Multidisciplinary Journal of TUM 2(1) 2023 45-55 DOI: https://doi.org/10.48039/mjtum.v2i1.46 **Original Article**

Occurrence of red blood cell abnormalities in donor blood donated at the regional blood transfusion centre, Mombasa, Kenya

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Abstract

During transfusion, components of blood including red blood cells, platelets and plasma are directly administered into the recipient to treat conditions such as anaemia and haemostatic deficiencies. In most cases, effective blood transfusions will positively impact patient prognosis. Red blood cell abnormalities greatly impact on the effectiveness of blood transfusion. This is because the efficacy of a red blood cell unit depends on the amount of blood delivered, the quality of cells and the life span of a given unit. The aim of this study was to establish the occurrence of red cell abnormalities in donor blood. This descriptive cross-sectional study was done at the regional blood transfusion centre, Mombasa and at the Technical University of Mombasa, Kenya. Consecutive blood samples were analyzed for selected red cell parameters. A total of 676 samples were analyzed. The study found that 31.07 % of the donor samples had one or more of the abnormalities assayed. There was a significant variation (t - 0.03, CI 95%) in the total red blood cell count. A significant Pearson's positive correlation was realized between the osmotic fragility and haemoglobin concentration ($r = 0.195$; $p < 0.001$). This shows that a significant proportion of donated red blood cells in the donor pool had intrinsic or extrinsic abnormalities. There is therefore need to develop strategies that may better help to filter out these abnormalities as well as investigate the effect of such abnormalities to the recipient.

Key Words: Transfusion, Red cell abnormalities, Regional blood transfusion centre, Kenya

Introduction

Blood transfusion is saving more lives since Landsteiner and his colleagues discovered blood group antigens and antibodies (Klein & Anstee, 2008). It has therefore become an integral part of clinical practice in most parts of the world (Janatpour & Holland, 2007). The World Health Organization (WHO) issued guidelines for transfusion practice which cover aspects of donation, storage, distribution and the clinical use of blood and blood products. These guidelines that were adopted by the World Health Assembly (WHA) are incorporated in blood bank practices in member states. Therefore, the WHO recommends that each country formulates specific guidelines to govern blood banking and transfusion service (Force, 2004; WHO, 2010, 2012). The most common factors assessed during donor selection are blood group serology and the presence or absence of transfusion transmissible infections (Yazdanbakhsh et al., 2012; Milkins et al., 2014). This is primarily in order to minimize transfusion transmissible infections and avoid adverse events occasioned by immunological and non-immunological agents (Woodfield et al., 2007; Pham et al., 2009) . Other factors assayed are the levels of haemoglobin and the general well-being of the donor. This group of tests help to ascertain

Published, January 2023

the donor's fitness and ability to donate without the occurrence of untoward events (Carson et al., 2012).

The provision of safe blood for transfusion is the primary goal of blood banking and transfusion services. It is with this in mind that transfusion scientists have suggested that red cell anomalies should be routinely assayed as a measure geared towards attaining the transfusion of safe blood (Ho et al., 2003; Chassé et al., 2016). Moreover, evidence of the presence of red cell membrane defects that promote formation of storage lesions that may adversely affect the outcome of transfusion have been documented (Renzaho et al., 2014; D'Alessandro et al., 2015; Tzounakas et al., 2016). These measures, if taken into consideration may help in attaining safe clinical use of blood and blood products, thereby minimize adverse reactions during transfusion.

Red cell membrane defects are common causes of haemolysis in most parts of the World (Barcellini et al., 2011). These are attributable to a host of abnormal environmental and hereditary causes (Surgenor, 2013). These abnormalities range from intrinsic defects (haemoglobin defects, defects on the cell membrane, defective enzymatic activity) to extrinsic defects (storage changes during component processing) (Hoffbrand et al., 2011). Haemoglobin defects are found in abnormalities such as thalassemia and sickle cell traits. These disorders and traits are common in Africa (Frederic B Piel et al., 2013) and Asia (Chen et al., 2018) while enzyme and membrane permeability defects occur to a great extent in the European and Australasian regions (Colah et al., 2010; Da Costa et al., 2013). The existence of sickle cell and thalassemia traits in the community suggests that individuals with these conditions may be recruited to donate blood (Horowitz & Confer, 2005; Tsaras et al., 2009). Such blood however contains unstable red cell components which may be haemolysed in the peripheral blood resulting into mild to severe defects to the recipient or donor (Hoffbrand et al., 2011; Kudale et al., 2014). During transfusion with blood from sickle cell trait, thalassemia traits, or red cells having other abnormalities, blood flow disorders and optical disturbances may occur (Chien, 1987). Red cell membrane anomalies resulting from enzyme defects include Glucose-6-Phosphate Dehydrogenase deficiency (G6PDd) and Pyruvate Kinase deficiency (PKd) (Howes et al., 2012). These have been reported to have clinical effects on exchange transfusion patients and individuals with chronic haemolysis and haemorrhagic disorders (Brunskill et al., 2015; Renzaho et al., 2014).

