Original Article

Assessment of ultrasound flow-mediated dilation of brachial artery in regular blood donors in a Nigerian Tertiary Hospital

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Abstract

Background: Iron is a pro-oxidant cofactor that may be linked to cardiovascular disease (CVD) progression and reduction of body iron stores have been hypothesized to reduce the risk of CV disease.

Aim: The aim of this study is to assess reduction in CVD risk susceptibility among regular blood donors compared with nondonors using ultrasound brachial artery flow-mediated dilation (BAFMD).

Settings and Design: A prospective comparative study designed to establish the difference between mean flow-mediated dilatation (FMD) in the patients who are regular blood donors compared with nondonors recruited from a Teaching Hospital donor clinic.

Materials and Methods: Data were collected over 7 months from December 2014 to June 2015. 100 eligible regular male blood donors, aged 21–50 years, were selected from a Teaching Hospital blood donor records and their BAFMD assessed. 50 nondonors/first time donors, of equivalent age group, consecutively were assessed for comparison. Serum markers of iron stores, markers of oxidative stress and other related cardiac risk factors were also assessed in all patients.

Results: BAFMD was significantly greater in regular blood donors when compared with nondonors (13.95% ± 7.02% vs. 8.20% ± 4.19%, $P = 0.000$). Serum ferritin was significantly decreased in regular blood donors when compared with nondonors (mean value 41.92 ng/ml ± 23.12 ng/ml vs. 61.97 ± 30.19 ng/ml, $P = 0.000$), but Hb did not differ between the groups. High FMD was significantly associated with high C-high-density lipoprotein and low C-LDL ($r = -0.215^{*}$, $P = 0.032$, $r = 0.188$, $P = 0.031$, $r = 0.193$, $P = 0.027$, $r = 0.0279$, $P = 0.002$, $r = 0.139$, $P = 0.084$). LDL was decreased in regular blood donors compared with nondonors.

Conclusion: The study provides prognostic information for assessing ultrasound BAFMD as a cardiac risk marker. Regular blood donors have enhanced cardiovascular function with increased flow-mediated dilation, decreased body iron stores, and decreased oxidative stress compared with nondonors.

Keywords: Blood artery flow-mediated dilation, blood donors, brachial artery, cardiovascular diseases, ultrasound

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INTRODUCTION

Cardiovascular disease (CVD) refers to the category of chronic diseases involving heart and blood vessels.[1] It is responsible for one-third of global deaths, claiming 17.1 million lives per year with over 80% taking place in low- and middle-income countries and 23.6 million deaths estimated by 2030.[1-3] CVD is the leading and increasing contributor to the global disease burden with high socioeconomic cost.[3,4]

Africa has witnessed increased urbanization and lifestyles, factors which have, in turn, raised the incidence of CVD.[3,5] Studies from South Africa have shown that 32.1% of men and 18.9% of women over 30 years had a 20% or higher risk of developing CVD in the next 10 years.[3,6-8] Total deaths in Nigeria as of 2005 are 2.01 million of which 11% are due to CVD with an estimated loss of 400 million dollars in national income.[4]

The risk factors for CVD are many and include the classic ones (hypertension, diabetes, hyperlipidemia, smoking, aging, etc.) and those discovered more recently (hyperhomocysteinemia, oxidative stress, infections, iron overload (IO), systemic inflammation, estrogen deficiency, local factors, and genetic predisposition).[5,9] Hypertension remains the most threatening risk factor, with prevalence ranging between 15% and 30% in adults.[3] Extrapolations from studies in Nigeria and elsewhere indicate that 5% of deaths could be due to hypertension, although the mortality rate is higher in developing countries than in developed ones.[3,6,8]

The Iron-Heart hypothesis first put forth by Sullivan in 1981 suggests that increased body iron stores are a risk factor for coronary heart disease and thus that iron depletion through phlebotomy or other means can reduce risk.[10,11]

This risk reduction can be assessed by studies involving endothelial cells whose homeostatic and vasodilator function mediated by nitric oxide have become a useful target for the indirect assessment for risk of CVD.[12]

Endothelial dysfunction is a cardinal feature of vascular disease states such as atherosclerosis and is associated with an increased risk of cardiovascular events. At present, endothelial dysfunction in the peripheral vasculature is an independent predictor of future cardiovascular events in patients with either established CVD or with a risk factor for vascular disease development.[12]

