EVALUATION OF IRON AND FOLATE DEFICIENCIES AS POSSIBLE CAUSES OF ANAEMIA IN UNFIT BLOOD DONORS

ÉVALUATION DES DÉFICITS EN FER ET EN FOLATE COMME CAUSES POSSIBLES D'ANÉMIE DANS LES DONNEURS DE SANG DE L'UNFIT

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ABSTRACT

BACKGROUND
Prospective blood donors are commonly deferred at the Blood Service of the University of Lagos Teaching Hospital, a tertiary health facility, majorly due to anaemia. Since experience shows that iron and folate deficiencies are common causes of anaemia in the population, it is desirable to investigate the iron and folate status of prospective donors who fail the blood donor fitness test to confirm whether or not, iron and folate deficiencies are part of the reasons for failure.

AIM AND OBJECTIVE
The objective of this study was to establish whether or not, iron and folate deficiencies are the causes of anaemia in prospective blood donors who fail the pre-donation haemoglobin test.

RESUME

CONTEXTE
Les donneurs de sang potentiels ajournés au Service de transfusion sanguine de l'hôpital universitaire de Lagos, un établissement de santé tertiaire, le sont principalement en raison de l'anémie. Puisque l'expérience montre que les carences en fer et en acide folique sont des causes courantes d'anémie dans la population, il est souhaitable d'examiner le statut en fer et en folate des donneurs potentiels qui échouent au test de condition physique du donneur de sang.

BUT ET OBJECTIF
L'objectif de cette étude était d'établir si les carences en fer et en acide folique sont la cause de l'anémie chez les donneurs de sang potentiels qui échouent au test d'hémoglobine avant le don.

MATERIALS AND METHODS

A total of 263 prospective donors were recruited, and, based on the Copper Sulphate specific gravity test set at a haemoglobin concentration cut-off of 12.5g/dl, they were divided into unfit (study n=153) and fit (control n=110) groups. Ten (10ml) of venous blood was drawn from each subject and distributed into sodium-EDTA specimen bottle (5ml) for estimation of full blood count within 2 hours of collection and plain disposable plastic tubes (5ml) for the estimation of serum ferritin, serum homocysteine and serum folate in both study and control groups.

RESULTS

There was a statistically significant decrease (p<0.05) in the haemoglobin (Hb) concentration and packed cell volume (PCV) of the study group compared to the control. There were 62 correctly rejected unfit donors who had haemoglobin level <12.5g/dL, of these, only 12 (19.36%) had serum ferritin level <15µg/L, 3 (4.8%), had homocysteine level >15µmol/L and 3 (4.8%) had folate level <3ng/ml, indicating iron and folate deficiency, respectively. There was no statistically significant association (P>0.05) between haemoglobin concentration and serum ferritin, homocysteine and folate levels within the study group.

CONCLUSION

The findings in this study suggest that in only about 20%, and in less than 5% of cases was iron and folate deficiency, respectively, the cause of anaemia, leading to correct rejection as blood donors. Therefore, other factors which may be associated with anaemia in prospective blood donors need to be further investigated.

MATÉRIELS ET MÉTHODES

Au total, 263 donneurs potentiels ont été recrutés et, sur la base de l'essai de densité de sulfate de cuivre fixé à une concentration de 12,5 g / dl, ils ont été divisés en inaptes (étude n = 153) et en bonne santé (contrôle n = 110) groupes. Dix (10 ml) de sang veineux ont été prélevés sur chaque sujet dans un tube de sodium-EDTA (5ml) pour la numération globulaire dans les 2 heures suivant la collecte et 5ml pour l'estimation du taux de ferritine sérique, de sérum homocystéine et de folate sérique dans les groupes d'étude et de contrôle.