The occurrence of intrinsic and extrinsic red cell abnormalities leads to the formation of red cell lesions. These lesions occur due to the decrease in intra-erythrocytic energy sources, making the cells less deformable and fragile leading to accelerated haemolysis and release of red cell microparticles (RMPs) (Kim-Shapiro et al., 2011). The presence of red cell defects increases the likelihood of accelerated lesion formation more so upon storage (Kim-shapiro et al., 2012). Upon transfusion, haemolysed cells and RMPs consume Nitric Oxide (NO) causing a decrease in NO bioavailability. This results in substantial changes in the rheological properties of the transfused blood (Adams et al., 2015; D'Alessandro et al., 2015). Other defects include citrate poisoning and Potassium ion leakage (Hess, 2010). The changes may cause clinically significant adverse effects to the transfused patient (Renzaho et al., 2014). The effects and severity depend on the patient condition and the storage time of the transfused unit (Papay et al., 2012).

Other red cell anomalies that may occur in donated blood include a variation in the red blood cell indices, variable haemoglobin concentration or total red cell counts (Mukherjee et al., 2010). Blood banks operate on the well set up attribute of storage testing and processing before dispatch (Greer et al., 2010; Janatpour & Holland, 2007). This gives room for laboratory testing to rule out TTIs and blood group serology (Schubert & Devine, 2010; Kubio et al., 2012; Wilkinson et al., 2012). This strategy may well be used to test blood for other abnormalities that may be associated with haemolysis and red cell membrane deformations that may affect the clinical efficacy of transfusion therapy (Koch et al., 2008; Antonelou et al., 2012; Gevi et al., 2012; Karon et al., 2012). The knowledge of the occurrence of these abnormalities is an essential tool towards mitigating any effects that may be associated with these abnormalities. Therefore, this study aimed at determining selected red cell abnormalities in blood donated at the Regional Blood Transfusion Centre, Mombasa.

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Materials and methods

This descriptive cross-sectional study assayed 676 units obtained at the Regional Blood Transfusion Centre (RBTC), Mombasa which is the main centre offering blood bank services to the coastal region of Kenya. The blood bank's annual collection is estimated at 12,000 units (KNBTS & ICF Macro, 2010). Convenient consecutive sampling was used to achieve the desired sample size. Sampling was done on various days during the blood donor campaigns in Mombasa County during the months of August through to November 2017.

Laboratory analysis and data collection

Four millilitres of blood were collected in Ethylene Diamine Tetra-acetic Acid (EDTA) vacutainers directly from the blood bag tubing. The blood was then analyzed for the set parameters. Blood cell count and osmotic fragility testing were done within 6 – 8 hours of collection. Samples were refrigerated at $4^0C \pm 2^0C$ to maintain red cell viability. All other assays were done within 24 hours of collection. Red cell count parameters including total red cell count, haemoglobin concentration, mean cell volume and packed cell volume were analyzed using the Medonic M 20M (Boule Medical AB, Sweden) haematology analyzer. Red cell lysis was determined by the use of increasingly hypotonic saline solution method. The haemolysis observed was detected using the Genesys™ 10S Vis UV spectrophotometer at 540 nm. The values obtained were expressed as a percentage of the 100 % lysis from a reference tube containing deionized water (Bain et al., 2017; Parpart et al., 1947). G6PD was determined using the methemiglobin method. Sodium nitrite was used to convert hemoglobin to hemiglobin which was then converted to methaemiglobin by addition of methylene blue. Samples were incubated with methylene blue for 90 minutes to stimulate the pentose pathway. Samples that were G6PD deficient were not able to reduce methaemoglobin. These were therefore lysed and retained the colour of the reagent. The presence of unstable haemoglobin was assayed using the Isopropanol solubility test. Freshly prepared haemolysates were incubated with isopropanol in tris-isopropanol buffer Flocculation indicated the presence of unstable haemoglobins.

