

## Age–Gender Specific Reference Values for Iron Indices Among Apparently Healthy Population in Abeokuta, South Western Nigeria.

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### ABSTRACT

**Introduction:** The knowledge of laboratory reference values is necessary because most available reference values for laboratory tests are outdated or adopted from the manufacturers of the diagnostic test derived from the populations living in Europe and United States.

**Objectives:** The study is aimed at determining the age-gender specific reference values for iron indices among apparently healthy individuals in Abeokuta, South-Western Nigeria.

**Methods:** A total of 385 subjects which comprised of 180 (46.75%) males and 205 (53.25%) females were enrolled in this study of within aged 18 and 77 years. The subjects for this study were recruited after the informed consent was obtained from every participant. Data were also collected with semi-structured self-administered questionnaire based on World Health Organization guidelines for students' substance use survey. The data generated were expressed as Median and 95 percentile (2.5 and 97.5 percentile distribution) and were used to construct the reference ranges. The study reference values were compared with the standard reference values using the one Wilcoxin Signed Ranked Test.

**Result:** The majority (80.78%) of these participants have tertiary education at the time of enrollment. Most of the subjects (88.05%) were Yoruba ethnicity, typically Abeokuta residents. The results showed that all the determined study reference values were significantly different from the standardized reference ranges ( $P < 0.01$ ).

**Conclusion:** The study showed significantly lower values of haemoglobin, red cell count, MCHC, MCH, MCV, haematocrit serum iron, TIBC, and significantly higher values of RDW and ferritin compared with the standardized reference values from the Caucasians. It is recommended to use this established reference values in Abeokuta rather than Caucasians values in order to prevent the wrong diagnosis of iron related diseases.

**Keywords:** Abeokuta, age-gender, iron indices, Reference values.

**Running Title:** Age–Gender Specific Reference Values for Iron Indices

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## INTRODUCTION

Iron is an essential micronutrient required for virtually every aspect of normal cell function [1]. Iron as a component of haemoglobin in the erythrocytes (red blood cells), is required for transporting oxygen around the body, in form ferritin and haemosiderrin, as a storage pool and myoglobin, for use of oxygen in the muscles and tissue [2]. The iron in the haem complexes of haemoglobin and myoglobin is stabilized in the ferrous state while the interaction with the adjacent globin protein enables it to bind reversibly to oxygen [3].

Body iron content is approximately 4.0g and 3.5g in men and women, respectively. In adults, most body iron is present in haemoglobin of circulating erythrocytes (60-70%) for oxygen transport and in muscle myoglobin (10%). The remaining body iron (20-30%) is found primarily in storage pools located in the liver and reticuloendothelial (macrophage) system as ferritin and haemosiderin (which is a degraded form of ferritin). Only about 1% of body iron is incorporated in the range of iron-containing enzymes and less than 0.2% of body iron is in the plasma transport pool where most of it is bound to transferrin [4].

Reference ranges for iron indices are essential for effectively diagnosing diseases, assessing and monitoring iron related disorders. Most available reference intervals for laboratory tests are outdated or adopted from the manufacturers of the diagnostic tests [5].

However, the common practice in Nigeria, is the use of reference ranges derived from populations living in Europe or United States [6], which may have been contributing to the misdiagnoses of iron-related problems or diseases in the country.

Health and disease can only be distinguished by accurate and reliable reference range of a particular test. The establishment of

reference ranges in this study will guide the physicians in the interpretation of iron-related laboratory investigations as against the use of Caucasian reference values and thereby improve the diagnoses of iron-related diseases and the clinical management of the patients in Abeokuta, South Western Nigeria. The Dietary Reference Values (DRVs) for iron intake estimate the amount of dietary iron that needs to be consumed to meet the systemic physiological needs for iron. There is a lack of good-quality data on body losses, systemic iron stores, the efficiency of iron uptake, and measuring the body's adaptation and functional use of iron to intakes to enable good estimations as seen in Table 1.

Iron indices are important health indicators widely used in clinical practice and therefore, accurate and appropriate reference ranges are crucial for the interpretation of the normalcy of results [7].

Body iron content is approximately 4.0g and 3.5g in men and women respectively. In adults, most body iron is present in haemoglobin (60-70%) in circulating erythrocytes where it is essential for oxygen transport and in muscle myoglobin (10%). The remaining body iron (20-30%) is found primarily in storage pools located in the liver and reticulo-endothelial (macrophage) system as ferritin and haemosiderin [8]. It is important to note that there are no single ideal biological measure for iron status; nevertheless, red cell parameters, serum ferritin, serum iron, TIBC and transferrin saturation or combination of these parameters were frequently used for biological iron indices [9].