There are many techniques for assessing endothelial function; these can be either invasive or noninvasive. For the assessment of preclinical disease, the ideal technique for measuring endothelial function must be noninvasive, reliable, reproducible, cheap, and easy to perform.[7]

Noninvasive methods of measuring endothelial function include ultrasound flow-mediated dilatation (FMD). Ultrasound FMD is the most widely used method for searching both small and large population studies of adults and pediatric patients susceptible to an acute cardiovascular event (defined as "vulnerable patient").[12]

In the invasive method, vasoactive agents are delivered via intra-arterial infusion, while the response is measured with high-resolution ultrasound or strain gauge plethysmography.[12]

This study aims to assess reduction in the susceptibility of cardiovascular risk among the population of people who donate blood regularly on the bias of those that have never donated blood, using a high-resolution ultrasound whose noninvasiveness, cheapness and availability serve as advantages over other imaging modalities.[7]

MATERIALS AND METHODS

Study design

A comparative study designed to establish the difference between mean FMD in the patients who are regular blood donors compared with nondonors recruited from a Teaching Hospital donor clinic. This was a bidisciplinary study conducted by the Radiology and Hematology units of a teaching hospital.

Duration of study

Recruitment into the study was carried out for 7 months with all the ultrasound scans done from December 2014 to June 2015.

Sample size

The study involved 100 regular blood donors and 50 age-matched controls. This figure was derived using the formula for statistically significant results.

$$n = \frac{(u + v)^2 (d_1^{\,2} + d_0^{\,2})}{(u_1 - u_0)^2}$$

Where n = sample size of each group
u = one-sided percentage point of normal distribution corresponding to 100%-power.

The power of this study was 90%, implying that at least 90% of the study population should have 33% increases in FMD.

v = percentage point of the normal distribution corresponding to (two-sided) significance level.
u_1 = expected mean (hypothetic mean).
u_0 = universal mean.

For sample size determination, we chose 33% increases in the level of FMD observed consistently in the patients as compared with the universal mean (as obtained from literature) as a good prognostic outcome of CVD risk reduction in the study population.[13]

Universal mean (u_0) from literature[13] is 5.89% ± 2.88%.

Thus expected subject mean (u_1) is 7.83% ± 3.83%

At 90% power u = 1.28

At significance level of 5% v = 1.96

$$n = \frac{(1.28 + 1.96)^2 (3.83^2 + 2.88^2)}{(7.83 - 5.89)^2}$$

$$n = \frac{241.06}{3.76}$$

$n = 64.11$

This was rounded up to 150 patients to broaden the base of this study, to account for possible attrition and to improve result reliability.

Inclusion criteria for regular blood donors were male adults between 21 and 50 years of age that have voluntarily donated at least twice within the past 2 years and gave informed consent.

Inclusion criteria for the control group were male adults between 21 and 50 years of age who had never donated blood and gave informed consent.

Exclusion criteria for regular blood donors and the control group were all females and male subjects <21 years and >50 years of age, hypertensive subjects, patients with a history of major bleeding events (including trauma and surgery,) within the past 2 years, diabetes mellitus, previous myocardial infarction, cancer or active chronic inflammatory disease, or alcohol and tobacco use within 6 months.

Clinical measurements taken include age and body mass index (BMI) and blood pressure. Two separate blood pressure readings were taken 30 min apart, and the higher value was taken as the blood pressure.

Laboratory studies conducted by the hematologists were on 15 ml of venous blood drawn from each subject. A volume of 5 ml of this was put into sodium ethylene diamine tetra-acetate (EDTA) specimen bottles. This sample was used for the full blood count, including the red blood cell indices and was analyzed within 2 h of collection. The remaining 10 ml of the blood was transferred to new plain screw-capped disposable plastic tubes and allowed to stand at room temperature until clotted and the clot retracted (about 2 h). This was then centrifuged and sera separated and transferred to plain cryotubes using a transfer pipette. The serum was aliquoted and stored at −80°C until analysis was performed. The full blood count was carried out on the EDTA anticoagulated samples using the Sysmex KX-21N hematology analyzer. Analysis of serum iron and lipids were performed using the Roche/Hitachi 902 autoanalyzer manufactured in Japan. Serum ferritin was determined by the enzyme linked immunoassay method (ELISA) from commercial assay kit, FERRITIN ELISA KIT manufactured by Biotech Laboratories, high-density lipoprotein (HDL)-C PLUS 3rd generation kit manufactured by Roche Diagnostics GMBH, Sandhofer Strasse 116, MANNHEIM, LDL-C PLUS 2nd generation kit manufactured by Roche Diagnostics GMBH, SANDHOFER STRASSE 116, MANNHEIM and TG KIT manufactured by Roche Diagnostics GMBH, SANDHOFER STRASSE 116, MANNHEIM were used for HDL, LDL, and triglyceride, respectively, at a University Teaching Hospital laboratory. The Friedewald formula was used to confirm the value of LDL.