Data analysis

Data was recorded and cleaned in Microsoft Excel for management and analysis. Descriptive statistics was done to determine means and standard deviations in the population. Chi-square distribution was used to determine the significance of associations between various observations. Kruskal-Walis test was used to test the correlation of independent variables. ANOVA distribution was used to predict within-group statistical significance of continuous variables. p- value of less than or equal to 0.05 were considered significant. All descriptive statistics and statistical tests were done using SPSS statistical software

Results

Donor characteristics

A total of 676 donor units were assayed during the study period. Out of these, 98 % (n = 660) had been obtained from male donors while only 2% (n = 16) were from female donors (Table 1).

Error! Reference source not found. Table 1. Percentage frequency of donors with reference to gender sampled over the study period

		Frequency	Percent (%)	Valid Percent (%)	Cumulative Percent (%)
Valid	Female	16	2.4	2.4	2.4
	Male	660	97.6	97.6	100.0
	Total	676	100.0	100.0	

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ABO blood type specificity and Overall abnormalities

The ABO and Rh blood groups frequencies were 46.01 % for type "O" as the highest and 4.14 % for type "AB" with the lowest frequency (Figure 1a). The study found that 31.06 % of the donor units had at least one abnormal parameter while 58.73 % had none of the abnormalities according to the parameters assayed (Figure 1b). Donor cells that had reduced G6PD activity but all other parameters within normal were 10.21 %.

Red cell abnormalities in donor blood.

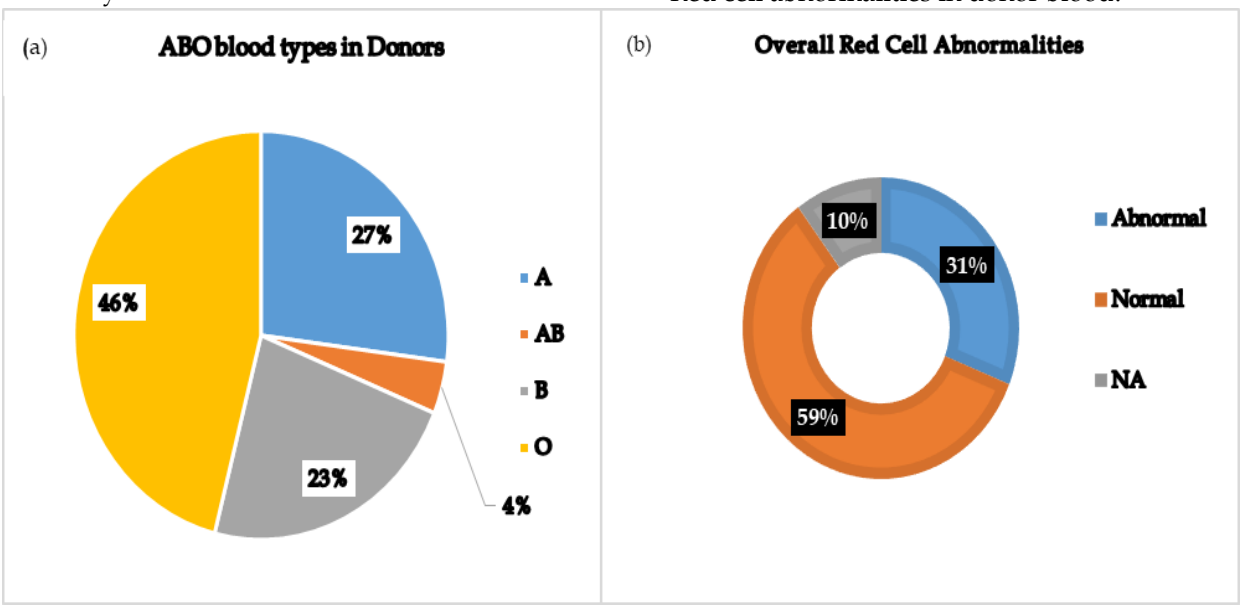


Figure 1(a-b) a. Distribution of ABO blood types amongst the assayed donors. b. overall proportion of donor cells with abnormalities

Haemoglobin stability

There were no unstable haemoglobins observed amongst the donor units assayed. No flocculation was observed earlier than 30 minutes in any of the units. At least 20 % of the donor cell Haemoglobin showed flocculation at 30 minutes while the rest of the Hb samples flocculated at between 40 and 70 minutes (Figure 2a).

Osmotic Fragility

Donor red cells that exhibited an increased resistance to lysis were 7 % while those that were more fragile than normal were 3.7 %. Of the red cells showing increased resistance, 0.3 % were highly resistant (figure 2b).