In recent years, extensive studies on apparently healthy population have worked out normal ranges for iron indices in adult. From the study carried out in Nigeria by Olawumi *et al.* [10] on reference values of haematological parameters for healthy adult in Ilorin, North Central Zone of Nigeria with

a descriptive cross sectional study, the iron indices differ from one individual to another due to several factors. The red blood cell count, haemoglobin concentration, PCV and MCHC were significantly higher among males than females. There was however no significant gender difference in the values of MCV and MCH. It was concluded that the normal reference value obtained in this study was notable different from those that are currently used in the hospitals in that locality. The study of Akpotuzor *et al.* [11] revealed a high degree of variability within adults serum iron, total iron-binding capacity and transferrin saturation fraction whether in rural or urban areas. The researchers observed that the adult males had higher serum iron levels, slightly lower total iron-binding capacities and higher transferrin saturation fraction levels than females. However, age showed no significant influence on these iron parameters in adult life for both genders.

According to Zacharski *et al.* [12] that observed different patterns of iron accumulation based on age, gender, and race. Serum ferritin levels reflected difference grades which are population-based in body iron stores, but the percentage of transferrin saturation does not always in most occasions. However, the study carried out in Malaysia by Hassan *et al.* [13] observed a positive association between Serum Ferritin and MCV but the correlation was not significant between Haemoglobin and Serum ferritin in Africa–American patients.

Fleming *et al.* [14] showed overall prevalence of high iron stores of 13.9% and 12.2% for male and female respectively, using a serum ferritin of  $>300\mu\text{g/L}$  in males and  $>200\mu\text{g/L}$  in females. However, Dosoo *et al.* [15] found significantly higher levels of serum Iron in Males compared to Females.

Diet has a greater influence on iron indices among apparently healthy individuals. Based

on the study carried out in Sweden by Hallberg *et al.* [16], the dietary reference values for healthy adults differ according to age and gender. The Reference Nutrient Intake (RNI) of  $8.7\mu\text{mol/L}$  and  $14.0\mu\text{mol/L}$  are expected for male and female respectively at the age interval of 19-50 years. However, the reference nutrient intake for individual above 50 years in respective of gender is  $8.7\mu\text{mol/L}$ . However, currently available reference values for iron indices in standard literature are often met with difficulty in interpretation of iron status among populations in developing countries [18]. This study is to determine the age-and gender-specific reference values for iron indices (red blood cell count, mean cell haemoglobin concentration, mean cell volume, haematocrit, red cell distribution width, serum ferritin, serum iron and total iron binding capacity) among apparently healthy individuals in Abeokuta, Southwestern, Nigeria. Health and disease can only be distinguished by accurate and reliable reference range of a particular test. This study was imminent because the knowledge of the reference ranges established through this study will guide the physicians in the interpretation of iron-related laboratory investigations as against the use of Caucasian reference values and thereby improve the diagnoses of iron-related diseases and the clinical management of the patients in Abeokuta, South Western Nigeria.

## MATERIALS AND METHODS

### THE STUDY AREA

The research was carried out in Abeokuta, Ogun State, Nigeria. Abeokuta is the largest city and State capital of Ogun State in South-West Nigeria [45]. It is situated on the east bank of the Ogun River, near a group of rocky outcrops in a wooded savanna, and 77 km north of Lagos by railway, or 130 km by

water. As of 2018, Abeokuta and the surrounding area had a population of 624,700. The geolocation is North and East hemisphere. The temperature is 78.6°F or 25.9°C. It has a latitude of 79°0.000'N and longitude of 32°0.000'E with altitude of 67m.

### **STUDY POPULATION**

This was a cross sectional study of 385 apparently healthy adult population of Abeokuta, Ogun State, South-Western, Nigeria, which included 180 males and 205 females in the age range of 18 and 77 years. Informed consent form was filled by each of the participants. Inclusion criteria for subjects selection are the non-pregnant and apparently healthy males and females resident in Abeokuta, Ogun State; including apparently healthy individuals that were not on haematinics or iron therapy, and non-menstruating women that agreed to the informed consent. Non-consenting apparently healthy individuals, pregnant women, individuals on treatment with iron tablets, patients with bleeding disorders, and patients with sickle cell diseases or haemoglobinopathies were excluded from subjects selection. Ethical clearance was obtained from the Department of Research and planning in Ogun State Ministry of Health, Abeokuta, Nigeria for the study. Structured questionnaires were administered to the prospective subjects and information, such as age, gender, marital status, educational status, ethnic group, occupation and medications were obtained from them.