Technique of ultrasound examination

All ultrasound examinations were performed by the researchers using a standardized approach to the measurements brachial artery flow-mediated dilation (BAFMD) based on report of the International Brachial Artery Reactivity Task Force described by Corretti *et al*.[14] The scans were done in the morning with the participants in a fasting state.

The TOSHIBA Nemio XG SSA-580A Diagnostic Ultrasound System manufactured in Japan, March 2010; equipped with vascular software for two-dimensional imaging, color, and spectral Doppler with a 7.5–10 MHz linear array transducer, attached to a high-quality mainframe, was used to acquire the images.

The patients were positioned supine and made to rest for 10 min with regular blood pressure monitoring until stabilization. The study was conducted, in a quiet room at controlled room temperature (23°C). An ultrasound coupling gel was applied along the medial aspect of the

arm at an approximate value of 5 cm above the antecubital fossa and the transducer placed therein, the brachial artery was imaged at this level and the longitudinal image acquired.

The brachial artery diameter (first baseline value, D1) was obtained by measuring the intima-lumen interface at the diastolic phase [Figure 1a]. Hyperemia was provoked with the sphygmomanometer cuff already positioned on the patient's right forearm and inflated up to 50 mmHg above systolic pressure for 5 min and then deflated. Postocclusion value (D_2) measured by the intima-lumen interface at the diastolic phase at 60 s was also obtained [Figure 1b].

The brachial artery FMD was thereafter calculated with the formula: BAFMD = $(D_2 - D_1)/D_1 \times 100$), and the results expressed in percentages.[2]

On the Doppler image flow velocity in cm/second was sampled through the brachial artery at an angle of 60° for Doppler spectral display.

Presence of vascular pathologies (peripheral arterial diseases) was checked and further evaluated by measuring the peak systolic velocity and end-diastolic velocity [Figure 2].

Ethical consideration

Ethical approval for the study was obtained from the Research and Ethics Committee of the Lagos University Teaching Hospital before commencement of recruitment into the study. In addition, written informed consent was obtained from each patient. All patients were assured of the confidentiality of the volunteered information and examination findings.

Statistical data analysis

The collected data was analyzed using Statistical Package for Social Science (SPSS®) for Windows, version 17.0.1; Chicago, IL 2007 and Microsoft Excel for Windows 2010. Data were expressed as the mean ± standard deviation. The comparison of the measurements from each group was determined using analysis of variance and the *post hoc* Bonferroni test. Linear and multiple regression analyses were performed with FMD as the dependent variable, and the following as independent variables: serum ferritin, C-HDL, C-LDL, baseline diameter and BMI; to determine their correlation and colinearity. Probability values of $P < 0.05$ were considered statistically significant.

RESULTS

A total of 150 patients were enrolled in the study. 100 were regular blood donors (study group) whereas 50 were nondonors (control group).

Table 1 shows the sociodemographic distribution of the patients in the study population. The mean age for the regular blood donors was 34.63 ± 8.21 years and for the nondonors was 35.12 ± 7.58 years. The mean BMI of the regular blood donors (25.29 ± 3.02 kg/m^2) was not significantly different from that of the non-donors (25.41 ± 3.26 kg/m^2). P =0.536. The highest frequency of blood donors was seen in the 31–35 age range making about 24.7% of the entire study population and the lowest in 46–50 age range, about 11.3% of the entire study population.

