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Sonographic Evaluation of Carotid Intima-Media Thickness and Iron Status of Regular and First-Time Blood Donors in a Tertiary Hospital in South-West Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Regular blood donation which decreases blood iron levels may protect against cardiovascular diseases (CVD). This protection against atherosclerosis can be assessed by Carotid Intima-Media Thickness (CIMT) a biomarker for atherosclerosis. This study sought to determine and compare the mean and maximum CIMT, the serum level of iron and plaque prevalence among regular and first-time blood donors in Lagos University Teaching Hospital (LUTH), Lagos.

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Methods: A comparative, cross-sectional study involving 214 randomly-selected blood donors (107 regular and first-time blood donors each). Using a structured proforma, sociodemographic, anthropometric, radiological and laboratory data were collected from blood donors in the two groups. high frequency linear probe was used to assess CIMT, and presence of plaque in both extra-cranial carotid arteries. CIMT was the distance between the intima–blood interface and the adventitia–media junction and iron study included serum total iron, ferritin, transferrin and total iron binding capacity. Comparison between the study groups was done by Chi-square test and Student t-test/Mann-Whitney U-test for categorical and continuous variables respectively. Level of significance was set at $p<0.05$.

Results: The mean and maximum CIMT of both carotid arteries in regular blood donors (mean right CIMT-0.49+/-0.11mm, maximum right CIMT-0.58+/-0.14mm, mean left CIMT-0.51+/-0.16mm, maximum left CIMT-0.60+/-0.27mm) were significantly($p<0.05$) lower than those of the first-time blood donors(Mean right CIMT-0.79+/-0.17mm, maximum right CIMT-0.93+/-0.25mm, mean left CIMT-0.83+/-0.31mm, maximum left CIMT-0.96+/-0.27mm). Median serum iron(10.5µmol/L; IQR:8.8-13.5µmol/L), ferritin(30.50µg/ml; IQR:16.2-600µg/ml) and transferrin(2.1g/L;IQR:0.9-2.3g/L) in regular blood donors were significantly lower($p<0.05$) than serum iron (16.1µmol/L; IQR:13.7-18.6µmol/L), ferritin(158.0µg/ml; IQR:115-365.2µg/ml) and transferrin(2.3g/L; IQR:2.1-2.4g/L) in first-time donors. Plaque prevalence was significantly higher (χ^2=5.81; p – 0.016) in first-time (11.2%) than regular (2.8%) donors.

Conclusion: Regular blood donation significantly lower CIMT values, and iron parameters. Regular blood donation may offer protection against atherosclerosis.

Keywords: Regular blood donation; serum iron; serum ferritin; carotid intima-media thickness; atherosclerosis.

1. INTRODUCTION

In 1981, Sullivan postulated the first theory that linked reduced blood iron levels and the risk of coronary heart disease.[1] Similar to Sullivan's proposition, other studies have shown that accumulated iron in the blood catalyzes free radical formation which leads to oxidative vessel wall damage [2,3]. Free iron also leads to the oxidative modification of lipoproteins and oxidized lipoproteins are thought to have pro-inflammatory properties which promote the initiation, progression, and destabilization of atherosclerotic plaque [4]. By these two oxidative mechanisms, free iron is believed to contribute to thickness of blood vessels. It is believed that iron is a pro-oxidant which promotes the formation of free radicals [1]. These free radicals, once formed, lead to injury of the arterial endothelium and subsequently atherosclerosis [1,2].

Regular blood donation which decreases blood iron levels is thought to be protective against cardiovascular diseases among blood donors [5]. It has been found that donating blood regularly, reduces endothelial dysfunction and slows down the progression of atherosclerosis [6]. Lowering body iron stores through blood donation may reduce one's risk for cardiovascular events [5,7]. Therefore, the benefits of regular blood donation are not limited to recipients of the donated blood but also extend to those who donated the blood. It has also been discovered that a decreased iron store resulting from the monthly menstrual flow in premenopausal women is associated with decreased risk of myocardial infarction when compared to males of same age and post-menopausal women [8].

High iron load which may be found in non-blood donors, men, and post-menopausal women, thus affects intima layer of arteries. This Intima-media thickness indicates the presence of atherosclerosis and predicts the possibility of future cardiovascular related diseases [9,10]. Increased carotid intima thickness is therefore a sensitive subclinical marker for atherosclerosis and a verified risk predictor of future CVD events like stroke and coronary artery disease [11].

Two major approaches of evaluating endothelial integrity involve the use of invasive and non-invasive techniques. Some examples of noninvasive techniques include carotid ultrasonography, ultrasound flow-mediated dilatation of brachial artery, flow-mediated magnetic resonance imaging and laser doppler flowmetry while the invasive method involves delivering vasoactive agents by intra-arterial infusion with subsequent measurement of the response using strain gauge plethysmography or high-resolution ultrasound imaging [12]. Radiological methods of measuring carotid intima thickness include ultrasonography, multi-detector computed tomography angiography and magnetic resonance imaging. However, while computerized tomography scan and magnetic resonance imaging are expensive, not readily available and require more technical expertise to use and maintain, ultrasonography is non-invasive, fast, reproducible, relatively cheaper, and readily available [12,13]. Carotid ultrasound is a non-invasive diagnostic imaging modality which evaluates the anatomy and function of the carotid artery. It is useful in providing information about intima-media thickness (IMT), diameter of carotid artery, the presence of plaque, blood flow and velocity [10]. The history of using ultrasonography to measure carotid intima thickness dates back to 1984 when Pignoli et al demonstrated a significant association between the histological features of common carotid artery and ultrasound examination findings of CIMT [14].