### **SAMPLE COLLECTION**

#### **Laboratory Procedure**

About 6 ml of whole blood was collected from each participant and out of which 2ml was put into EDTA anticoagulated tube while the remaining 4 ml was put in a plain container. The EDTA anticoagulated blood

was used for red cell parameters (Haemoglobin, Mean Cell Volume, Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentration, Red Cell Distribution Width and Red Blood Cell count). The red cell parameters were analyzed using Swelab Alfa 3 – part fully automated haematology analyzer. However, the sample collected into the plain tube was allowed to clot and the serum obtained. The serum was tested for serum iron and total iron binding capacity using colorimetric method while serum ferritin concentration was determined using a human ferritin enzyme immunoassay kit- ACCU Bind ELISA Microwell ferritin kit [46].

#### **Red Cell Indices**

Swelab Alfa 3-part haematology analyzer (serial No: 4711, software version: 2.7.5) was used for the estimation of Hb, MCV, MCH, MCHC, RDW and RBC count.

#### **Procedure**

The following steps were used when analyzing the red cell parameters

- (1) The reagent needed was checked for the number of samples to be processed.
- (2) The power switch was on at the left side of the unit. Self-check, auto rinse and background check was automatically performed and the Ready (ready for analysis) appeared.
- (3) Quality control blood materials (low, normal and high) were performed so as to verify that the instrument was performing within the specified ranges.
- (4) If the result of the quality control falls within acceptable ranges, input the blood samples.
- (5) Every sample was identified and the sample introduced into the system for analysis.

### Estimation of Serum Ferritin

Serum ferritin was determined using the product code 2825300 – Ferritin (FTL) Human ELISA Kit manufactured by Monobind Inc. Lake Forest, CA 92630, U.S.A.

### Procedure

Approximately, 0.025 millilitre (25µl) of the appropriate serum reference, control, and specimen were pipetted into the assigned wells. 0.100 millilitre of the ferritin biotin reagent was added to each well. The microplate were gently swirled for 30 second to mix and cover. They were incubated for 30 minutes at room temperature. The content of each microplate was discarded by decantation. 350 µl of wash buffer was added to each well, aspirated and repeated 2 times. 0.100 millilitre (100µl) of the ferritin enzyme conjugate was added to each well. It was incubated for 30 minutes at room temperature. The content of the microplate was discarded by decanting and blotting the plate dry with absorbent paper. 350 µL of wash buffer was added and decanted. This was repeated twice. It was incubated for 15 minutes at room temperature. 0.050 millilitre of the stop solution was added to each well and mixed gently. The absorbance in each well was read at 450nm in the microplate reader.

### Estimation of Serum Iron

Serum Iron was determined using Iron Ferene fluid 5+1 test (Order No. IF01000120) by colorimetric method manufactured by Centronic GmbH Am Kleinfield 11, 85456 Wartenberg/Germany.

### Procedure

Approximately, 1.0 millilitre of reagent was pipetted into each of the 3 test tubes labelled blank, standard and test. 0.05 millilitre of sample was added into the test tube labelled test. 0.05 millilitre of standard reagent was added into the test tube labelled standard.

0.05millilitre of distilled water added into the test tube labelled blank. The tubes were mixed and incubated at room temperature for 10 minutes. The absorbance of the standard (Ac) and samples (Ax) against reagent blank was read.

The result was calculated as

$$\text{Serum iron } (\mu\text{mol/L}) = \frac{\text{Absorbance of sample} \times 30}{\text{Absorbance of standard}}$$

### Estimation of Total Iron Binding Capacity (TIBC)

Serum Total Iron Binding Capacity was determined using colorimetric method (Order No: IF08000050 manufactured by Centronic GmbH Am Kleinfield 11, 85456 Wartenberg/Germany).

### Procedure

Approximately, 500µL of Reagent 1 (FeCl<sub>3</sub> – 89.5mmol) was added into a vial. 250µL of the sample was added to reagent 1. The reagent 1 and the sample were mixed and allowed to stay for 5-30minutes at 25°C. 1 spatula of reagent 2 (MgCO<sub>3</sub>) was added and allowed to stay for additional 30 -60minutes. The mixture was shake within this period occasionally for about 5 times. The mixture was centrifuged for 10 minutes at 4000U/min. The clear supernatant was then used as for Iron determination.