The laboratory values of the participants are shown in Table 2. Serum ferritin (a marker of iron stores) was significantly decreased in regular blood donors when compared with nondonors (41.92 ± 23.12 vs. 61.97 ± 30.19, P = 0.000). This difference in serum ferritin was also consistent in all the age ranges. Despite

Table 1: Sociodemographic distribution of subjects

Age group (years)	Frequency (%)		Total
	Study group	Control group	
Total	100 (100)	50 (100)	150 (100)
Age range			
21-25	15 (15)	5 (10)	20 (13.3)
26-30	18 (18)	10 (20)	28 (18.7)
31-35	26 (26)	11 (22)	37 (24.7)
36-40	17 (17)	12 (24)	29 (19.3)
41-45	12 (12)	7 (14)	19 (12.7)
4650	12 (12)	5 (10)	17 (11.3)
Mean BMI per age group (kg/m^2)			
Age group (years)	**Study group**	**Control group**	**Total (%)**
21-25	24.35±2.43	21.88±0.71	23.73±2.38
26-30	24.41±3.51	24.62±2.57	24.48±3.16
31-35	26.23±3.57	26.85±4.56	26.42±3.84
36-40	24.47±2.38	25.31±2.45	24.82±2.41
41-45	26.90±1.85	28.24±5.53	27.40±2.57
46-50	25.25±2.57	25.88±0.97	25.44±2.21

BMI – Body mass index

Table 2: Laboratory values of subjects

Age group (years)	Serum ferritin (ng/ml)		Total
	Study group	Control group	
21-25	35.63±24.42	76.56±28.82	45.87±30.17
26-30	47.66±27.50	56.14±32.15	50.69±28.94
31-35	42.09±23.36	47.51±28.34	43.70±24.66
36-40	40.33±16.61	74.08±36.22	54.30±30.97
41-45	34.08±14.11	61.15±22.59	44.05±21.72
46-50	50.85±27.90	62.93±20.53	54.40±25.94
Comparison of mean HB concentration, PCV, and red cell indices in the study population			
Mean HB, PCV, red cell indices	**Study group**	**Control group**	***P***
Haemoglobin (g/dl)	13.07±2.36	12.98±1.30	0.403
PCV (%)	41.00±7.10	38.74±4.14	0.219
MCH (pg)	26.78±8.42	27.30±2.42	0.643
MCV (fl)	80.32±7.44	85.23±6.25	0.002
MCHC (g/dl)	31.73±2.19	32.62±1.15	0.036

PCV – Packed cell volume; MCH – Mean cell hemoglobin; MCV – Mean cell volume; MCHC – Mean cell hemoglobin concentration

severely reduced iron stores in the high-frequency donors, hemoglobin (Hb), and hematocrit did not differ in regular donors versus nondonors. Mean corpuscular volume was significantly lower and red cell distribution width was significantly greater in high-frequency blood donors when compared with low-frequency blood donors. Mean corpuscular Hb concentration did not differ between groups.

The mean HDL of the regular blood donors (1.44 ± 0.41) was not significantly different from that of the nondonors (1.48 ± 0.39), $P = 0.576$ [Table 3]. Low-density lipoprotein (LDL) was however reduced in the regular blood donors when compared with that of the nondonors (2.20 ± 1.13 vs. 2.68 ± 0.86, $P = 0.009$).

Flow-mediated dilation was significantly increased in the regular blood donors when compared with that of the nondonors (13.95% ± 7.02% vs. 8.20% ± 4.19%, $P = 0.000$). Estimates of differences in flow-mediated dilation between regular blood donors and nondonors were consistent across age ranges [Table 4] with 75th of the regular donors having an FMD >15% [Figure 1a and b].

In a multivariate analysis of the study group higher FMD showed inverse weak correlation with ferritin, LDL and BMI ($r = -0.215$ $P = 0.016$, $P = 0.027$ and $r = -0.139$ $P = 0.084$), positive weak correlation HDL ($r = 0.188$ $P = 0.031$) and baseline diameter ($r = 0.279$, $P = 0.002$), whereas in the control group, lower FMD revealed significant positive weak correlation LDL ($r = 0.292^{x}$, $P = 0.020$), higher BMI ($r = 0.326$ $P = 0.005$) and baseline diameter ($r = 0.240$, $P = 0.047$), while the correlation with HDL ($r = -0.047$, $P = 0.374$) and lower serum ferritin (-0.196; $P = 0.087$) were not statistically significant [Table 5].

Table 6 depicts the effect of blood donation on regular blood donors. The study group (regular blood donors) was divided into two, based on the number of times they had donated in the last 3 years (2 units/year donations; >3 units/year donations in the last 3 years) The FMD, serum ferritin, and LDL showed significant differences in these two groups ($P = 0.024$, $P = 0.032$ and 0.045), respectively. There was no significant difference noted with HDL ($P = 0.325$).