RÉSULTATS

Il y avait une diminution statistiquement significative (p <0,05) de la concentration d'hémoglobine (Hb) et de l'hématocrite (groupe hématocellulaire) du groupe d'étude par rapport au groupe témoin. Il y avait 62 donneurs inaptes correctement rejetés qui avaient un taux d'hémoglobine <12,5g / dL, seulement 12 (19,36%) avaient un taux sérique de ferritine <15µg / L, 3 (4,8%), avaient un taux d'homocystéine> 15µmol / L et 3 (4,8%) avaient un taux de folate <3ng / ml, indiquant une carence en fer et en folate, respectivement. Il n'y avait pas d'association statistiquement significative (P> 0,05) entre la concentration en hémoglobine et les taux sériques de ferritine, d'homocystéine et de folate dans le groupe d'étude.

CONCLUSION

Les résultats de cette étude suggèrent que dans seulement 20% des cas, et dans moins de 5% des cas, la carence en fer et en acide folique était la cause de l'anémie, conduisant à un rejet correct en tant que donneurs de sang. Par conséquent, d'autres facteurs qui peuvent être associés à l'anémie chez les donneurs de sang potentiels doivent être étudiés plus en détail.

INTRODUCTION

Blood donor deferral is largely the consequence of anaemia in prospective blood donors in developing countries[1, 2]. Prospective donors may feel healthy enough to donate blood, but may fail the donor fitness test and thus be rejected as donors. The copper sulphate test is still used to assess fitness for blood donation in many blood centres in the developing countries, including some in Nigeria[3] and those who fail this test, often inquire about the reasons they are considered unfit despite feeling healthy.

Nutritional anaemia results from dietary deficiency of nutrients such as iron, vitamins (especially folate and B_{12}) and protein which are essential for red blood cell formation. Nutritional anaemia is still prevalent, especially in females, in developing countries[1]. The common causes of nutritional anaemia in adults are reported to be iron, folate and vitamin B_{12} deficiencies[4] while deficiencies of vitamin A, C, E, and B_6 and mineralized copper are not common[5]. Iron deficiency is the most widespread single nutrient deficiency in the world and it is responsible for 75% of anaemia.[6]

Low haemoglobin level is primarily used as an indicator of anaemia; and a minimum pre donation haemoglobin concentration of 12.5 g/dL is required for blood donation[7]. Hence, most blood banks consider donors with haemoglobin level <12.5 g/dL as unfit and are rejected as blood donosr[7]. In order to generate information for counselling unfit blood donors, it is desirable to establish the role of iron and folate deficiency as common causes of failure of the pre-donation haemoglobin check. While other causes of anaemia

in prospective blood donors such as frequent blood donation, heavy menstrual or other forms of blood loss, or underweight are easy to detect[8,9] nutritional lack of iron and folates requires laboratory evaluation[10]. This study was conducted to evaluate the role of iron and folate deficiency in the relatively low haemoglobin levels found in prospective blood donors who fail the pre-donation fitness test.

MATERIALS AND METHODS

This was a case-control study at the donor clinic of the Lagos University Teaching Hospital, Nigeria, for which approval was obtained from the hospital ethical committee. A total of 263 prospective donors were recruited and they were stratified into fit and unfit, based on the outcome of the Copper Sulphate specific gravity test. Structured questionnaires were administered to each prospective donor to obtain information on demography, health status, dietary history, menstrual pattern, exercise and social and behavioural history. The inclusion criteria were all healthy adults aged 18 – 60 years, weighing at least 50kg. Study subjects included prospective donors who were rejected only for failing the copper sulphate test. Other exclusion criteria included women in on-going menstruation, and persons with haemolytic anaemia such as sickle cell disease. Also excluded were persons with transfusion transmissible infections such as HIV, viral hepatitis, HTLV, and Syphilis., Persons who had had major surgery in the last one year, and women who are pregnant or less than six weeks post-partum were also excluded, as well as persons on medication with aspirin, finasteride and haematinics, or have received blood transfusion in the previous three months.

- **Sampling Method**

  A systematic sampling method was used in selecting subjects for the study. For every 2 unfit donors one was selected, to make 153 for the study. Of the fit donors, 1 of every 10 was selected to make 110 as the control group. This brought the total number of samples to 263.