Mean cell volume

PCV for the red blood cells assayed had 3 % of the donor units below the normal reference range (34 % to 50 %). There was no donor blood having TRBC above 6.4 cells x 10^{12} Cells/l, however, 4.4% of the donors had TRBC count below the normal range (3.8 – 6.4 x 10^{12} cells/l).

MCV assayed and recorded as Normal (80fl – 100fl), Low MCV (<80fl) and High MCV (>101fl). A high proportion of donor blood had low MCV (9.91 %) as compared to those with high MCV (4.14 %). A cumulative 14.05 % of the donor red cells had MCV values outside of the normal range (Figure 2a).

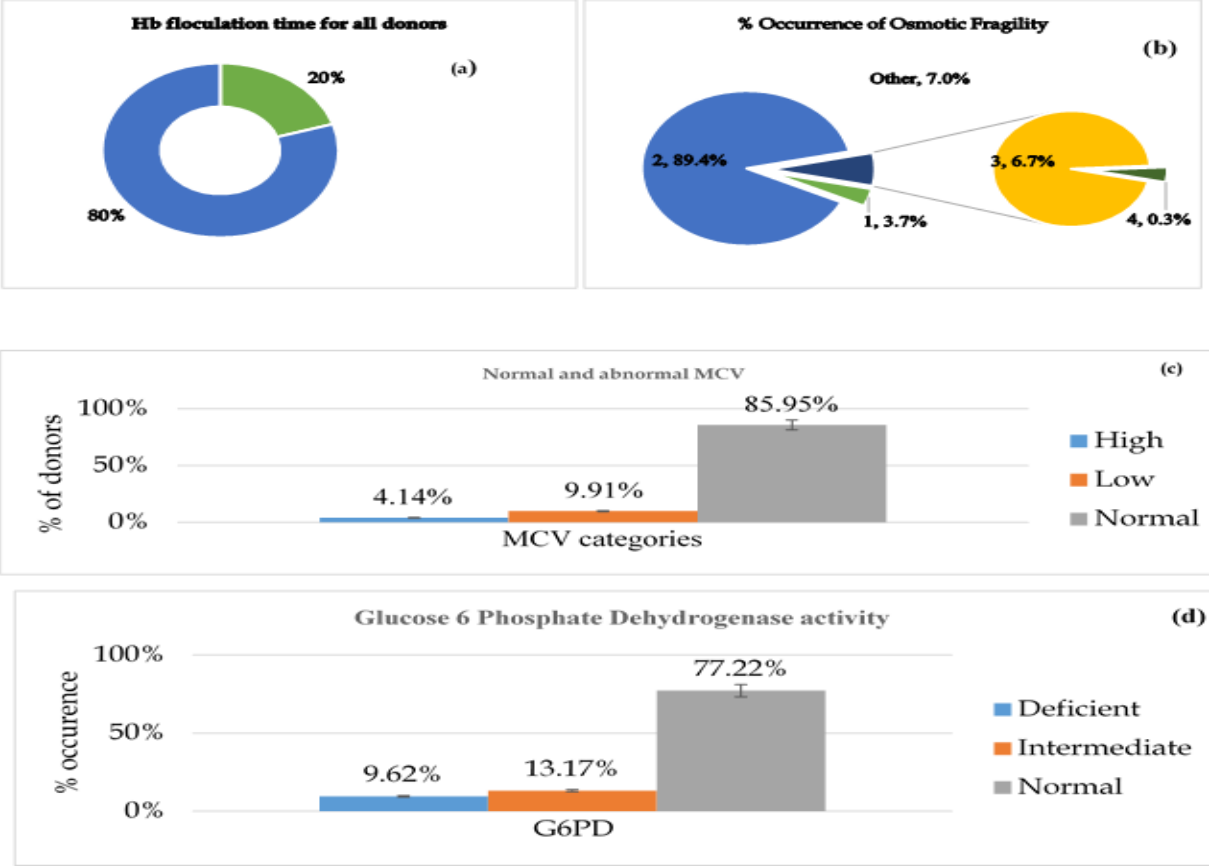
Glucose 6-phosphate Dehydrogenase Deficiency

The study found that 9.61 % of the donors had G6PD deficiency while 13.17 % had reduced enzyme activity and were therefore likely to be heterozygous for the G6PD deficiency gene. Cumulatively 22.78 % of the donor red cells did not have normal G6PD activity (Figure 2d).