DISCUSSION

At the beginning of the 20th century, CVD was responsible for 10% of all deaths worldwide; today, that figure has risen to 30%, with 80% occurring in developing countries. CVD, with a mortality toll of 17.5 million, is the leading cause of death globally.[1,4,15] Voluntary blood donors have been shown to have a reduced risk of having this disease because of the protective effect of decreased iron stores with a resultant increase in their BAFMD thereby improving vascular function.[13,16]

Ultrasound has been proven to be the imaging modality of choice because it is noninvasive, cheap, reproducible,

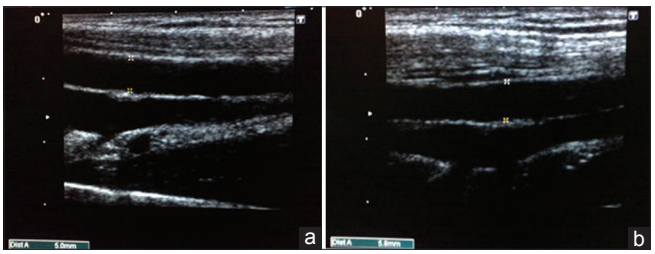

Figure 1: (a) B mode prehyperaemic sonogram of the arm taken 5 cm proximal to the cubital fossa showing the lumen-intima interface diameter measured as D1. (b) B mode posthyperaemic sonogram of the arm taken 5cm proximal to the cubital fossa showing the lumen-intima interface diameter measured as D2

Table 3: High and low density lipoprotein values in the study population

Age group (years)	Mean HDL (mmol/L)		Total	Total mean per group
	Study group	Control group		
21-25	1.53±0.46	1.40±0.28	1.50±0.42	df - 1, *F* - 0.315, *P* - 0.576
26-30	1.41±0.35	1.37±0.37	1.40±0.35	Study group: 1.44±0.41
31-35	1.38±0.40	1.51±0.34	1.42±0.38	Control group: 1.48±0.39
36-40	1.47±0.51	1.55±0.34	1.51±0.43	
41-45	1.31±0.34	1.28±0.28	1.30±0.31	
46-50	1.58±0.33	1.79±0.71	1.64±0.46	
Age group (years)	**Mean LDL (mmol/L)**		**Total**	**Total mean per group**
	Study group	**Control group**		
21-25	2.12±0.80	2.69±0.60	2.27±0.78	df - 1, *F* - 6.913, *P* - 0.009
26-30	2.18±1.31	2.84±0.76	2.42±1.17	Study group: 2.02±1.13
31-35	2.20±1.11	2.60±1.13	2.32±1.11	Control group: 2.68±0.86
36-40	2.44±1.08	2.41±0.79	2.43±0.95	
41-45	2.04±1.38	2.96±0.67	2.38±1.24	
46-50	2.15±1.13	2.76±1.30	2.33±1.23	

LDL – Low-density lipoprotein; HDL – High-density lipoprotein

nonionizing, and easy to perform. This study is a prospective study which aims to assess the cardiovascular risk in voluntary blood donors and nondonors using BAFMD.[7,14]

With advancing age, there is a decline in FMD which is associated with an increased incidence of CVD.[17,18] This was also seen in this study which revealed a decline in FMD in the 36–40, 41–45 and 46–50 age range. These findings were at variance with studies by Wray *et al*.[19] and Jensen-Urstad and Johannsson who found a preserved FMD in the older age group.[20] They attributed their findings to lack of normative FMD reference value for comparison;[14,21] their studies also included much younger men <20 years and women.

Table 4: Mean flow mediated dilatation distribution in the study population

Age group (years)	Mean FMD		Total	*P*
	Study group	Control group		
21-25	14.09±7.71	7.51±4.35	12.45±7.50	0.090
26-30	11.46±7.14	8.16±3.20	10.28±6.17	0.180
31-35	14.85±8.36	11.05±4.39	13.72±7.55	0.164
36-40	14.45±5.64	6.15±1.95	11.02±6.08	0.000
41-45	15.12±7.02	6.72±4.39	12.02±7.34	0.011
46-50	13.74±4.57	9.69±6.59	12.55±5.37	0.164

df - 1, *F* - 28.483, *P* - 0.000. Mean study group: 13.95±7.02. Mean control group: 8.20±4.19. FMD – Flow mediated dilatation

Table 5: Correlation of flow mediated dilatation with serum ferritin, high density lipoprotein, low density lipoprotein and body mass index