Atherosclerosis is a chronic progressive disease of arteries whereby areas of damaged endothelium promote the entry of inflammatory cells which initiates the process of plaques formation [12,13,15]. Over time, atherosclerotic lesions can lead to severe narrowing of arteries which can cause distal ischemia for organs supplied by affected arteries [16]. It can also trigger thrombotic occlusion of the major arterial supply to tissues such as the brain, heart, limbs, and other affected organs. It is well recognized as a major cause of cardiovascular diseases globally [16]. Over 80% of cardiovascular death globally occur in low- and middle-income countries [17]. In Nigeria, there are more deaths attributed to CVD than other non-communicable diseases and in south-western Nigeria, cardiovascular diseases are projected to remain the single leading cause of death [17-19].

Prompt availability of blood and blood products for transfusion is crucial for saving lives globally. Life threatening surgical and medical conditions as well as some surgical interventions may require blood transfusion to save the lives of patients. In developing countries, there is still a need to bridge the gap between the continuous demand for blood and supply of blood and its products in health facilities [20]. The shortfall in supply of blood is related to the challenges of getting first time blood donors and maintaining regular donors in blood banks in low- and middle-income countries (LMIC) [21].

Evaluating the common carotid intima media thickness (CIMT) is useful in detecting atherosclerotic changes; thus CIMT is a marker of atherosclerosis and a surrogate marker of vascular disease in the general population [11,22]. A study carried out in Netherlands reported a slight decrease in carotid intima thickness in frequent blood donors and it was concluded that regular blood donation may protect against accelerated atherosclerosis [22]. Furthermore, future cardiovascular events can be predicted by determining the plaque morphology, evaluating the surface of plaques, detecting the presence of plaque ulceration and arterial stenosis [23]. Hence, this study sought to determine and compare the mean and maximum CIMT and the serum level of iron among regular and first-time blood donors in Lagos University Teaching Hospital (LUTH), Lagos. Carotid intima media thickness was defined as the distance between the intima–blood interface and the adventitia–media junction. It further investigated the occurrence of plaque in regular blood donors and first-time blood donors. A plaque was defined as a localized wall thickness which is at least twice the thickness of adjacent IMT [24]. We hypothesized that there is no difference in the mean and maximum CIMT of regular blood donor and first-time blood donors in Lagos University Teaching Hospital (LUTH), Lagos, Nigeria.

2. METHODOLOGY

2.1 Study Area

The study took place in the blood bank of Lagos University Teaching Hospital (LUTH). The blood bank was founded in 1962; currently has a blood donor unit, screening unit, compatibility testing unit and blood fractionation unit. The blood donor unit is very active with about 45-50 units of blood collected on a daily basis, amounting to an average of 12,000 units are collected annually. About 2,000 – 2,500 units are donated by voluntary blood donors and the rest are donated by replacement donors. The donor recruitment unit keeps an electronic register of schools, churches, companies or organizations whose staff or worshippers voluntarily donate to LUTH's blood bank at least twice a year.

2.2 Study Design

This is a comparative cross-sectional study which compared the Carotid intima media thickness and iron parameters of regular returning blood donors and first-time blood donors in the blood bank of LUTH over a period of 8 months (December 2020 – July 2021).

2.3 Study Population

The study population included Regular blood donors in the blood bank in LUTH, who were male and aged 21 – 50 years. Regular blood donors were donors who have donated at least twice annually for the last four consecutive years. The first-time donors were age-matched, male donating blood for the first time in life. Donors with hypertension (defined as systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥ 90mmHg or donor on antihypertensive drugs), history of major bleeding events (including trauma and surgery) within the past two years, Diabetes mellitus (defined as FBS >7.0mmol/L and/or on any blood glucose lowering drugs), previous myocardial infarction, cancer or active chronic inflammatory disease, renal or thyroid disease and individuals on cholesterol lowering drug, and who were using tobacco within 6 months preceding the study were excluded from the study.

2.4 Sample Size Determination

Sample size was calculated using sample size formula for estimating mean difference between 2 groups as shown below [25,26].

$$n = 2 \times \left(\frac{\left(Z_{(\alpha)} + Z_{(1-\beta)}\right) \times S}{(\bar{X}_d - \bar{X}_n)} \right)^2$$

Where n is the minimum sample size required for the study; $Z_{(\alpha)}$ is the statistic that defines the level of confidence desired in the study (1.96 for significance level of 95% confidence interval). $Z_{(1-\beta)}$ defines the power of the study (1.28 for a power of 90%); X_d is mean carotid intima thickness of regular blood donors (0.83mm) [22] while X_n is the mean carotid intima thickness in sub-clinical atherosclerosis (0.9mm) [27]. S is the standard deviation (0.15). A minimum sample size of 97 persons per group was obtained after substitution. A total of 214 persons (107 persons in each group) were involved in the study after adjusting minimum sample size for non-response using a non-response rate of 10%.