Figure 2 (a-d) (**a).** Haemoglobin flocculation time for the donors (**b)**. Proportion of donor cells showing osmotic resistance and fragility of assayed donor red blood cells. (**c)**. Proportion of donors with normal and below or above normal

Published, January 2023

mean cell volume (**d).** Proportion of donors with normal and deficient glucose 6 phosphate dehydrogenase.



Statistical significance of red cell values

There was a statistically significant variation in total red blood cell count (CI 95%, p – 0.05). Other red cell values did not return statistically significant variations (Table 2; p > 0.05 all cases).

Table 2. Statistical analysis of red cell values in donor blood sampled over the study period

	Hb g/dL	PCV %	TRBC x10⁶/µlitre	MCV
Mean	13.3712	39.8797	4.6194	84.8302
Standard Error	0.0397	0.1374	0.0194	0.3268
Median	13.5000	39.4000	4.6100	83.8942
Mode	13.5000	38.8000	4.6100	80.2966
Standard Deviation	1.0317	3.5732	0.5042	8.4969
Sample Variance	1.0644	12.7680	0.2542	72.1973
Confidence Level (95.0%)	0.0779	0.2698	0.0381	0.6417

Published, January 2023

Mean Cell Volume in relation to Osmotic fragility

The Pearson's correlation coefficient showed a significant relationship between osmotic fragility and mean cell volume. The study found that cells with an increased mean cell volume tended to be more osmotically fragile (Table 3).

Table 3. Statistical relationship between osmotic fragility and mean cell volume

		Mean_cell_volume	Osmotic_fragility
Mean_cell_volume	Pearson Correlation	1.000	0.195
	Sig. (2-tailed)		0.001
	Sum of Squares and Cross-products	49385.824	380.994
	Covariance	73.164	0.564
	N	676.000	676.000
Osmotic_fragility	Pearson Correlation	0.195**	1.000
	Sig. (2-tailed)	0.001	
	Sum of Squares and Cross-products	380.994	77.148
	Covariance	0.564	0.114
	N	676.000	676.000

Correlation is significant at the 0.01 level (2-tailed).

Relationship between osmotic fragility and G6PDd and Hb value

There was a significant relationship between the Hb concentration and osmotic fragility (p = 0.05). However, there was no significant relationship between abnormal osmotic fragility and G6PDd states (Table 4**Error! Reference source not found.**). The study found that the concentration of haemoglobin differs in the G6PD states.

Table 4. Test for significance between Hb concentration and **G6PDd**

S/N	Distribution	Test	p-value
1	The distribution of osmotic fragility is the same across categories of G6PDd	Independent-samples Kruskal-Wallis Test	0.285
2	The distribution of Zscore (Hb_concentration) is the same across categories of G6PDd	Independent-samples Kruskal-Wallis Test	0.033

Discussion

The study assayed 676 samples from units of blood collected at the Regional Blood Transfusion centre of Mombasa in Kenya. More males than females made the majority of the blood donors. This has been observed in Nigeria (Felix et al., 2017) and other developing countries and may be as a result of misconceptions about blood donation and low Hb levels in women of child bearing age resulting from iron deficiency anaemia. The overall proportion of red cell abnormalities was 31.07 % while 10.21 % could not be categorized as abnormal as these only had reduced G6PD activity with all other parameters normal. The current study selectively assayed red blood cell indices that included haemoglobin

estimation, packed cell volume, total red cell counts, mean cell volume. Up to 10.21 % of the donated units had haemoglobin levels lower than the national guidelines for Hb levels at donation which is set at 12.5g/dL. A similar study by Rajab et al., (2005), found that in Western Kenya, 16.5 % of the donors had low Hb levels compared to 3.4 % of donors in Nairobi region. The lower Hb concentration may be attributed to low altitude and high malaria endemicity in both western Kenya (Kisumu) and coastal Kenya (Mombasa) against the relatively malaria free Nairobi region. There were 12.4 % of the donors with abnormal mean cell volume when compared to the WHO classification of anaemia by MCV which categorizes normocytic cells as those with MCV within 80-100fL±2, microcytic cells as those having MCV lower than 80fL±2 and macrocytic cells as those with MCV greater than 100fL±2. This current study observed that microcytosis occurred in 9.91 % (>80fL±2) of the donors while 4 % were macrocytic (<102fL). These findings correspond to findings by Rajab et al., (2005?) for microcytosis in Kisumu (12.4%) and macrocytosis in Nairobi (4.1%). The variation in total red blood cell count was statistically where about 4.4 % of the donors had counts lower than the normal reference range. These variations may have occurred as a result of inadequate screening of blood donors. This study found that 10.6 % of the donors had abnormal osmotic fragility patterns. At least 7% of these exhibited high resistance to lysis while 3.6 % were abnormally fragile. In a Norwegian study, about 0.9 % of blood donor red cells were found to be osmotically more fragile than normal while in Germany, a prevalence of 1.1% for osmotically fragile cells was observed (Godal & Heistø, 1981). These studies show that abnormal cell variations occur in much lower frequencies in the two countries than in Kenya.