Correlation studies	*r*	*P*
FMD and serum ferritin		
Study group (*n*=100)	−0.215[x]	0.016
Control group (*n*=50)	−0.196	0.087
FMD and HDL		
Study group (*n*=100)	0.188	0.031
Control group (*n*=50)	−0.047	0.374
FMD and LDL		
Study group (*n*=100)	− 0.193	0.027
Control group (*n*=50)	0.292[x]	0.020
FMD and BMI		
Study group (*n*=100)	−0.139	0.084
Control group (*n*=50)	0.326[xx]	0.005
FMD and D1		
Study group (*n*=100)	−0.279	0.002
Control group (*n*=50)	0.240	0.047

[x, xx]are statistically significant. FMD – Flow mediated dilatation; HDL – High density lipoprotein; LDL – Low density lipoprotein, BMI – Body mass index, D1 – Baseline diameter, [x]Strength of the correlation

BMI as a traditional cardiovascular risk factor decreases with activities like exercise and blood donation that would result in an increased vascular function. The BMI in the study group was lower when compared with control. This was similar to the outcome of a prototype study by Fernández-Real[22] (BMI nondonors kg/m^2 27.4 ± 3.6 regular donors/27 ± 2.6). Similar findings but significant was noted by Adias *et al*.,[23] (BMI values of 24.4 ± 2.4 kg/m^2 in the first time donors was significantly higher than the 21.7 ± 1.7 kg/m^2 obtained in repeat donors $P < 0.001$). This was contrary to the findings of Zheng *et al*.[13] and Ascherio *et al*.[24] that noted the BMI of the high frequency donors was higher than the low-frequency donors. This might be due to the fact that both the study and control group were donors.

Iron measured as serum ferritin is an essential but potentially harmful nutrient. In men, iron stores, assessed by serum ferritin concentration,[6] rise after adolescence. It contributes to many important physiologic functions in the body; however, it increases biological markers of oxidative stress, cytotoxicity, and lipid peroxidation in biological systems. Various literature have suggested that the catalytic role of iron in lipid peroxidation may account for the formation of atherosclerotic lesions and loss of endothelial wall integrity.[23] More than three decades ago, it was proposed that iron depletion protects against CVD and that this effect may explain the remarkably low incidence of cardiovascular disorders in menstruating women.[25,26] This study revealed that regular blood donation and increased frequency of >3 U/year ($P = 0.032$) as a subset with the study group significantly depleted iron stores measured as serum ferritin. These were similar to the outcome of studies by Klipstein-Grobusch *et al*.,[27] Zheng *et al*.,[13]

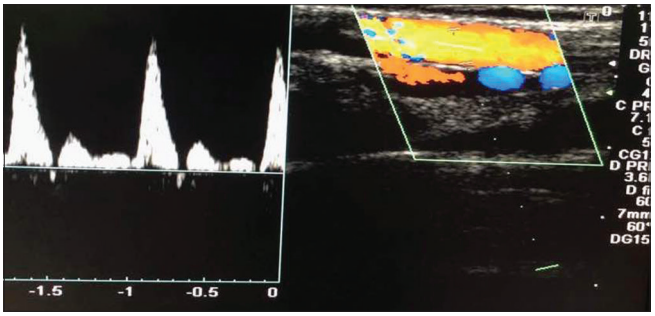

Figure 2: Triplex ultrasound scan of the brachial artery

Table 6: Effect of frequency of donation on flow mediated dilatation, serum ferritin and lipid profile

	2 U/year in the last 3 years (*n*=67)	3 U/year in the last 3 years (*n*=330)	*P*
FMD	13.30±7.02	15.34±6.18	0.024
Serum ferritin (ng/ml)	41.27±18.31	40.16±28.90	0.032
HDL (mmol/l)	1.48±0.42	1.38±0.38	0.325
LDL (mmol/L)	2.02±1.14	2.42±0.97	0.045

FMD – Flow mediated dilatation; HDL – High density lipoprotein; LDL – Low density lipoprotein; U/year – Units of blood per year