### Calculation:

$$\begin{aligned} \text{Serum TIBC } (\mu\text{g/dl}) &= \text{Change in Absorbance} \times 3183 \\ \text{Serum TIBC } (\mu\text{mol/l}) &= \text{Change in Absorbance} \times 570 \end{aligned}$$

### STATISTICAL ANALYSIS

The data generated were expressed as Median and 95 percentile (2.5 and 97.5 percentile distribution) were used to construct reference ranges. The study

reference values were compared with the standard reference values using the one Wilcoxon Signed Ranked Test. Age specific reference values were displaced as box plots using Graph pad Prism version 5.0. Data were considered significant at  $P < 0.05$ .

## RESULTS

### Demographic Details of Enrolled Subjects

A total of 385 subjects which comprised 180 (46.75%) males and 205 (53.25%) females were enrolled in this study. Subjects were aged between 18 and 77 years. The majority (80.78%) of these participants had tertiary education at the time of enrolment. 40.78% were students, 39.22% were skilled while 20.00% were unskilled. Most of our subjects (88.05%) were of Yoruba extraction, typical of Abeokuta residents. Other details are cited in Table 2.

### Reference Values of Iron Indices among Apparently Healthy Male Subjects in Abeokuta

The reference values of iron indices among apparently healthy male subjects in Abeokuta were determined and compared with that of standard reference values obtained from Dacie and Lewis [17]. Results from Table 3 shows that all the determined study reference values were significantly different from that

of the standardized reference ranges ( $p < 0.01$ ). Specifically, the haemoglobin concentration, red blood cell count, mean cell haemoglobin, mean cell haemoglobin concentration, mean cell volume, haematocrit, serum iron, and total iron binding capacity showed significantly lower median values than the median of the standardized reference values while red cell distribution width (Study *Mdn*: 14.60%, Standard *Mdn*: 12.80%,  $p = 0.00$ ) and ferritin (Study *Mdn*: 171.20 ng/ml, Standard *Mdn*: 118.00 ng/ml,  $p = 0.00$ ) were observed to be significantly higher than that of the standard value. Other details are cited in Table 3.

### Reference Values of Iron Indices among Apparently Healthy Female Subjects in Abeokuta

The determined reference values for the female subjects were significantly lower than of the standardized values. Hb, RBC count, MCH, MCHC, MCV, haematocrit, serum iron and TIBC ( $p < 0.01$ ), while red cell distribution width (Study *Mdn*: 14.90%, Standard *Mdn*: 12.80%,  $p = 0.00$ ) and ferritin (Study *Mdn*: 70.00 ng/ml, Standard *Mdn*: 67.00 ng/ml,  $p = 0.00$ ) were significantly higher than that of standardized reference ranges ( $P < 0.00$ ). Other details are cited in Table 4.

**Table 1: Dietary reference values for iron-mg/day ( $\mu\text{mol}/\text{day}$ ) (Adapted from Hallberg *et al.*, 1995).**

Age	Lower reference nutrient intake (LRN)	Estimated average requirement (EAR)	Reference nutrient intake (RNI)
0-3 months	0.9 (15)	1.3 (20)	1.7 (3)
4 – 6 months	2.3 (40)	3.3 (60)	4.3 (80)
7 – 9 months	4.2 (75)	6.0 (110)	7.8 (140)
10-12 months	4.2 (75)	6.0 (110)	7.8 (140)
1-3 years	3.7 (65)	5.3 (95)	6.9 (120)
4-6 years	3.3 (60)	4.7 (80)	6.1 (110)
7-10 years	4.7 (80)	6.7 (120)	8.7 (160)
11-14 years (male)	6.1 (110)	8.7 (160)	11.3 (200)
c11-14years (Female)	8.0 (140)	11.4 (200)	14.8 (260)
15-18 years (male)	6.1 (110)	8.7 (160)	11.3 (200)
15-18years (Female)	8.0 (140)	11.4 (200)	14.8 (260)
19-50 years (males)	4.7 (80)	6.7 (120)	8.7 (160)
19-50years (females)	8.0 (140)	11.4 (200)	14.8 (260)
50 + years	4.7 (80)	6.7 (120)	8.7 (160)

1 $\mu\text{mol}$  = 55.9  $\mu\text{g}$

**Table 2: Demographic Details of Enrolled Subjects**

Parameter	Frequency	Percentage (%)
Total No. of subjects	385	100.00
• Male	180	46.75
• Female	205	53.25
Age Range (years)	18 – 77	
Educational Status		
• Primary	8	4.10
• Secondary	66	34.10
• Tertiary	311	80.78
Occupation		
• Student	157	40.78
• Skilled	151	39.22
• Unskilled	77	20.00
Ethnicity		
• Yoruba	339	88.05
• Hausa	19	4.94
• Igbo	8	2.08
• Others*	19	4.93

