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Response of Cellular Elements to Frequent Blood Donations among Male Subjects in Calabar, Nigeria

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ABSTRACT

Abuse of blood donation is common in Calabar and Nigeria. Research on blood donors has focused on the erythrocyte and iron-related parameters, without considering the other cell lines of the donors. This study examined the effect of frequent blood donations on leucocytes and thrombocytes. One hundred and eighty four subjects aged 18-35 years were used for this study. The subjects were divided into 5 groups thus; 35 (19%) control group, 32 (17.4%) of first time donors, 35 (19%) of second time donors, 41 (22.3%) of third time donors and 41 (22.3%) of fourth time donors. Their full blood counts were evaluated using complete automated cell counter and values obtained were statistically analyzed. Results showed that 63% of the donors were commercial blood donors. Haematocrit (Hct) of first ($41.9 \pm 0.66\%$), second ($40.1 \pm 0.47\%$), third ($39.1 \pm 0.54\%$) and fourth ($33.3 \pm 0.56\%$) time male donors decreased progressively as number of times of donation increased, compared with control ($43.9 \pm 0.55\%$). Total White Blood Cell (WBC) count significantly ($p < 0.001$) decreased in first, third and fourth time donors, compared with control. Monocytes, granulocytes and platelet counts decreased progressively with repeated blood donations. Lymphocytes increased progressively following repeated blood donations. We therefore, conclude that, serial blood donation decreases Hct, WBC (monocyte and granulocyte) and platelet counts in males and increases lymphocyte count.

Key words: Anaemia, blood donors, leucocyte, thrombocyte

INTRODUCTION

A blood donor can be defined as one who gives blood for transfusion purposes. An adult who is in good condition and has no illness is suitable as a donor. Transfusion services have particular rules which outline the guideline for the protection of the donor and the recipient (Ranney and Rapaport, 1997). A blood donor must be between the ages of 18 and 65 years of either sex and they should conform to the national standard of fitness as laid down by the Act of Parliament in UK (Milman, 1996). The donation of blood is accepted commonly 2 or 3 times in 12 months and the loss is excess in expectant age females than males (Hewitt and Wagstaff, 1989). Iron lost can be restored in males when they donate blood up to 5 units yearly, while females may likely become

iron deficient if they donate more than 1 unit per year (Hermosa *et al.*, 1996). The rate of erythropoiesis is coordinated with iron availability by iron regulation of erythroid differentiation. Iron deficiency reduces the responsiveness of erythroid progenitors to erythropoietin, apparently through an iron-aconitase-isocitrate pathway (Bullock *et al.*, 2010). With a dearth of iron, decreased erythroid utilization for red blood cell production helps preserve the supply of iron for vital functions in other tissues. This protection is incomplete. Iron deficiency, even in the absence of anemia, is associated with decreased physical endurance and work capacity, fatigue and impairments in attention, concentration and other cognitive functions (Falkingham *et al.*, 2010). In the United States, according to Code of Federal Regulations, the requirements for whole blood donation, a minimum acceptable hemoglobin concentration of 12.5 g dL^{-1} and a minimum interval between donations of 8 weeks are identical for women and men. Neither self-selected and administered iron supplements nor the usually recommended iron-rich components of the diet have appreciable effects on the risk of iron-deficient erythropoiesis. This observation suggests that recommendations typically given at blood centers to donors deferred because of low hemoglobin are ineffectual in preventing iron deficiency (Delaney *et al.*, 2011). The US Food and Drug Administration is currently considering changes in the hemoglobin and haematocrit standard and in the interval between donations to better protect donors (FDA., 2010).

Hemoglobin and haematocrit are important measurements in the diagnosis and treatment of anaemia and polycythaemia, while red cell indices give facts about the hemoglobin status and size of red cells which are serviceable in elucidating etiology of anaemias. Leucocyte and platelets are useful in the assessment of sepsis as well as haemostatic status, respectively (Mamoury *et al.*, 2003). Ferrokinetic studies in the analyzed population were not useful for evaluating iron reserves and even were misleading in the diagnosis. A low ferritin concentration had been described as a good parameter for diagnosing iron deficiency anaemia (Munoz *et al.*, 2011).