- **Specimen Collection and Preparation**

  Ten (10) ml of venous blood was drawn from each subject. Five (5ml) was put into sodium EDTA specimen bottle for estimation of full blood count within 2 hours of collection using the Haematology auto-analyzer (Sysmex KX21®). The remaining 5 ml of blood was transferred to plain disposable plastic tubes and allowed to stand at room temperature until clotted. This was centrifuged and sera transferred into plain cryotubes. The sera were stored at -72°C and used for the estimation of serum ferritin, serum homocysteine and serum folate. Serum ferritin was measured using a chemiluminescence assay (Beckman Diagnostics, Access B, Fullerton, CA, USA). Serum homocysteine was measured using a flouresence polarization immunological assay (Abbott Diagnostics, AxSYM System®, Abbott Park, IL, USA). Serum folate was determined using a chemiluminescent immunological assay (Diagnostic Products Corporation, Immulite 2000, Los Angeles, CA, USA).

- **Statistical Analysis**

  Data obtained was analysed using Statistical Package for Scientific Solution (SPSS) software version 11. Values of the haematological parameters were expressed as mean ± standard error of mean. Comparison between the mean values was done using students' t-test. Categorical variables were compared using the Fisher's test and $P < 0.05$ was considered as statistically significant.

RESULTS

A total of 263 subjects were recruited into the study. Of this, 153 were unfit donors (study group) while 110 were fit donors (control group). The ages of the subjects were between 18 and 60 years. 42 (27.45%) Of the unfit donors were females while 14 (12.73%) of fit donors were females. Unfit donor status was thus more common in females $P = 0.003$ (Table 1). The mean haemoglobin (13.44 ± 1.07 g/dL) and the packed cell volume (40.55 ± 5.05%) for the control group was significantly higher ($P<0.05$) than that of the study group 12.54 ± 1.47 g/dL and 37.91 ± 4.57%, $P=0.000$. The mean cell volume (MCV) and the Mean Cell Haemoglobin (MCH) were not significantly different ($P>0.05$) see table 2. However, the red cells of the study group appeared to be more dehydrated with MCHC of 32.93 ± 1.77 g/dL as compared with 31.31 ± 1.59 g/dL for the control, $P=0.02$.

The mean Haemoglobin of males in the control group (13.52 ± 0.99) was expectedly higher than of males that were found unfit 12.63 ± 1.46 g/dL $p= 0.0000$. Similarly females in the control group had higher haemoglobin than females that were unfit but this did not reach significant level $p = 0.236$.

Of the 153 donors classified as unfit by CuSo4 test only 62 (40.53%) had Hb <12.5 g/dL resulting in false rejection of 91 (59.47%) donors. On the other hand, 15 (13.63 %) of the fit donors according to CuSO4 test had Hb level below 12.5 g/dL. Of the 62 correctly rejected unfit donors only 12 (19.36) had serum ferritin level <15 µg/L. On the other hand, of the 91 falsely rejected unfit donors who had haemoglobin >12.5 g/dL 8(8.7%) also had serum ferritin <15 µg/L .The difference in the proportion that had serum ferritin below 15ug/L. In the two groups however fell slightly short of the statistically significant level. $P = 0.057$. Of the 62 correctly rejected unfit donors who had haemoglobin <12.5 g/dL only 3 (4.8 %) had homocysteine level >15 µmol/L.while of the 91 falsely rejected unfit donors only 3 (3.3 %) also had homocysteine >15 µmol/L**.** Thus hyperhomocysteinaemia was not more common in both the correctly and the falsely rejected blood donors than in the fit donors, $P=0.63$, and 0.31 respectively (Table 5).

Of the 62 correctly rejected donors only 3 (4.8 %) had folate level <3 ng/ml. While out of the falsely rejected donors who had haemoglobin >12.5 g/dL only 3 (3.3%) also had folate <3 ng/ml. Low level folate was thus not more common in both correctly and falsely rejected donors compared with fit donors (Table 6).