Orimadegun *et al*.,[28] which also reported reduced incidence of CVD among their own study population. Studying iron stores of Nigerian blood donors Adediran *et al*.[29] and Usanga[30] also noted a significantly lower ferritin levels in regular blood donors compared with nondonors and healthy controls, respectively; men in the study group had a lower mean serum ferritin (48.57 ± 45.17 ng/mL) than men in the control group (145.49 ± 87.74 ng/mL, $P = 0.00$) and Linpisarn *et al*.[31] in which serum ferritin level was significantly lower in those who donated three times per year compared to the first time donors among the Thai population. These were consistent with the findings of Milman and Kirchhoff[32] Meyers *et al*.,[33] Mackintosh, and Jacob.[34] Contrary to our finding, Akpotuzor *et al*.[35] concluded that there was no observable difference in biochemical iron parameters between regular donors and healthy controls. This was attributed to regional differences in the iron parameters. High anti-oxidant and anti-inflammatory activities of HDL are associated with protection from CVD. Mean HDL levels in the control group were higher (1.48 ± 0.39) than in the study group (1.44 ± 0.41) but not statistically significant. Low HDL levels were also noted in other similar studies by Zheng *et al*.,[13] Fernández-Real *et al*.[22] and Jadrić *et al*.[10] This similar observation was also documented among the regular blood donors in the Nigerian population by Adias *et al*.[23] This low HDL though within normal range might be due to hidden negative lifestyle such as alcohol consumption and cigarette smoking among the study population. However, a similar study in Chennai in the Indian population by Bharadwaj[36] reported higher HDL level in recent regular donor's reason being that the past donors had some amount of negation due to the harmful effect of smoking at least up to 1 year from the date of blood donation.

Low-density lipoprotein (LDL) as an oxidative stress maker is known to play an important role in the pathogenesis of atherosclerosis, and CVD,[23,36] and low level of LDL have also been associated with reduced risk of CVD among the regular blood donors.[37] This study documented a significantly low LDL ($P = 0.000$) in the study group compared with the control group. Several studies have also reported similar findings in their patients[13,22] This was contrary to the outcome of a similar study by Jadrić *et al*.[10] who reported significantly higher LDL in regular blood donors. This is because his control groups were women in the reproductive age group who had lower LDL levels as a result of significant iron loss during menstruation.

Flow-mediated dilation (FMD) in the brachial artery is a noninvasive biomarker of vascular function that has been previously reported to be related to endothelium-dependent vasomotion in the coronary circulation and to be significantly associated with clinical outcomes in CVD populations.[13,20] Findings of enhanced vascular function in association with regular blood donation, suggests that regular blood donation is associated with reduced cardiovascular risk,[4] and this was observed in this study. This finding of enhanced FMD in association with regular blood donation in this study group on the bias of the control group ($P = 0.000$) is consistent with the findings of the similar study of Zheng *et al*.[13] ($P = 0.0003$). Patel *et al*.[38] studied people with diabetes and reported that brachial artery flow-mediated dilation was also greater in high-frequency donors before and during oral glucose tolerance testing. Nishizaka *et al*.[39] in a related study of hyperaldosterone and vascular reactivity noted that FMD was significantly lower in 36 patients with hyperaldosteronism (1.8% ± 1.3% vs. 3.9% ± 1.9%, $P\_=0.0001$) compared with the 44 patients without hyperaldosteronism. Dalli *et al*.[40] in a similar study among men with risk factors of CVDs and men with acute myocardial infarction on the bias of healthy men demonstrated a significantly increased FMD in healthy men (5% ± 2.6% and 7.8% ± 3.1%, respectively $P < 0.0001$). Similarly, The BAFMD was significantly compromised in the group with risk factors as compared with the control group, regardless of sex as seen in the study of Cristiane *et al*.[2] In retrospective data analysis of 68 patients undergoing coronary angiography by Suessenbacher *et al*.,[41] an absolute improvement in FMD 3% appears to be related to a lower risk of future cardiovascular events, whereas a single FMD measurement was not associated with clinical outcome during a mean follow-up period of up to 4 years. Furthermore in a related work by Chan *et al*.,[37] an impairment of FMD is associated with cardiovascular events following the measurement of FMD in 152 patients with CAD ($P = 0.012$.) Modena *et al*.[42] tested the prognostic role of reversible endothelial dysfunction in 400 postmenopausal mild-to-moderate hypertensive women. In 250 (62.5%) patients, FMD had significantly improved to 10% (mean FMD in this group: 13.9% ± 2.6%) after 6 months of treatment, which was associated with fewer events compared to patients with no change in FMD (5.9% vs. 21.3%). The mean FMD value in the group without improvement was 7.1% ± 2.5%. Interestingly, FMD values in both groups are comparable to the values measured in our study population: 13.9% ± 4.3% in regular blood donors (population of improved FMD with less risk of CVDs) and 8.20% ± 2.9% in nondonors (population of increase in the risk of cardiovascular events).