2.5 Sampling Technique

Participants in the regular blood donor group were selected using the systematic random sampling technique. Using the blood bank donation register, a list of regular blood donors who satisfy the inclusion criteria was generated. This list served as the sampling frame for the recruitment. One hundred and seven male donors were recruited using a sampling interval of 3. When a selected donor declined to participate in the study, the next donor on the list was recruited for the study and the selection process returned to the initial sequence until the sample size is completed.

Simple random sampling technique was deployed to select first-time blood donors. The average daily attendance of first-time blood donors in the LUTH blood banks is 15 to 20 donors daily. Five computer-generated random numbers (between 1 and 20) were generated every research day using Microsoft Excel software. First time donors with serial numbers corresponding to the computer-generated random number were recruited for the study. When a donor declined participation, next donor arriving (who is not among computer generated numbers) would be recruited as a replacement. This was done every research day until sample size is completed.

2.6 Study Instrument

Study Questionnaire was a structured, self-developed questionnaire with 4 sections. Section 1 explored sociodemographic features, past medical and surgical history, and blood donation history among participants. Section 2, 3 and 4 records results from anthropometric measurements, Radiological findings, and Laboratory investigation, respectively.

2.7 Data Collection

A written informed consent was obtained from regular and first-time blood donors after explaining the objective, procedure, safety and benefit of the research to them. The study questionnaire was administered on all study participants to obtain sociodemographic data and anthropometric measurement before clinical and radiological examination. After radiological examination, blood samples were taken for laboratory examinations. The weight and height of all subjects was measured using a weighing scale and stadiometer respectively. Body mass index was calculated from the subjects as weight in kilograms divided by height in meters squared. Blood pressure was taken from subjects. Two separate blood pressure readings were taken 30 minutes apart and the higher value was taken as the blood pressure. Only subjects with systolic blood pressure below 140mmHg and diastolic blood pressure below 90mmHg were recruited for the study. Laboratory studies were conducted by the hematologists. Iron studies were carried out for both regular and first-time donors. About 10mls of venous blood was drawn from each subject for iron studies. The 10 ml of the blood was transferred to new plain disposable plastic tubes which had screw caps and was thereafter allowed to stand at room temperature until clotted and the clot retracted (about 2 hours). This was centrifuged, and sera separated and transferred to plain cryotubes using a pipette. The serum was aliquoted and stored at −80°C until analysis is carried out. Analysis of serum iron was performed using an auto analyzer.

2.8 Carotid Doppler Ultrasound Examination

A high-resolution grey scale ultrasound scan was done first using a Toshiba Xario 200 TUS-X200 with a linear high frequency (7.2-14MHz) transducer probe. Participants laid in supine position with the head closer to the ultrasound machine. A resting period of at least 2 minutes was observed. Next, the participant's head was rotated to 45^0 in order to tilt the head to the opposite direction of the carotid artery being measured. The head of the participant rested comfortably on the couch while the neck is slightly hyperextended [23,28]. Adequate amount of ultrasound coupling gel was applied on the participant's neck, along sternocleidomastoid muscle. The linear transducer was placed on the participant's neck at the supraclavicular level. The area of interest which is the carotid artery was sought for before proceeding superiorly. A cross sectional view of the carotid artery was done before turning 90^0 for longitudinal view of the artery. The four segments of the carotid artery which include the common carotid artery, the bulb, external and internal carotid arteries were identified. Thereafter, the presence or absence of plaque in the common carotid artery was recorded. The intima-media thickness of the distal common carotid artery (measured 10mm from the carotid bifurcation), carotid bulb and proximal internal carotid artery (10 mm distal to carotid bulb) were measured. The IMT was represented as two parallel echogenic lines giving a double line pattern. (Fig. 1). This represents the interface between the junction of vessel lumen and the intima and the media-adventitia interface [23,28]. Three measurements each of the far wall IMT was taken from the distal common carotid artery, carotid bulb and proximal internal carotid artery on both sides and the average was recorded (Fig. 2). Subclinical atherosclerosis was defined by carotid intima thickness greater than 0.9mm [29].