In Nigeria, blood donation is abused. Several donors donate blood as many as 5 times in a year for pecuniary benefits. Furthermore, Nigerians, like most Africans have peculiarities in their blood indices that are quite different from Caucasians (Ezeilo, 1972, 1974). These peculiarities include lower RBC and WBC counts and neutropenia when compared to the Caucasians (Ezeilo, 1972, 1974). The reasons for the lower hematological indices were reported to be genetic and dietary. For instance, foods rich in thermally oxidized palm oil reduce red cell count in the rat (Mesembe *et al.*, 2004; Essien *et al.*, 2014; Ani *et al.*, 2014, 2015). Thus, the consequences of frequent blood donations may be more severe in Africans than the Caucasians. This study is therefore, aimed at assessing the effect of frequent blood donations on leucocytes and thrombocytes aside the erythrocyte parameters among frequent male blood donors in Calabar, Nigeria.

MATERIALS AND METHODS

Research site and subject grouping: Research area was University of Calabar Teaching Hospital (UCTH) Blood Donor Bay and approval was obtained from the Health Research Ethical Committee (HREC) of the hospital before the collection of samples commenced. Permission was obtained using informed consent from the donors. Bio-data and medical history were obtained using a questionnaire before blood donation. Four hundred and fifty donors came to UCTH bleeding bay during the 4 months (August-November) of gathering samples in 2011. They all donated blood once or repeatedly within the period of 2 months of sample collection. After selection of donors using questionnaire. A total of 184 subjects were recruited. The donors were 18-49 years of age. Furthermore, 75% of the 184 participants were commercial donors (those who donate for monetary

benefit). They were divided into 5 groups, thus; 35 (19.0%) control subjects, 32 (17.4%) first time donors, 35 (19.0%) second time donors, 41 (22.3%) third time donors and 41 (22.3%) fourth time donors.

The inclusion criteria were as follows:

- The donor's packed cell volume $>0.400 \text{ L L}^{-1}$
- Those who donated blood in a previous period of less than 2 months (for control group and first timers)
- Sero-negative HIV 1 and 2, hepatitis B and C and *Trepanoma pallidum*

The exclusion criteria were as follows:

- Donors taking iron supplements or had gone through a major surgery in the past 3 years were excluded
- Donors with history of recent blood transfusion in the past 2 years were excluded

Collection of blood samples: Two milliliters of venous blood was collected from donors by venepuncture between 8:00-11:00 am GMT and it was then dispensed into EDTA sample bottle in a concentration of 2 mg mL^{-1} of blood. The blood sample was used for full blood count to obtain haematocrit, leucocyte count, leucocyte differential and platelet counts using complete automated cell counter (ERMA INC. Tokyo PCE-210, 5.10 version).

Data analysis: Analysis of variance (ANOVA) was utilised alongside *post hoc* multiple comparisons. Computer software SPSS (version 18) and microsoft excel were used. Values are expressed as Mean $\pm$ Standard error of mean. Chi-squared analysis was also used for percentages and proportions of the male blood donors. Values of $p < 0.05$ were considered significant.

RESULTS

Age distribution of subjects: Table 1 shows the age distribution of subjects employed for this study. Subjects aged 18-25 years formed 45.7% of the total number of subjects recruited. Subjects aged 26-35 years formed 47.3% of the total number of subjects recruited, while subjects >36 years of age formed 7.1% of the total number of subjects recruited for this study. Table 1 also shows that subjects in the control group, first, second, third and fourth time donor groups formed 19.0, 17.4, 19.0, 22.3 and 22.3% of the total number of subjects, respectively.

Comparison of haematocrit (Hct) of the different groups of subjects: Mean Hct was 43.9 ± 0.55 , 41.9 ± 0.66 , 40.1 ± 0.47 , 39.1 ± 0.54 and $33.3 \pm 0.56\%$, for control group, first, second, third and fourth time blood donors, respectively as shown in Fig. 1. The mean values of Hct decreased

Table 1: Age distribution of repeated blood donors (N = 184)

Age (years)	Control	1st time	2nd time	3rd time	4th time	Percentage
18-25	25	15	14	15	15	45.7
26-35	7	15	20	21	24	47.3
≥ 36	3	2	1	5	2	7.1
Total (%)	35 (19.0%)	32 (17.4%)	35 (19.0%)	41 (22.3%)	41 (22.3%)	184 (100%)