\*Others represent Nigerian ethnic groups such as Ijaw, Fulani and Ishekiri

**Table 3: Reference Values for Iron Indices among Apparently Healthy Male Subjects in Abeokuta**

Parameter	Reference Value [ <i>Mdn</i> (p2.5 – p97.5)]		<i>p</i> -value
	This Study n = 180	Standard <sup>a</sup>	
Hb (g/dl)	12.30 (10.30 – 14.80)	14.50 (11.00 – 18.00)	.00**
RBC ( $\times 10^9/l$ )	4.75 (3.61 – 6.71)	5.00 (4.00 – 6.00)	.00**
MCH (pg)	26.70 (22.30 – 32.60)	29.50 (24.50 – 34.50)	.00**
MCHC (g/dl)	31.40 (28.00 – 53.36)	33.00 (30.00 – 36.00)	.00**
MCV (fl)	82.30 (66.81 – 112.10)	92.00 (74.00 – 110.00)	.00**
Haematocrit (%)	41.30 (34.00 – 49.03)	45.00 (35.00 – 55.00)	.00**
RDW (%)	14.60 (13.15 – 16.40)	12.80 (10.40 – 15.20)	.00**
Ferritin (ng/ml)	171.20 (21.01 – 365.50)	118.00 (16.00 – 220.00)	.00**
Serum Iron ( $\mu\text{mol/l}$ )	17.00 (5.11 – 33.00)	19.50 (11.00 – 28.00)	.00**
TIBC ( $\mu\text{mol/l}$ )	39.80 (22.71 – 74.00)	58.20 (44.80 – 71.60)	.00**

Key: *Mdn* = median, p2.5 = 2.5<sup>th</sup> percentile, p97.5 = 97.5<sup>th</sup> percentile, n = total number, *p*-value = error probability, Hb = haemoglobin, RBC = red blood cell, MCH = mean cell haemoglobin, MCHC = mean cell haemoglobin concentration, MCV = mean cell volume, RDW = red cell distribution width, TIBC = total iron binding capacity, \*\*Significant difference observed using one sample Wilcoxon signed ranked test,  $p < .01$ , <sup>a</sup>Standard reference values of all parameters were obtained from Bates and Lewis (2011).

**Table 4: Reference Values for Iron Parameters among Apparently Healthy Female Subjects**

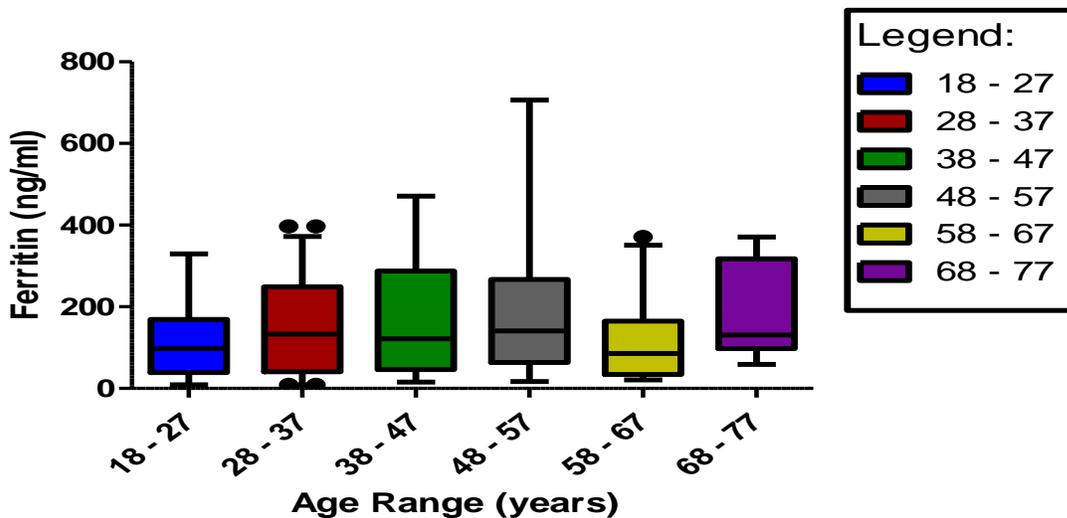
Parameter	Reference Value [ <i>Mdn</i> (p2.5 – p97.5)]		<i>p</i> -value
	This Study n = 205	Standard <sup>a</sup>	
Hb (g/dl)	11.00 (9.20 – 14.50)	13.00 (10.50 – 15.50)	.00**
RBC ( $\times 10^9/l$ )	4.14 (3.09 – 7.20)	4.30 (3.30 – 5.30)	.00**
MCH (pg)	26.60 (23.10 – 34.50)	29.50 (24.50 – 34.50)	.00**
MCHC (g/dl)	31.30 (28.00 – 34.60)	33.00 (30.00 – 36.00)	.00**
MCV (fl)	85.50 (72.50 – 107.70)	92.00 (34.00 – 110.00)	.00**
Haematocrit (%)	36.60 (31.00 – 44.80)	41.00 (31.00 – 51.00)	.00**
RDW (%)	14.90 (13.00 – 22.90)	12.80 (10.40 – 15.20)	.00**
Ferritin (ng/ml)	70.00 (9.200 – 266.70)	67.00 (10.00 – 124.00)	.00**
Serum Iron ( $\mu\text{mol/l}$ )	15.00 (6.00 – 31.80)	16.30 (6.60 – 26.00)	.00**
TIBC ( $\mu\text{mol/l}$ )	35.00 (18.90 – 79.60)	58.20 (44.80 – 71.60)	.00**