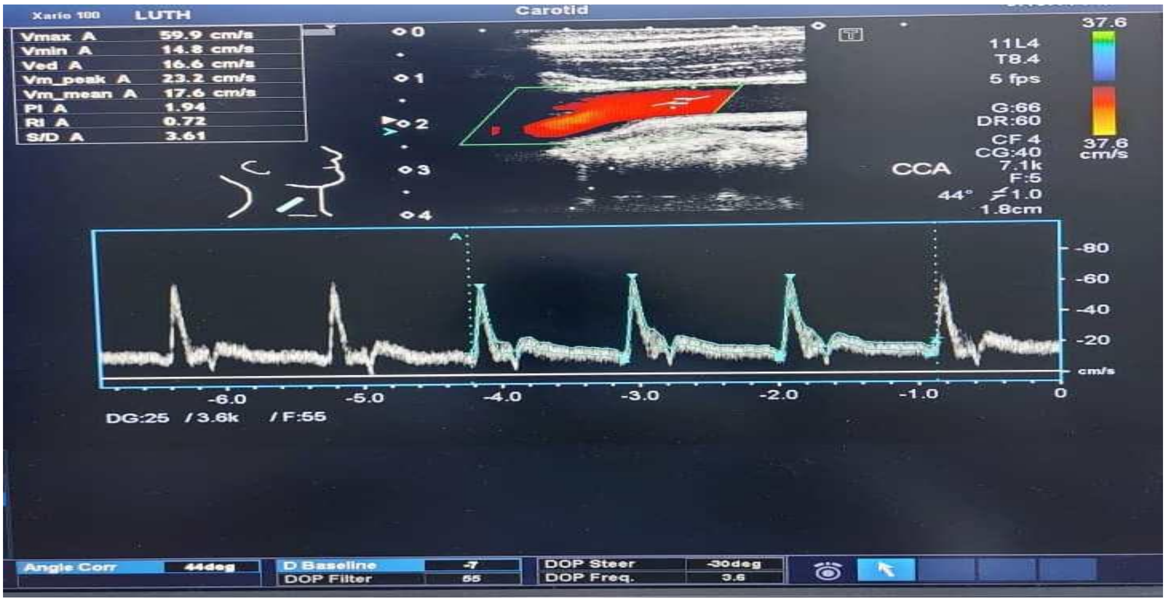

Fig. 1. Probe placement for evaluating the common carotid, external carotid and internal carotid arteries with corresponding B Mode

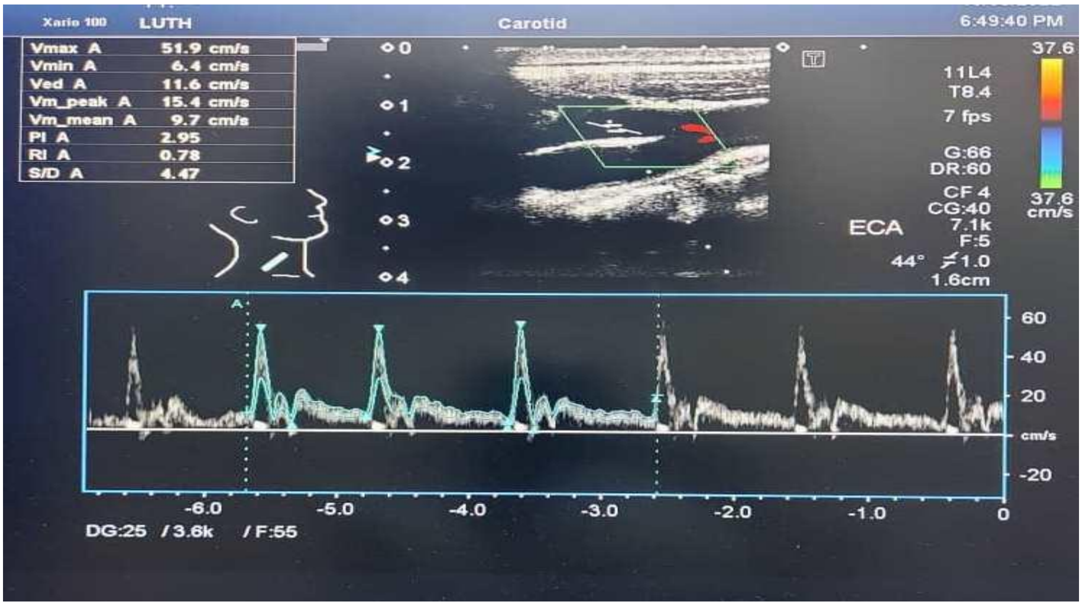

Fig. 2. Probe placement for evaluating the common carotid, external carotid and internal carotid arteries with corresponding Colour doppler patterns

2.9 Data Management

All completed study questionnaire were checked for completeness and correctness after every research day. Data from questionnaire were entered into statistical package for social sciences (SPSS) version 25.0 which was used for data analysis after data cleaning. Categorical variables like educational status, donor status, presence and absence of plaque were analyzed and summarized as frequencies and percentages. Continuous variables like age, weight, height, body mass index, laboratory results (Serum Iron, Ferritin, Transferrin, Transferrin saturation, and Total iron binding capacity), and carotid intima-media thickness (IMT in common carotid-CCA, internal carotid-ICA, external carotid arteries-ECA and the bulb) were tested for normality using the Shapiro-Wilk's test of normality. Continuous variables in the study were summarized using mean and standard deviations when they are normally distributed. However, those continuous variables that were skewed (iron parameters) were summarized using median and interquartile range. The difference between the two study groups (Regular donors and first-time donors) for categorical variables like age groups, donor status, educational status, and BMI categories was investigated using the Chi-square test of proportion. The difference between the intima media thickness in the common carotid artery, internal carotid artery, the bulb, the external carotid artery, mean CIMT and maximum CIMT among regular donors and first-time donors was investigated using a student's t-test because variables they were normally distributed while serum iron parameters were compared using the Mann-Whitney U-test since they were skewed variables. Level of significance was set at p-value <0.05.

2.10 Ethical Consideration

Approval to undertake this study was obtained from the Research Ethics committee of the Lagos University Teaching Hospital (LUTH), Lagos. Participating in this study was voluntary after subjects have gone through the subject information sheet; they were allowed to ask questions about the study and answers were provided clear any doubt. Written consent was obtained from all participants after they showed willingness to participate. This study was conducted in a manner that respected the confidentiality of participants' information.