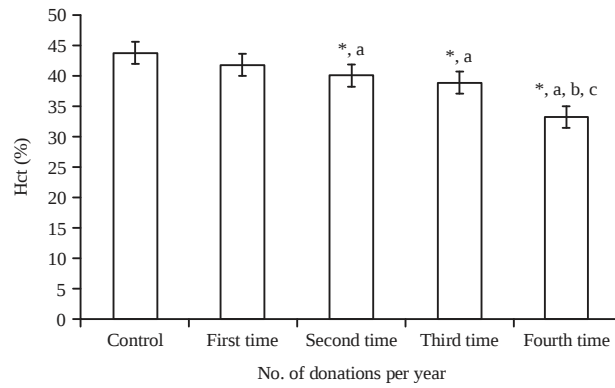


Fig. 1: Haematocrit (Hct) of repeated blood donors. Values are Mean $\pm$ SEM. *p<0.05 vs control, a: p<0.05 vs. first time, b: p<0.05 vs. second time and c: p<0.05 vs. third time

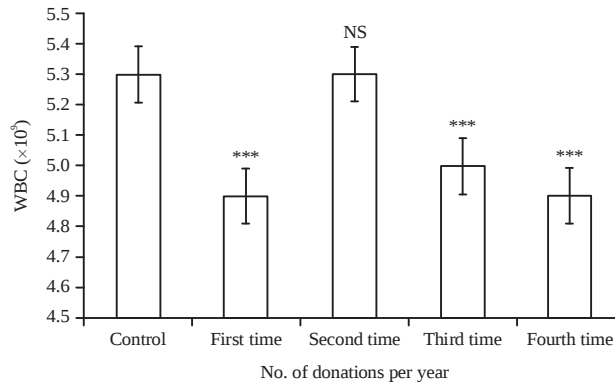


Fig. 2: White blood cell counts following repeated blood donation. Values are Mean $\pm$ SEM. ***p<0.001 vs. control, NS: Not significant vs. control

significantly (p<0.05) from 43.9% for the control group to 33.3% in fourth time donors progressively as the number of donations increased within a year. There was also progressive decrease in Hct from second to fourth time donations. Haematocrit was significantly (p<0.05) reduced in fourth time donors, compared with first, second and third time donors (Fig. 1).

Comparison of leucocyte (WBC) count in the different groups: Figure 2 shows leucocyte count of donors following repeated blood donation. The mean WBC count of male blood donors were $5.3 \pm 0.18 \times 10^9$, $4.9 \pm 0.21 \times 10^9$, $5.3 \pm 0.33 \times 10^9$, $5.0 \pm 0.19 \times 10^9$ and $4.9 \pm 0.23 \times 10^9$ for control group, first, second, third and fourth time donors, respectively. The first, third and fourth time donors had white blood cell counts that were significantly (p<0.001) lower than that of the control group.

Comparison of the percentage of lymphocyte in the different groups: The percentage of lymphocytes in leucocyte differential count is shown in Fig. 3. Lymphocytes increased progressively following repeated blood donation. Percentage of lymphocyte was significantly (p<0.001) increased in third ($39.7 \pm 1.59\%$) and fourth ($41.3 \pm 1.35\%$) time donors, compared with control ($33.7 \pm 1.52\%$).

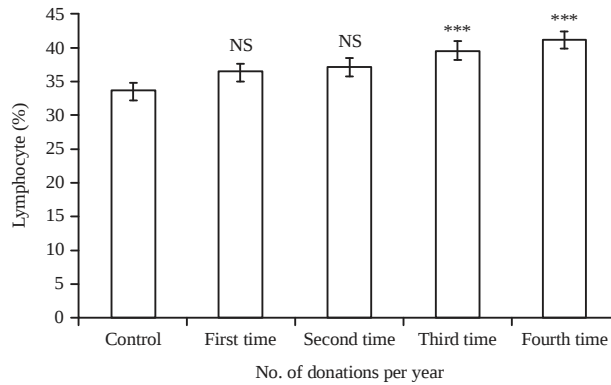


Fig. 3: Lymphocyte count following repeated blood donation. Values are Mean±SEM. ***p<0.001 vs. control, NS: Not significant vs. control