Abnormal lysis patterns of RBCs occur due to microcytosis and macrocytosis. Owing to the variations in both MCV and G6PD activity osmotically resistant cells will tend towards microcytosis while osmotically fragile cells are likely to be macrocytes. These macrocytes may have been as a result of G6PD deficiency states that lead to spherocytosis and increasing fragility. Unstable haemoglobin occurs in individuals having HbS, HbH and other abnormal molecules. These haemoglobins are inherently prone to abnormal lysis. This lysis may be demonstrated as a precipitate when these unstable proteins are mixed with isopropanol. Lysis may occur within 5 minutes for homozygous individuals or 30 minutes in the hemizygous-heterozygous persons (Carrell & Kay, 1972). In our study, 20 % of donor cells lysed after 30 minutes but below 40 minutes showing heterozygosity for unstable haemoglobins. A study in western Kenya found that 17% and 38% of school going children were sickle cell trait and α-thalassemia minor traits respectively (Suchdev et al., 2014).

The activity of G6PD enzyme was qualitatively assayed. About 1 in every 10 donors was deficient in the enzyme G6PD (Wigina et al., 2018). A point prevalence of 22.8% for all forms of G6PDd was found amongst the donors' assayed. Our study found that 13.17% of the donors had reduced G6PD activity and were most likely heterozygotes. This compares with the 16.4% proportion of heterozygotes found in the Tanzanian population (Mwakasungula et al., 2014) A study in Yemen found that among healthy male donors, 7.2% had G6PDd in the capital city of Sanaa (Al-nood et al., 2012). In Kenya the estimated prevalence for G6PDd is in the range of 10% to 13% (Howes et al., 2012). The current study compares favourably with the results from Nigeria by Akanni et al. (2010)who found a prevalence of 19.5% in blood donors in Osogbo, Nigeria (2010) and Nguetse et al. (2016) who reported that 73% of the study subjects in selected African countries had normal G6PD activity (2016). However these findings do not correspond with estimates by Howes et al. (2012) which indicate that prevalence of G6PDd in Kenya is about 13 % (Howes et al., 2012). The association of G6PDd with health conditions is a major area of study today. Nigerian study also associated neonatal jaundice to the deficiency showing 47 % of the jaundiced neonates in the study were G6PD deficient (Akanni et al., 2010). It has also been found that altered G6PD activity may play a critical role in severe pulmonary hypertension (Chettimada et al., 2014). Results from the current study show that that the presence of G6PD deficiency is widespread in the general population and could be associated with the high malaria endemicity in the study population.

Conclusion

Red blood cell abnormalities were found to be present in the donated red blood cells. In our current study, 31.05% of the donor cells exhibited one or more of the assayed abnormalities. There was a statistically significant variation in the total red blood cell counts and osmotic fragility. The study also found a marginal association between osmotic fragility and mean cell haemoglobin. Glucose 6-phosphate dehydrogenase deficiency is present among donors visiting the regional blood transfusion centre, Mombasa. The screening of G6PDd in donated blood using the methaemoglobin reduction method was able to yield results that point to the existence of homozygous and heterozygous G6PDd states in the population. The relationship between the parameters tested yielded marginal correlations between haemoglobin concentration and G6PD deficiency categories donors.

Recommendations

The study recommends that a multicentre study on the effects of red blood cell abnormalities should be carried out to determine the effects of these abnormalities on recipients. It is also recommended that the detection of G6PDd in donor blood should be incorporated as a screening method at the blood bank and that blood so screened be labelled as either G6PDd deficient or non-deficient. Finally, the hospital blood banks should determine the osmotic fragility of donor blood before transfusion. This may be limited to blood given to patients with chronic blood needs i.e. transfusion dependent patients.

Acknowledgement

Authors greatly acknowledge the logistical support of Technical University of Mombasa, Jomo Kenyatta University of Agriculture & Technology, and the Regional blood transfusion centre, Mombasa. We also thank Prof. Ochieng Odalo for his contribution towards the successful completion of this study.

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