TABLE 1: Sex distribution of respondents

Sex	Study (Unfit Donors)	Control (Fit Donors)	Total
Male	111	96	207
Female	42	14	56
Total	153	110	263

TABLE 2: Comparison of mean haemoglobin concentration, packed cell volume, and red cell indices in study and control subjects

Variable	Study Group n=153	Control Group n=110	P-Value
Haemoglobin (g/dL)	12.54 ± 1.47	13.44 ± 1.07	0.00*
PCV (%)	37.91 ± 4.57	40.55 ± 5.05	0.00*
MCV (fl)	83.84 ± 7.89	84.11 ± 8.57	0.79
MCH (pg)	27.57 ± 2.77	27.96 ± 8.19	0.58
MCHC (g/dL)	32.93 ± 1.77	31.31 ± 1.59	0.02*

* Significant P-value (p<0.05)

TABLE 3: Comparison of mean haemoglobin in test and control between sexes

VARIABLE		STUDY GROUP Male=111, Female=42	CONTROL GROUP Male=96, Female=14	P-VALUE
HAEMO-GLOBIN (g/dL)	Male	12.63 ± 1.46	13.52 ± 0.99	0.000**
	Female	12.31± 1.51	12.85 ± 1.33	0.236
	P-value	0.2326	0.025**	

TABLE 4: Comparison of ferritin levels in anaemic and non- anaemic subjects

SUBJECTS (Donors)	Ferritin	HAEMOGLOBIN				
		Anaemia (<12.5 g/dL)	Normal (≥12.5 g/dL)	χ^2	Df	P
Study (UNFIT by CuSO$_4$ Test)	Abnormal (<15 µg/L)	12 (19.3 %)	8 (8.7 %)	3.621	1	0.057
	Normal (≥15 µg/L)	50 (80.7 %)	83 (91.3 %)			
Control (Fit by CuSO$_4$ Test)	Abnormal (<15 µg/L)	0 (0.0 %)	13 (13.7 %)	0.574	1	0.449
	Normal (≥15 µg/L)	15 (100.0 %)	82 (86.3 %)			

** Significant P-value (p<0.05)

TABLE 5: Comparison of homocysteine levels in anaemic and non-anaemic subjects

SUBJECTS (Donors)	Homocysteine	HAEMOGLOBIN				
		Anaemia (<12.5 g/dL)	Normal (≥12.5 g/dL)	χ^2	Df	P- value
Study (UNFIT by CuSO$_4$ Test)	Abnormal (>15 µmol/L)	3 (4.8 %)	3 (3.3 %)	0.233	1	0.630
	Normal (<15 µmol/L)	59 (95.2 %)	88 (96.7 %)			
Control (Fit by CuSO$_4$ Test)	Abnormal (>15 µmol/L)	1 (6.7 %)	2 (2.1 %)	1.016	1	0.313
	Normal (<15 µmol/L)	14 (93.3 %)	93 (97.9 %)			

**Significant P-value (p<0.05)

TABLE 6: Comparison of folate levels in anaemic and non-anaemic subjects

SUBJECTS (Donors)	Folate	HAEMOGLOBIN				
		Anaemia (<12.5 g/dL)	Normal (≥12.5 g/dL)	χ^2	Df	P- value
Study (UNFIT by CuSO$_4$ Test)	Abnormal (<3 ng/L)	3 (4.8 %)	3 (3.3 %)	0.411	1	0.522
	Normal (>3 ng/L)	58 (95.2 %)	88 (96.7 %)			
Control (Fit by CuSO$_4$ Test)	Abnormal (<3ng/L)	0 (0.0 %)	3 (3.2 %)	0.487	1	0.485
	Normal (>3 ng/L)	15 (100 %)	92 (96.8 %)			