3. RESULTS

3.1 Sociodemographic Characteristics and Type of Donor Among Participants

3.1.1 Sociodemographic characteristics of returning and first-time blood donors

A total of 214 participants completed the study, 107 Regular/returning donors and 107 first-time donors. The mean age of participants was 31.9years with a standard deviation of 7.4years (Table 1). The mean age of regular donors was 32.0 ± 7.5years, while for first-time donors, it was 31.8 ± 7.4 years with no significant difference (t-test = 0.16;p – 0.876) between the two study groups. (Table 1). One hundred and thirty-four participants (62.6%) attained tertiary level of education, while 80 (37.4%) had only secondary level of education. Educational status was similarly distributed between the 2 groups in the study (χ^2 = 0.08; p – 0.777). The mean height, weight and body mass index of participants were 1.71 ± 0.08m, 71.9 ± 13.9kg and 24.2 ± 4.9kg/m^2, respectively. The mean height (t-test = 1.03; p – 0.305), mean weight (t-test = 0.31; p – 0.760) and mean body mass index (t-test = 0.83; p – 0.409) were not significantly different between the two study groups (Table 1).

3.1.2 Type of donors among returning donors and first-time blood donors

Table 1 also show that slightly above half (51.9%) of the participants were replacement donors, and 103 participants (48.1%) were voluntary donors. Among the regular donor group, majority of participants were voluntary donors (66.4%), while among the first-time donors, majority were replacement donors (70.1%). This observed difference in the donor status between the study groups was statistically significant (χ^2 = 28.34; p – 0.001).

3.1.3 The mean and maximum carotid intima thickness of returning and first-time blood donors

The mean right intima media thickness of the carotid artery (CIMT) of study participants was 0.64 ± 0.21mm, while the mean left CIMT was 0.66 ± 0.30mm (Table 2). Among participants in the regular donor group, the mean right CIMT was 0.49 ± 0.1mm while among first-time donors, it was 0.79 ± 0.17mm showing that the right CIMT is significantly (t-test = 14.34; p – 0.001) higher in first-timer donors than in returning donors (Table 2). The mean left CIMT showed a similar trend, (0.83± 0.31mm Vs 0.51±0.16mm) with left CIMT significantly (p-0.001) higher in the first-time donor than regular donor participants (Table 2).

The maximum right and left CIMT among study participants were 0.76±0.27mm and 0.77±0.31mm respectively. Among regular donors, the maximum right and left CIMT were 0.58±0.14mm and 0.60±0.27mm respectively. The first-timer donor had mean maximum right and left CIMT of 0.93±0.25mm and 0.96±0.27mm respectively, showing a significant difference (p <0.05) between the two study groups (Table 2). Furthermore, Table 2 shows the intima media thickness in the different parts of the right and left carotid artery in the regular and first-timer donor groups. All parameters were significantly higher (p<0.05) in the first-time donors than regular done.

3.1.4 The level of blood iron parameter in returning and first-time blood donors

Table 3 shows that the median serum iron, ferritin, and transferrin in the total study population were 13.3µmol/L (IQR: 10.1 – 17.1µmol/L), 70.0ng/ml (IQR: 29.0 – 152.3ng/ml) and 2.2g/l (IQR: 1.9 – 2.4g/l) respectively. The total iron binding capacity (TIBC) and transferrin saturation (TS) were 56.1 µmol/L (IQR: 48.1 – 59.5 µmol/L) and 26.2% (IQR: 19.3 – 36.6%) respectively.

Among the regular blood donors, serum iron was 10.5µmol/L (IQR: 8.8 – 13.5µmol/L), while in the first-time donors it was 16.1µmol/L (IQR: 13.7 – 18.6µmol/L) with a significant difference shown by Mann-Whitney U test result of 1122.0 and a pValue of 0.001 (Table 3). Median serum ferritin, transferrin, TIBC and TS were significantly greater (p<0.05) in the first-time blood donors than in the returning blood donors.

3.1.5 Presence and number of plaques among returning and first-time blood donors

There were 15 participants (7.0%) who had atherosclerotic plaque along the course of the carotid artery, 3 were in the returning donor group amounting to 2.8% of the group while 12 were in the first-time donor group making up 11.2% of the group of first-time donors. The difference in distribution of atherosclerotic plaque between the study groups was significant ($\chi2$ = 5.81; p – 0.016). The number of plaques found in the participant was also significantly ($\chi2$ = 5.86; p – 0.043) different between the two groups. Nine participants (8.4%), and 3 participants (2.8%) of the first-time donor group had 1 plaque and ≥2 plaques respectively, along the course of the carotid artery, while only 2 participants (1.9%) among the regular donors had 1 plaque (Table 4).