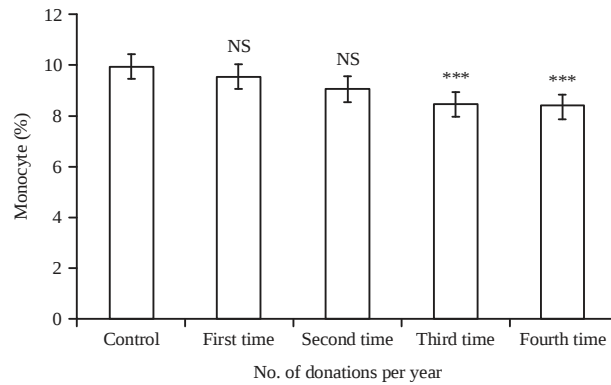


Fig. 4: Percentage of monocyte following repeated blood donation. Values are Mean±SEM. ***p<0.001 vs. control, NS: Not significant vs control

Comparison of the percentage of monocytes in the different groups: The percentage of monocytes in the different groups were 10.0 ± 0.65 , 9.6 ± 0.83 , 9.1 ± 0.83 , 8.5 ± 0.99 and $8.2 \pm 0.77\%$ for control group, first, second, third and fourth time donors, respectively (Fig. 4). There was a significant ($p < 0.001$) decrease in the percentage of monocytes in third and fourth time donors when compared with control group.

Comparison of the percentage of granulocytes in the different groups: Figure 5 shows a progressive decrease in percentage of granulocytes with increase in the frequency of blood donation. The percentage of granulocyte was significantly ($p < 0.001$) reduced in first ($53.9 \pm 1.99\%$), second ($53.6 \pm 1.88\%$), third ($51.8 \pm 1.67\%$) and fourth ($50.5 \pm 1.56\%$) time donors when compared with that of the control group ($56.3 \pm 1.79\%$).

Comparison of platelet count in the different groups: The mean platelet count of male blood donors were $237 \pm 9.55 \times 10^9$, $241 \pm 9.83 \times 10^9$, $243 \pm 9.78 \times 10^9$, $202 \pm 9.67 \times 10^9$ and $209 \pm 7.77 \times 10^9$ for control group, first, second, third and fourth time donors, respectively. The platelet count was significant ($p < 0.01$) reduced in third and fourth time donors, compared with control (Fig. 6).

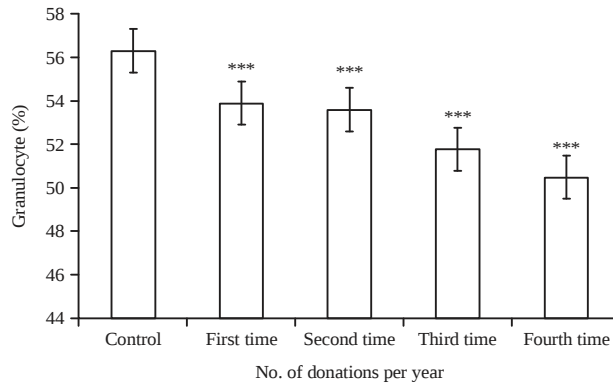


Fig. 5: Percentage of granulocyte following repeated blood donation. Values are Mean±SEM. ***p<0.001 vs. control

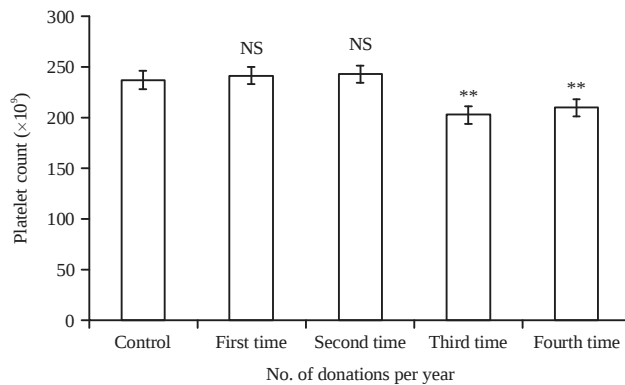


Fig. 6: Platelet count following repeated blood donation. Values are Mean±SEM. **p<0.01 vs. control, NS: Not significant vs control

DISCUSSION

The age distribution of the subjects in the different groups was not significantly different from each other. Since age affects haematological indices, the similarity in ages ruled out the effect of age on the results obtained. The percentages of the total number of subjects at various times of blood donation were also similar. They ranged from 17.4-22.3% of the total number of 184 at each time of blood donation (Table 1). The similarity in percentages of blood donors at various times of blood donation ruled out statistical bias when comparing groups. However, 45.7% of the total blood donors were in the 18-25 years group and 47.3% were in 26-35 years group. Only a few (7.1%) were above 36 years old. The reason for younger subjects, that is, persons below 36 years donating blood is not certain. Since most of the donors were paid, blood donation may be promoted by youth unemployment because statistics show that 40 million Nigerians (mostly youths) are unemployed (SURE-P, 2013).