The findings of Modena *et al*.[42] and our findings suggest that FMD may be used to individualize risk factor

management. In variance to the above findings, Fathi *et al*.,[43] did not find an association between FMD and cardiovascular events in a high-risk population. Possible explanations for this discrepancy include differences in the study population and inter-individual variation of FMD. The latter problem might be overcome when serial measurements are performed.

FMD showed a weak inverse correlation with BMI in the study group. This is similar to the work done by Benjamin *et al*.[44] on a clinical correlate of FMD and Framingham heart study which showed FMD% to be inversely related to BMI. It is also consistent with similar studies by Ziccardi *et al*.[45] and Perticone *et al*.[46] associating endothelial dysfunction with obesity. Peña *et al*.,[47] in a similar study found FMD was significantly related to BMI. This was at variance with the study by Nishizaka *et al*.[39] where no association was observed between FMD and BMI. This would probably be due to the fact the study population was more of people that had nontraditional risk factors like low-density lipoproteins.

Other authors have also researched the possibility that body iron stores may be related to factors associated with CVD. Meroño *et al*.[48] analyzed the lipid and lipoprotein metabolism and novel markers of CVD in 20 male patients with iron overload, versus 20 sex- and age-matched healthy controls, as well as their relationship with ferritin concentration and insulin resistance. IO was diagnosed based on: transferrin saturation >45%, ferritin concentration >500 μg L − 1 and homozygosity for HFE gene C282Y or H63D mutations, or increased iron liver stores assessed by semi-quantitative grading in liver biopsies. The main findings of this study included the presence of the so-called atherogenic dyslipidemia in most patients with IO, apart from an increased oxidized LDL concentration and higher (cholesteryl ester transfer protein) and lipoprotein-associated phospholipase A2 (Lp-PLA2) activities, in comparison with age- and sex-matched controls. This is similar to results of Berge *et al*.[49] who evaluated serum ferritin, sex hormones and cardiovascular risk factors in healthy women and found that ferritin significantly correlated inversely with both total cholesterol and LDL.

Blood donation has not been documented to result in the reduction of hemoglobin and packed cell volume (PCV),[13] this would be accounted for by the laboratory standard of not phlebotomizing an anemic subject. The mean Hb and PCV were not significantly different in the control and study group (P = 0.403 and 0.219), respectively, a finding consistent with laboratory criterion of Lagos University Teaching Hospital that excludes anemic patients from the donation. This is similar to the observations of Zheng *et al*.,[13] who found in their study that there was no significant difference in the Hb in the high-frequency blood donors and first-time donors. These were similar to the observation of study by Uche *et al*.,[50] and Szymczyk-Nuzka and Wołowiec *et al*.[51] who reported a normal Hb and PCV in 151 regular male donors who had given over 10 units of whole blood with the frequency of 4–6 units per year. Flesland *et al*., also reported no significant difference in the Hb concentration of regular blood donors and first-time donors.[52] This finding is in contrast to the work of Djalali *et al*.,[53] Jeremiah and Koate,[54] and Okpokam *et al*.,[55] who reported a significantly lower Hb and PCV in regular blood donors when compared with healthy controls. This was attributed to poor economic status of these donors as they donate for monetary gains, poor nutrition status as well as no iron supplement after each donation.[55]

CONCLUSION

The findings in this study support a potential link between blood donation and reduced cardiovascular risk and further suggest that ultrasound assessment of BAFMD may be a useful ideal technique for measuring endothelial function since it is noninvasive, reliable, reproducible, cheap, and easy to perform.

Limitations

The results of the current study are only applicable to a relatively healthy cohort of men without co-existing vascular disease; a population of particular interest with respect to improving primary prevention and risk detection. While both serum ferritin and FMD have been promoted as biomarkers that may be predictive of CVDs risk, the current study could not follow-up the patients to know how many of the study population later developed CVD. Sampling once at 60 s might be also be a limitation.

Recommendations

There is no doubt that a test, which detects silent cardiovascular risk, would allow early intervention by lifestyle change, or medication and primary prevention could start as early as childhood. This is a long run could dramatically reduce the looming socioeconomic burden of CVD on the health system and society at large. Ultrasound BAFMD should be done regularly as a cardio risk marker in our population. Regular blood donation should also be incorporated.

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Conflicts of interest

There are no conflicts of interest.

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