4. DISCUSSION

The Carotid intima media thickness (CIMT) is a key surrogate marker for atherosclerosis and an established strong predictor for cerebrovascular and coronary complications [13]. This study sought to assess and compare CIMT in returning and first-time blood donors and explore the relationship between regular blood donation and carotid intima media thickness as an indicator of early stages of atherosclerosis. The study reported a mean CIMT of 0.64±0.21mm and 0.66±0.30mm in the right and left carotid arteries respectively in the total study participants. This finding is similar to results from studies done by Ajiboye et al, by Umeh et al, and Soneye et al who reported mean CIMT of 0.69±0.21mm, 0.67±0.11mm and 0.65±0.06mm respectively [28-30]. Other studies by Okeahialam et al, Paul et al, and Adaikkappan et al reported higher measures of CIMT, 0.91mm, 0.75mm, and 0.74mm respectively [31-33]. All these findings are from apparently healthy normal individuals in the different studies, which implies CIMT values vary in normal individuals in different population and this thickness may be a reflection of multiple interrelated factors not just subclinical stages of atherosclerosis in normal individuals. This is because CIMT is known to correlate with factors like age, genetic and even environmental factors that promote the development of atherosclerosis [31]. Okeahialam et al suggested that environmental factors contributing to high CIMT among Africans may include prevalent infection and psychological stress which are associated with the increased production of proinflammatory cytokines seen in atherogenesis [31]. Furthermore, Naqvi et al posit that the disparity in CIMT measurement may arise from the lack of uniformity in the methodology in CIMT studies [34]. This calls for standardization of methods/procedures in the sonographic assessment of CIMT and also standardization of instrumentation used in CIMT studies. There also might be a need to determine normal values/thresholds for CIMT in evaluating individuals for atherosclerotic risks using the CIMT thresholds defined for different locality as expressed by Okeahialam et al. [31].

Table 1. Sociodemographic characteristics, anthropometric measures, and donor type among Study participants

Characteristics	Total N = 214 (%)	Study groups		Chi-square	pValue
		Returning donor N = 107 (%)	First time donor N = 107 (%)		
Age Group					
21 – 30 years	113 (52.8)	55 (51.4)	58 (54.2)	0.86	0.652
31 – 40 years	66 (30.8)	32 (29.9)	34 (31.8)		
41 – 50 years	35 (16.4)	20 (18.7)	15 (14.0)		
Mean Age ± SD in years	31.9 ± 7.4	32.0 ± 7.5	31.8 ± 7.4	0.16	0.876
Educational Status					
Secondary	80 (37.4)	41 (38.3)	39 (36.4)	0.08	0.777
Tertiary	134 (62.6)	66 (61.7)	68 (63.6)		
Body Mass Index					
Normal	154 (72.0)	76 (71.0)	78 (72.9)	0.15	0.927
Overweight	28 (13.1)	14 (13.1)	14 (13.1)		
Obese	32 (15.0)	17 (15.9)	15 (14.0)		
Mean height ± SD in m	1.71 ± 0.08	1.72 ± 0.06	1.70 ± 0.09	1.03	0.305
Mean weight ± SD in kg	71.9 ± 13.9	72.3 ± 13.7	71.7 ± 14.1	0.31	0.760
Mean BMI ± SD in Kg/m^2	24.2 ± 4.9	24.5± 4.5	24.0 ± 5.2	0.83	0.409
Donor status					
Voluntary Donor	103 (48.1)	71 (66.4)	32 (29.9)	28.34	0.001*
Replacement Donor	111 (51.9)	36 (33.6)	75 (70.1)		

Table 2. Carotid ultrasound findings (grey scale imaging) among participants in the two study groups

Parameter	Mean ± Standard deviation			Student's t-test (pValue)
	Total	Returning donor	First-time donor	
Intima media thickness in mm				
Right CCA	0.57 ± 0.19	0.45 ± 0.12	0.70 ± 0.18	10.43 (0.001*)
Right ICA	0.60 ± 0.20	0.46 ± 0.12	0.74 ± 0.17	12.09 (0.001*)
Right Bulb	0.74 ± 0.27	0.56 ± 0.15	0.93 ± 0.25	11.24 (0.001*)
Right ECA	0.56 ± 0.21	0.41 ± 0.10	0.73 ± 0.23	4.42 (0.001*)
Mean Right CIMT	0.64 ± 0.21	0.49 ± 0.11	0.79 ± 0.17	14.34 (0.001*)
Max. Right CIMT	0.76 ± 0.27	0.58 ± 0.14	0.93 ± 0.25	12.41 (0.001*)
Left CCA	0.63 ± 0.61	0.47 ± 0.14	0.82 ± 0.97	3.22 (0.002*)
Left ICA	0.64 ± 0.55	0.54 ± 0.85	0.76 ± 0.17	2.12 (0.036*)
Left Bulb	0.85 ± 0.76	0.66 ± 0.65	1.06 ± 0.98	3.00 (0.003*)
Left ECA	0.62 ± 0.20	0.48 ± 0.13	0.79 ± 0.14	5.21 (0.001*)
Mean Left CIMT	0.66 ± 0.30	0.51 ± 0.16	0.83 ± 0.31	9.42 (0.001*)
Max. Left CIMT	0.77 ± 0.31	0.60 ± 0.24	0.96 ± 0.27	9.96 (0.001*)

Table 3. Serum iron parameter and lipid profile among participants

Parameter	Median (IQR)			U test (pValue)
	Total	Returning donor	First-time donor	
Iron study				
Iron (µmol/L)	13.3 (10.1 – 17.1)	10.5 (8.8 – 13.5)	16.1 (13.7 – 18.6)	1122.0 (0.001*)
Ferritin (µg/ml)	70.0 (29.0 – 152.3)	30.5 (16.2 – 60.0)	158.1 (115.0 – 365.2)	336.0 (0.001*)
Transferrin (g/L)	2.2 (1.9 – 2.4)	2.1 (0.9 – 2.3)	2.3 (2.1 – 2.4)	8284.5 (0.001*)
TIBC (µmol/L)	56.1 (48.1 – 59.5)	52.6 (23.8 – 58.3)	57.7 (53.8 – 60.8)	8284.5 (0.001*)
TS (%)	26.2 (19.3 – 36.6)	22.5 (18.2 – 42.2)	27.5 (21.9 – 33.2)	6344.4 (0.007*)