Key: *Mdn* = median, p2.5 = 2.5<sup>th</sup> percentile, p97.5 = 97.5<sup>th</sup> percentile, n = total number, *p*-value = error probability, Hb = haemoglobin, RBC = red blood cell, MCH = mean cell haemoglobin, MCHC = mean cell haemoglobin concentration, MCV = mean cell volume, RDW = red cell distribution width, TIBC = total iron binding capacity, \*\*Significant difference observed using one sample Wilcoxon signed ranked test,  $p < .01$ , <sup>a</sup>Standard reference values of all parameters were obtained from Bates and Lewis (2011).

**Comparison of Ferritin, Serum Iron, and Total Iron Binding Capacity Concentration among Apparently Healthy Subjects of various Age Groups in Abeokuta.**

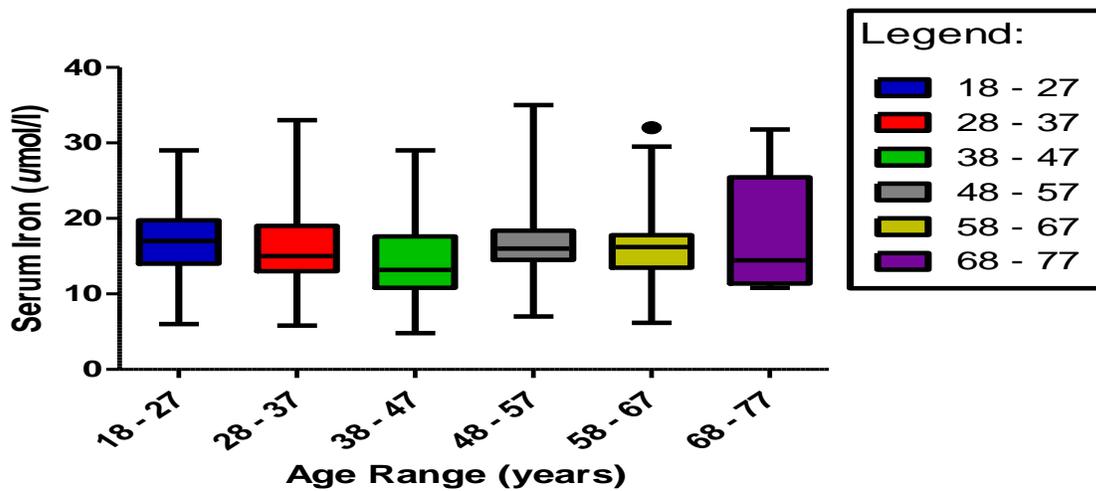
Figures 1- 3 compare the ferritin, serum iron, and total iron binding capacity (TIBC) concentrations among apparently healthy subjects of various age groups in Abeokuta. Ferritin concentrations were observed to rise from 18 years (n = 64, *Mdn* = 97.80 ng/ml), peaking at the 48 to 57 years age group. The lowest ferritin value was observed in the 58 to 67 years age group (Figure 1). The median

serum iron concentration trended with a progressive drop from 18 years to 47 years. This value elevated at the 48 to 57 years age group and finally declined at the 68 to 77 years group (Table 2). TIBC also showed a final and sharper decline at 68 to 77 years age group (Figure 3). However, the highest median TIBC values were observed in the 38 to 47 years group.



