing the G6PD deficient phenotype [7]. The deficiency of G6PD enzyme is an X-linked recessive in-born error of metabolism [2]. This condition predisposes individuals especially males to acute red blood cell haemolysis and neonatal jaundice. The condition occurs in the presence of exogenous factors such as consumption of fava beans, oxidative stress due to infections, anaemia and certain drugs among them dapsone, methylthioninium chloride (methylene blue), nitrofurantoin, phenazopyridine, primaquine, rasburicase and tolonium chloride (toluidine blue) [8]. G6PDd is the most common genetically determined red blood cell enzyme deficiency in the world [1]. Currently about 160 different variants of G6PDd have been isolated and it is estimated that 400 million people are affected worldwide [1]. The disease presentation in most individuals is largely asymptomatic in the absence of exogenous triggers [9]. In severe variants however, G6PD deficiency may result in non-spherocytic haemolytic anaemia even without oxidative stress [10]. The manifestations of severe haemolysis due to G6PDd start early and has been implicated as a common cause of severe neonatal jaundice [11]. Enzyme deficiencies have been implicated in hemolytic symptoms after transfusion and are viewed as potentially fatal to patients transfused with such blood [12,13]. The reaction of nitrites with haemoglobin may be exploited to screen donors for G6PD. Sodium nitrite converts the haemoglobin to haemiglobin via an autocatalytic reaction that involves the formation of methaemoglobin and nitrate as the stable end product [14]. The current study aimed to determine the occurrence of G6PD deficiency amongst blood donors at the Regional Blood Transfusion Centre – Mombasa and the correlation between occurrence of deficiency and ABO blood type or haemoglobin concentration.

Methods

This was a cross-sectional study conducted in Mombasa Kenya. Samples were collected from donors at the Regional Blood Transfusion Centre (RBTC) Mombasa and analysis done at the Technical University of Mombasa department of medical science laboratories. Ethical review was obtained from the Pwani University Ethical review board under reference number ERC/MSc/009/2017. A no objection statement was also obtained from the Director, Kenya National Blood Transfusion Service.

A total of 676 samples were drawn from donors recruited and bled by the RBTC staff. These blood samples were assayed for G6PD activity using the Methaemoglobin reduction method. One milliliter of blood was added to a 0.1mL combined nitrite, dextrose and methylene blue reagent and the two mixed by inversion. Methylene blue was then added to stimulate the pentose phosphate pathway. Control tubes comprising of one milliliter of blood without reagents and another containing sodium nitrite without methylene blue were also mixed. All the tubes were then incubated at 37^0C for 90 minutes, 0.1mL of the mixtures were then obtained and subjected to lysis using 10mL of distilled water.

The presence of various degrees of lysis was representative of the G6PD activity of the sample. Normal blood takes the color of the normal reference tube (clear red), G6PD deficient blood takes the color of the deficient reference tube (brown). Intermediate reactions will show the presence of heterozygote G6PD conditions. ABO blood type was assayed using microtiter plates and reagents ACCUCARE™ (Lab-care diagnostics, India) blood type using the anti "A" and anti "B" sera these were used to correlate with the findings of the G6PDd to check for association. Haemoglobin concentration was estimated using the Medonic TM haematology analyzer. Consent was inferred after the donors signed the donor health assessment questionnaire. All information obtained was treated with confidentiality in conformity to the Kenya National Blood Transfusion Service (KNBTS) guidelines on donor records. Prior permission to use the samples was obtained from the director of KNBTS. The study was approved by the Pwani University Ethical Review Board (PU-ERB).

Results

Overall G6PD deficiency

A total of 676 samples were assayed for G6PDd. Donor samples showing complete deficiency in G6PD activity were 9.6% of the total donors assayed. Intermediate reactions were observed in 13.2% samples. The total number of donors having G6PDd was 154 (22.8%) of the donor population. The proportion of normal and abnormal blood samples based on G6PD enzyme activity was 77.2% and 22.8%, respectively (fig. 1).

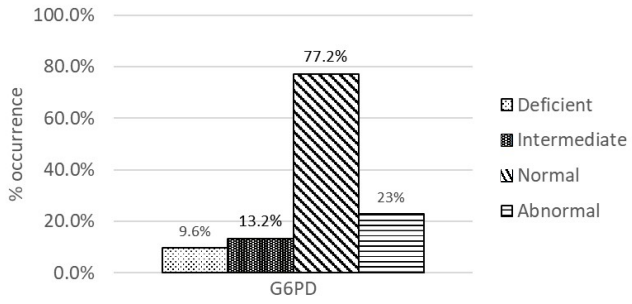
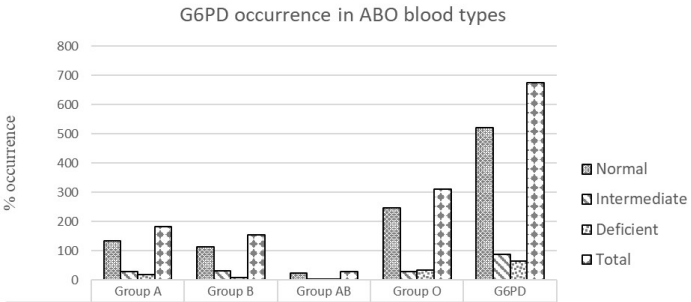


Figure 1: Proportions of G6PD activity in the blood samples collected at the Regional Blood Transfusion Centre (RBTC) Mombasa, Kenya.