Haematocrit significantly decreased from the first to the fourth time donors. This therefore, indicates that, repeated blood donation shortly after 3 months can significantly lower Hct. Since the interval between one donation and another was about 2 months, it is likely that this period was not sufficient for erythropoiesis to compensate for the decline in this parameter. The blood samples analyzed in this study were collected after blood was donated. This finding is

consistent with earlier reports by Akpotuzor *et al.* (2008), Okpokam *et al.* (2012), Nubila *et al.* (2014) and Rigas *et al.* (2014). The decrease in PCV found in donors that donated blood 2-4 times could proceed to iron deficiency anaemia. Okpokam *et al.* (2015) in a study conducted in Calabar reported that 57.6% of repeated blood donors (2-4 times year⁻¹) presented with iron deficiency without anaemia and 32.6% of the same group of donors presented with anaemia. The authors concluded that hemoglobin synthesis may have been utilizing the available iron in iron stores. Erhabor *et al.* (2013) and Adediran *et al.* (2013) also reported a high prevalence of iron deficiency among repeated blood donors in Sokoto, Northern Nigeria.

There was a decrease in white blood cell count of first, third and fourth time blood donors and also in platelet count of third and fourth time blood donors when compared with those of the control group. In general, it was observed that in third and fourth time blood donors, the leucocyte count (monocyte and granulocyte) and thrombocyte decreased even though their values were within the normal range, but significantly lower than that of control in this study. The control group values observed is consistent with a recent study in Benin by Nubila *et al.* (2014) and Erhabor *et al.* (2014). Moreover, lymphocyte count was slightly raised. These results indicate that repeated blood donation does not only affect red blood cell count and its indices, it also affects other blood cells, notably white blood cells and platelet counts. The reason for white blood cell (except lymphocyte) and platelet decrease is unknown. Since all the blood cells have a common origin in the bone marrow, it is likely that repeated blood donation affects the common progenitor cells in the bone marrow to stimulate or activate haemopoiesis. In general, the reduction in WBC count can put these donors at an increased risk of various infections other than malaria and sepsis. Repeated blood donation may predispose to infections owing to low white blood cell count, while the slightly raised lymphocyte may be caused by viral and chronic granulomatous infection. It may also predispose to thrombocytopenia owing to low platelet count. Low platelet count may predispose to bleeding tendencies. Benedict *et al.* (2012) reported that mean values of erythrocyte, leucocyte counts, average cellular volume, average corpuscular hemoglobin and average corpuscular. Hemoglobin were significantly reduced in paid donors when compared with unpaid donors, while platelet count showed no significant difference. Jeremiah *et al.* (2011) reported that Hct, absolute leucocyte count, monocyte, erythrocyte count and hemoglobin concentration were significantly reduced in serial blood donors, compared to 1st time donors, while thrombocytopenia was not observed in serial blood donors. The PCV and platelet count was significantly lower among commercial remunerated blood donors in Sokoto, Northern Nigeria and there was a need to re-emphasize or formulate policies on ways to seriously and innovatively attract and retain voluntary non-remunerated blood donors. However, in this present study, third and fourth time blood donors also had significantly lower platelet counts compared to control group. This may therefore be a peculiar feature of subjects in Calabar, Nigeria.

CONCLUSION

Repeated (third and fourth timers) blood donation has shown in this study to decrease Hct, WBC (monocyte and granulocyte) and platelet counts, but increase lymphocytes when compared to the control. There is need to maintain the frequency of blood donation that has been considered safe because all blood components are affected by repeated blood donations. Complete blood count should be carried out on all prospective blood donors. Also, there is need to have a facility for whole blood fractionation to enhance the use of blood components (platelet apheresis) which will reduce the need for more people donating repeatedly. More research and advanced tests especially in platelet functions should be carried out on donors to ascertain their health status.

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