**Statistically significant*

It is important to note, that the Left CIMT is slightly greater than the right CIMT (though not significant) in seemingly healthy normal individual in this study. This was also documented in Ibadan by Soneye et al, in Italy by Guarini, and Sweden by Rosfors [30,35,36]. A possible reason for a thicker left CIMT than the right CIMT maybe because the left carotid artery is a direct branch of the artic arch and it is exposed to higher haemodynamic changes from the aorta as suggested by Wendelhag, Wiklund and Wikstrand [37]. However, Okeahialam et al in Jos reported same mean readings with varying measures of dispersion for right and left CIMT, [31]. which is different from the findings of this study. It is thought that the non-significant difference in the index study and similar findings between the left and right CIMT in the Jos study by Okeahialam may suggest the sufficiency of measuring either the right or left carotid artery instead of both in the risk assessment for cardiovascular and coronary artery diseases.

After disaggregating the study population into regular blood donor and first-time donor it was found that the CIMT of regular blood donor (0.49 ± 0.1mm) in this study was significantly higher than that of first-time donors (0.79 ± 0.17mm). In a similar manner, a significant difference between donors and non-donors was reported by Engberink et al. [22] in Netherlands, although values of CIMT reported by Engberink for donors (0.82mm) and non-donors (0.85mm) were higher than values reported in this study for donors (0.49mm) and non-donors, known as first-time donors in this study (0.79mm) respectively. The possible explanation for the difference in the CIMT reported in the two studies is the age of participants recruited for the two studies. While the study in Netherlands by Engberink et al[22] was conducted among elderly participants who were 60 years and older, the index study was conducted among individuals who were 50 years and below. This buttress the assertion earlier made that varying factors including age may influence CIMT [31]. With age, comes diverse factors like hypertension, Diabetics that may contribute to cardiovascular risk explaining the higher values of CIMT seen in the elderly [22]. Despite the difference in the values reported and the different age groups involved in the two studies, the mean CIMT in regular blood donors was significantly ($p<0.05$) lower than that in non-blood donors (first-time donors) in both studies. This suggests that blood donation may offer some protection against atherosclerosis or its progression among regular blood donors.

Iron study was carried out by assessing serum iron, serum ferritin which is the main body iron store and transferrin, the main iron transporter in the body [9]. The total iron binding capacity (TIBC) which indicates the maximum amount of iron needed to saturate plasma or serum transferrin; and transferrin saturation were also assessed in the study. The values of the body iron store parameters obtained in the returning blood donors were significantly lower than values reported among first time donors. A similar observation was reported by Oboke et al in Lagos [5] and Engberinka et al in Netherland except for total iron which was higher in non-donors in the Netherland study [22]. The serum total iron in the index study was lower than the Dutch study in both the regular (10.5µmol/L Vs 18.5µmol/L) and first-time donors (16.1µmol/L Vs 18.2µmol/L) [22]. However, while serum iron was significantly higher in first-timer donor in the index study, the difference in serum iron in the study in Netherlands was non-significant but slightly higher among regular donors [22]. The contrasting results observed in these two study may be related to the age of participants and environmental differences in the study locations. The index study was among adults less than 50 years in Sub-saharan Africa, while the Netherland study was among the elderly (above 60 years) in Europe [22]. Nonetheless, iron has been known to contribute to the risk of atherosclerosis through its redox potential and the role it plays in endothelial dysfunction [2,4]. Serum Iron exist as Fe^{2+} and Fe^{3+} which are both important for many biochemical reactions, however, electron transfer between the two states also enhance the production of highly reactive oxygen species (ROS) such as peroxyl radicals, alkoxyl radicals, thiyl radicals, sulfonyl radicals, thiyl peroxyl radicals, and disulfides. These free radicals modify lipids, and proteins leading to impaired cell generation and endothelial dysfunction [5,8,10].

Table 4. Presence and number of plaques among participants in the two study groups

Characteristics	Total N = 214 (%)	Study groups		Chi-square	pValue
		Returning donor N = 107 (%)	First time donor N = 107 (%)		
Presence of Plaque					
Yes	15 (7.0)	3 (2.8)	12 (11.2)	5.81	0.016*
No	199 (93.0)	104 (97.2)	95 (88.8)		
Presence of Plaque in Right CCA					
Yes	5 (2.3)	0 (0.0)	5 (4.7)	5.12	0.024*
No	209 (97.7)	107 (100.0)	102 (95.3)		
Presence of Plaque in Left CCA					
Yes	3 (1.4)	1 (.9)	2 (1.9)	0.34	0.561
No	211 (98.6)	106 (99.1)	105 (98.1)		
Presence of Plaque in Right ICA					
Yes	1 (0.5)	0 (0.0)	1 (.9)	1.01	0.316
No	213 (99.5)	107 (100.0)	106 (99.1)		
Presence of Plaque in Right Bulb					
Yes	6 (2.8)	1 (0.9)	5 (4.7)	2.74	0.098
No	208 (97.2)	106 (99.1)	102 (95.3)		
Presence of Plaque in Left Bulb					
Yes	6 (2.8)	2 (1.9)	4 (3.7)	0.69	0.408
No	208 (97.2)	105 (98.1)	103 (96.3)		
Number of Plaque					
No plague	199 (93.0)	104 (97.2)	95 (88.8)	5.86	0.043*
1 plague	11 (5.1)	2 (1.9)	9 (8.4)		
≥ 2 plagues	4 (1.9)	1 (0.9)	3 (2.8)		