Correlation between ABO blood type and G6PDd occurrence



	Group A	Group B	Group AB	Group O	G6PD
Normal	134	115	25	248	522
Intermediate	29	31	1	28	89
Deficient	20	8	2	35	65
Total	183	154	28	311	676

Figure 2. Correlation between the ABO blood types and the occurrence of glucos 6 phosphate dehydrogenase deficiency in the donor population

The test results showed that there was a marginal correlation in the likelihood of having G6PDd between blood type AB and type A. Blood type A individuals had a higher likelihood of having G6PD deficiency compared to blood type AB individuals. The Chi square test for association showed that there was a correlation between ABO blood type and occurrence of G6PD deficiency (p-value 0.003989) A marginal significance of AB in reference to Blood type A was observed. Donors with Blood type AB were more likely to be normal compared to those of Blood type A (coef=1.09, s.e =0.63. p-value=0.08).

G6PD in relation to Haemoglobin concentration

Table 1: relationship between haemoglobin and G6PD condition in donors

	HB<12.0	HB>=12.0
NORMAL	10.5%	89.5%
INTERMEDIATE	7.9% 9	2.1%
DEFICIENT	10.8%	89.2%
ABNORMAL	9.1% 9	0.9%

Analysis of variance (ANOVA) showed that at least one of the G6PD groups had a different Haemoglobin concentration than the others with a calculated F-value of 0.0426 (CI 95%) this was not however reflected in the pairwise comparisons.

Discussion

The current study shows a point prevalence of 9.6% for homozygous G6PD and 13.2% showing heterozygosity. A point prevalence of 22.8% for all forms of G6PD was found amongst the donors' assayed (figure 1). Studies worldwide have shown that the G6PD deficiency offers either partial or full resistance to P. falciparum infection and has therefore been found in polymorphic proportions in malaria endemic areas [15]. These studies show that the condition is predominant in Asia and sub-Saharan Africa [4]. A study in Yemen found that among healthy male donors, 7.2% had G6PDd in the capital city of Sanaa. In Kenya, the estimated prevalence for G6PDd is in the range of 10% to 13% [15]. The current study compares favourably with the results from Nigeria by Akanni et al., who found a prevalence of 19.5% in blood donors in Osogbo, [16] and Nguetse et al., who reported that 73% of the study subjects in selected African countries had normal G6PD activity [17]. However these findings do not correspond with estimates by Howes et al which indicate that prevalence of G6PDd in Kenya is about 13% [15]. This may be due to the high prevalence rate of malaria in the coastal region. G6PDd has been shown to have a high prevalence in malaria endemic zones [15]. The association of G6PDd with health conditions is a major area of study today. Akanni et al have associated neonatal jaundice to the deficiency showing 47% of the jaundiced neonates in the study were G6PD deficient [16]. It has also been found that altered G6PD activity may play a critical role in severe pulmonary hypertension [18]. This study established that G6PD condition of a donor was associated with ABO blood types (figure 2). A marginal significance was established between the A and AB Donors. A person with Blood type "A" was more likely to be deficient than a person with blood type "AB" at a p-value 0.03989 (coefficient of variance=1.09, Standard error=0.63. p-value=0.08). The relationship between G6PD deficiency and haemoglobin concentration was difficult to establish (Table 1) even though an ANOVA model revealed that there is indeed a difference between the G6PD condition and the Hb levels of the donor (F-value 0.0426, CI 95%). This correlation is not however reflected in the pair wise comparisons. Future studies to incorporate a larger sample size needs to be done to establish this relationship.

Conclusion

Glucose 6 phosphate dehydrogenase deficiency is present in donated blood at the regional blood transfusion center, Mombasa. ABO type "A" individuals seem to have a marginally higher probability of having G6PD deficiency in comparison to the "AB" blood type. The percentage occurrence of G6PD is higher than has been previously estimated. There is therefore need to determine the presence of this condition in donors for documentation and reference, and in neonates when jaundice of unknown origin is encountered. The effect of G6PD deficient blood to the recipients is still a grey area that should be investigated further. There was a marginal statistically significant relationship between haemoglobin concentration of normal and G6PD deficient donors. This study was not however able to conclusively determine the relationship between G6PD and haemoglobin concentration. Further studies are needed to establish the relationship.

Conflicts of Interest

The authors declare no conflict of Interest.

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