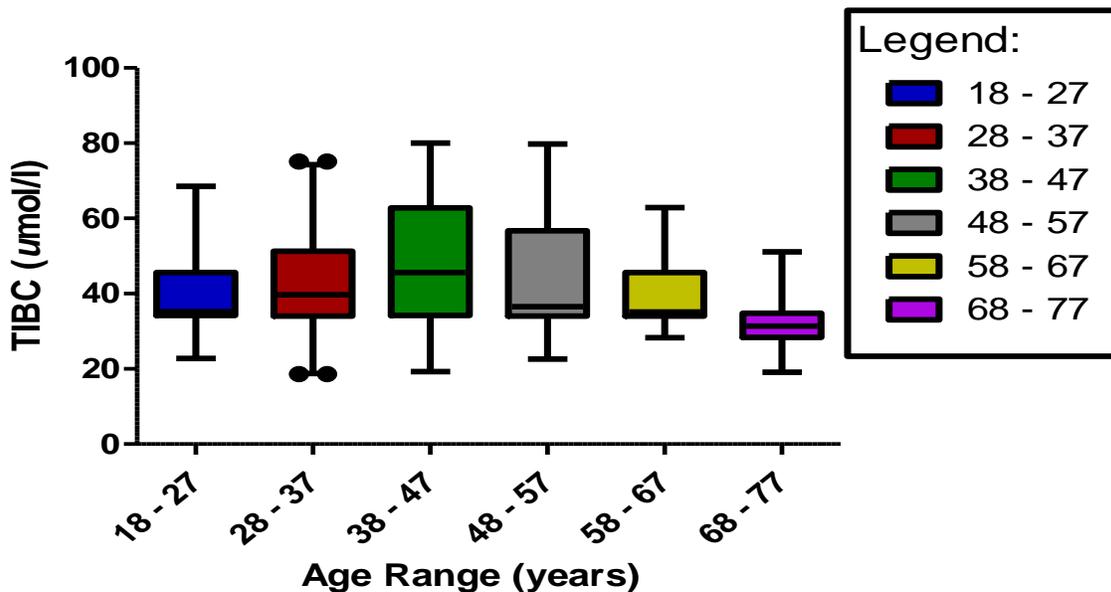
**Figure 1: Comparison of Ferritin Concentration among Subjects of various Age Groups in Abeokuta.**

*Key:* The outer lines of the boxes represent inter-quartile ranges, the middle line represents the median values, the whiskers on either ends of the boxes represent the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles, respectively. The black dots represent outliers. Group specifics: 18 – 27 years (n = 64, *Mdn* = 97.80 ng/ml), 28 – 37 years (n = 110, *Mdn* = 133.20 ng/ml), 38 – 47 years (n = 73, *Mdn* = 121.60 ng/ml), 48 – 57 years (n = 72, *Mdn* = 140.95 ng/ml), 58 – 67 (n = 50, *Mdn* = 85.70 ng/ml), 68 – 77 (n = 16, *Mdn* = 131.30 ng/ml)



**Figure 2: Comparison of Serum Iron Concentration among Subjects of various Age Groups in Abeokuta.**

*Key:* The outer lines of the boxes represent inter-quartile ranges, the middle line represents the median values, the whiskers on either ends of the boxes represent the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles, respectively. The black dots represent outliers. Group specifics: 18 – 27 years (n = 64, *Mdn* = 17.00 µmol/l), 28 – 37 years (n = 110, *Mdn* = 15.00 µmol/l), 38 – 47 years (n = 73, *Mdn* = 13.20 µmol/l), 48 – 57 years (n = 72, *Mdn* = 16.00 µmol/l), 58 – 67 (n = 50, *Mdn* = 16.20 µmol/l), 68 – 77 (n = 16, *Mdn* = 14.45 µmol/l)



**Figure 3: Comparison of Serum Total Iron Binding Capacity (TIBC) Concentration among Subjects of various Age Groups in Abeokuta.**

*Key:* The outer lines of the boxes represent inter-quartile ranges, the middle line represents the median values, the whiskers on either ends of the boxes represent the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles, respectively. The black dots represent outliers. Group specifics: 18 – 27 years (n = 64, *Mdn* = 35.20 µmol/l), 28 – 37 years (n = 110, *Mdn* = 39.70 µmol/l), 38 – 47 years (n = 73, *Mdn* = 45.60 µmol/l), 48 – 57 years (n = 72, *Mdn* = 36.50 µmol/l), 58 – 67 (n = 50, *Mdn* = 35.10 µmol/l), 68 – 77 (n = 16, *Mdn* = 31.35 µmol/l)

## DISCUSSION

Three hundred and eighty-five (385) subjects were enrolled in this study and comprising 180 (46.75%) males, and 205 (53.25%) females. The subjects were predominantly of Yoruba ethnicity (88.05%), with 80.78% of them having tertiary education. This is an indication that they were likely aware of the importance of a balanced diet that is nutritionally rich in bioavailable iron. It is no wonder that the reference value of ferritin concentration of our subjects were significantly higher than that obtained from standardized literature (116.00–220.0 ng/ml) [19] (Table 3).