**Significant P-value (p<0.05)

DISCUSSION

The purpose of this study was to determine the iron and folate status of prospective blood donors who failed the donation fitness test. The study evaluated a total of 263 subjects, 153 unfit (study group) and 110 fit subjects (control group). Blood donors in our centre are classified as fit and unfit according to the outcome of the Copper Sulphate Specific Gravity method which uses a haemoglobin level of 12.5g/dL as cut-off point. The large number of 'unfit' donors who had haemoglobin levels above 12.5g/dl,

and who were therefore falsely rejected, and the few (15/110) who passed the test but had haemoglobin levels below 12.5g/dl and were thus falsely accepted, confirms the subjectivity and unreliability, even in good hands, of the copper sulphate system as a screening test for blood donation. [11] It is also not clear how far, if at all, the higher MCHC in the unfit donors in this study affected the outcome of their copper sulphate test However, our conclusions on the iron and folate status of unfit prospective donors was based on the analysis of the results of the 62 correctly rejected donors.

In this study, the observed predominance of male donors within the study group (males 72.5%; females 27.5%) and control group (males 87.3%; females 12.7%) is in agreement with several studies[3, 7] which reported low population of females among prospective and recruited blood donors. This could be due to a general misconception that women are not eligible as blood donors, though pregnant and lactating women are correctly regarded as unfit for blood donation. [12]

In the present study, the higher haemoglobin and PCV in the control group as compared with the study group is not unexpected. [2, 12] The observed mean Haemoglobin of the male fit donors (13.52 ± 0.99) which was significantly higher than that of female fit donors (12.85 ± 1.33) (p=0.0062) is also not unexpected.[1]However, the mean Haemoglobin of male unfit donors (12.63 ± 1.46) was not significantly higher than that of female unfit donors (12.31 ± 1.51) (p=0.2326). This should also be expected since both sexes in the unfit donors were categorised as anaemic. Consequently, the rate of deferral for females would be higher than for males.

The body iron store can be quantified using serum ferritin level as a biomarker.[9] Only 19.3% of the 62 correctly rejected unfit blood donors who had Haemoglobin level <12.5 g/dL may be said to have iron deficiency anaemia as their serum ferritin levels were below the normal of <15 µg/L.. Our finding in this study is comparable to the study of Jeremiah et al.[9] who reported iron-deficiency anaemia in 12.0% of their study population in Port-Harcourt, Nigeria. The slight difference could be attributed to the characteristics of study population and the difference in the definition of iron-deficiency anaemia which was set at haemoglobin <11.0 g/dL and serum ferritin <12.0 ng/ml.

Folic acid deficiency (folate <3 ng/ml) was found in 3 (4.8%) of 62 unfit blood donors who had Haemoglobin <12.5 g/dL. Thus, folate deficiency may be regarded as an uncommon cause of anaemia in unfit blood donors. Sub-normal folate level was also documented in 3 (3.3%) of 91 unfit blood donors in this study who had Haemoglobin level > 12.5 g/dL. The significance of this finding may be debatable since sub-normal or near normal folate levels have been documented in non-anaemic individuals[13] who present with other complications of folate deficiencies such as vascular defects,[14] neural tube closure defect,[15] the HELLP syndrome[16] and increased predisposition to colonic cancers.[17]

The observed abnormal serum homocysteine levels (homocysteine >15 mmol/L) in 3 (4.8%) of 62 correctly rejected unfit blood donors was found to be similar to the number of correctly rejected blood donors 3 (4.8%) who were folate deficient (i.e. <3 ng/ml). This supports the well-established belief that serum folate concentration is inversely proportional to the serum homocysteine concentration.[10]

CONCLUSION

The evidence from this study reveals that iron and folate deficiencies may not be the major causes of anaemia in unfit prospective blood donors Therefore, efforts should be made to evaluate other factors which may be associated with the pattern of anaemia in deferred blood donors. It is also recommended that the copper sulphate test be discontinued as a pre-donation screening procedure, and be replaced with the more objective actual measurements of haemoglobin concentration

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