**Statistically significant*

Iron is transported, bound to proteins (transferrin), and stored intracellularly within a macro-protein structure, ferritin to ensure the number of free ions that initiate the formation of free radical is kept minimal. The average Transferrin value in this study was 2.2g/L (IQR: 1.9 – 2.4g/L) which was within normal limits of 2.0 – 3.6g/L in both the regular donors and first-time donors. Transferrin saturation, among regular donors was 30.0% from the study by Engberink et al and 22.5% from the index study. Among first-time donors, Engberink et al reported 33.0% and the present study showed 27.5% for Transferrin saturation. Though the study by Engberink et al reported higher values of transferrin saturation than the index study, the two studies were unanimous in reporting a higher value in the first-time groups than the regular donor groups. Ferritin showed a similar trend among regular donors and non-donors but its values showed a high variability between and within studies. The value of Ferritin among regular blood donors was 30.5µg/L, 41.9µg/L and 44.0µg/L; and among first-time donors, it was 158.1µg/L, 61.9µg/L and 124.0µg/L in the index study, a study by Oboke et al and the Engberink et al, respectively [5,22]. This illustrates a high variability in the value of Ferritin involved in the metabolism of iron in the body. Furthermore, the variability of ferritin within each study was further depicted by the skewness in the values of Ferritin reported in the Index and Netherland studies, hence it was presented using median and interquartile range. This variability could be explained by the knowledge that ferritin function as acute phase protein that is produced when acute or chronic inflammation occurs in patients, this is seen even in the presence of iron deficiency [4,38]. Lastly, total iron binding capacity (TIBC) was comparable among the first-time donors in the index (57.7µmol/L) and the Netherlands study (56.0µmol/L), while TIBC in the two studies were different for the regular donor group. The index study reported TIBC of 52.6µmol/L while the Netherland study found TIBC of 63.0µmol/L among regular donors [22]. Overall, the index study, the Lagos study by Oboke et al and the Dutch study by Engberink et al concluded that blood donors have lower iron profile than non-blood donors. Regular blood donors seem to be exposed to less oxidative stress from iron metabolites.

Relationship between iron stores and blood donation have been demonstrated in several studies, after Sullivan's first postulation in 1981 [1]. Sullivan proposed that the lower incidence of cardiovascular diseases in premenopausal women compared to postmenopausal women and men may be attributable to the lower iron store in the premenopausal women implying that iron is a risk factor for cardiovascular diseases [1]. Salonen et al and Meyers et al in 1998 and 2002 respectively reported that lowering iron stores by regular blood donation resulted in a significant decrease in cardiovascular risk [39,40]. Earlier in 1992, Salonen et al in a prospective randomized study, reported a higher likelihood of myocardial infarction in participants with Ferritin concentration greater than 200µg/L than in those with Ferritin concentration less than 200µg/L further supporting the link between iron stores and atherosclerosis [41]. Studies in diseases of iron overload like β-thalassemia major, also suggest a relationship between iron load and atherosclerosis reflected by increased CIMT [42-45].

The index study in the assessment of atherosclerosis risk further evaluated the presence of plaque in the carotid arteries of regular blood donors and first-time donors. The study reported a prevalence of 7.0% for plaque formation among participants, this was lower than the global average reported among persons aged between 30 and 79 years in 2020 [46]. The range of age under consideration in the global report may have accounted for the difference in prevalence of plaque. Nevertheless, plaque formation was significantly higher among first-time blood donor (11.2%) than regular donors (2.8%) in the index study. The plaque assessment in this study was in line with the new American Society of Echocardiography's recommendation while assessing for subclinical atherosclerosis [47]. CIMT evaluation combined with plaque assessment increases predictive ability of CIMT examination in risk evaluation especially for a first atherosclerotic cardiovascular disease (ASCVD) event [34,47]. This became imperative when the American College of Cardiology/American Heart Association (ACC/AHA) designated the carotid IMT as class III evidence level in assessing atherosclerotic cardiovascular disease not recommended for use in clinical practice for routine measurement risk [47]. Different parts of the CIMT have been found to predict different aetiologies of atherosclerosis, while intima thickness (IT) is useful in changes due to aging,[47-50] medial thickness is compactible with medial hypertrophy seen in hypertension [47,51,52]. CIMT and carotid plaque reporting is more accurate with prediction and prognosticating for several cardiovascular diseases [10,34,47].

5. CONCLUSION

This study found CIMT, a known predictor of atherosclerosis, was significantly lower among regular blood donor than first-time donors. All body iron status parameters were also significantly higher among first-time donors than regular donors. Furthermore, plaque prevalence was significantly higher among first-time donor than regular donors. The foregoing may suggest higher risk of atherosclerosis among first-time donor and a level of protection conferred by regular blood donation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT AND ETHICAL APPROVAL

Consent and Ethical approval are very important in this study. Ethical Approval was obtained from the Medical Research Ethics Committee of Lagos University Teaching Hospital before commencement of the study. A written informed Consent was obtained from each participants before recruiting them into the these a study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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