It is of interest to document also that the values obtained from this study were on most occasions lower than the standardized values [20, 21]. The median values of most indices of this study population tended to be lower compared to the standardized values [19]. Similar observations have been made on most red cell parameters in studies carried out in other African countries [22, 23]. These lower values for areas in sub-saharan Africa have been attributed to factors such as poor dietary iron intake and/or parasitic infections such as malaria, worm infestations in our population and this needs to be further addressed [24, 25]. Also, the difference could be due to environmental or genetic factors or combination of both or several other factors [23].

Cellular exposure to chemicals or its metabolite may cause a loss or requisite functional iron from intracellular sites. Following to exposure of environmental pollutants such as herbicides, chlorophenoxy and dioxins, may cause an immediate loss of functional iron from normal intracellular sites (e.g. altered expression of DMT1 and TFR1) follows exposure to these pollutants [1].

From the study carried out by Yu *et al.* [26], it was observed that genetic factors play a

vital role on iron status. The ability of individual to recover normal iron levels varies, which indicates that there are contributing factors in addition to dietary iron intake or supplementation. In recent decades, various studies have reported the associations between genetic variants and iron status. Single Nucleotide Polymorphisms (SNPs) are associated with parameters of iron status [27,28,29]. The SNPs rs7385804 in the TFR2 gene is associated with iron status and red cell indices while variants in the TMPRSS6 gene are associated with decreased serum iron and increased in serum ferritin [26].

This study has further shown significantly lower values of red cell parameters compared to the established standardized reference values of the Caucasian origin. Specifically, haemoglobin concentration, red blood cell count, mean cell haemoglobin, mean cell haemoglobin concentration, haematocrit were significantly lower than that of the standardized values for both genders. However, red cell distribution widths for males and for female were observed to be significantly higher than that of the standardized values. This is in agreement with the observations of Subhashree *et al.* [30].

For majority of iron parameters measured in the study, the values differ significantly between genders. Higher red cell indices levels were observed in males than females in this study and these are consistent with previous reports [5, 21, 7]. The reasons for these differences have been attributed to factors such as the one that influence androgen hormone on erythropoiesis and menstrual blood loss in females [9, 31]. To this end, we found a clear gender difference among the age groups for all parameters. However, it has been argued in the literature that decision whether to use different ranges for different population groups (in the case

males and females) should be based on P-values [10].

Based on the 2.5<sup>th</sup> percentile of the current study, it was reported that the lower reference range limit for haematocrit were 34% and 31% for men and women respectively compared to the lower reference values of the standardized literature of 35% and 31% for males and females respectively as reported by Dacie and Lewis[19]. This finding is in line with the finding of the study conducted by Kibaya *et al.* [31] which determined references ranges for rural population in Kericho, Kenya.

In contrary to our findings, Kone *et al.* [32] observed higher values of haemoglobin in East Africa with median values 15g/dL and 12.8g/dL for males and females, respectively as against 12.3g/dL and 11.0g/dL for males and females, respectively in this study. The reason for these higher values in this region of black population compared to this study and other West Africa studies [24, 25, 10] could be attributed to the impact of the high altitude in those regions leading to decreased oxygen in the air, and increased haematopoiesis in these populations.

The different values of RBC indices (MCV, MCH and MCHC) established in this study were compared to the standardized reference values and were observed to be statistically significant. It was observed that the mean values of this study for MCH, MCHC and MCV were found to have relatively low values in both genders than the standardized values. Similar study was carried out by Geoffrey *et al.* [33]. However, the 97.5<sup>th</sup> percentile intervals for this study tended to be higher in MCV and MCHC. Contrary to our study, Nejat *et al.* [34] reported an increase in MCV, MCH and MCHC when compared to our study. The difference to our findings may be attributed to relatively higher altitude of Asmara (2230m) and Kintampo (60-150m). This reported result signifies the importance

of altitude as a contributory factor to specific RBC indices. The finding of Kabasakal *et al.* [35] is in keeping with this study.

In this study, the mean value of serum ferritin in males is significantly higher than females mean value. The higher value in males is consistent with the known observation of gender difference in the storage of iron [36]. The value of ferritin among male subjects in this study is significantly higher than the standardized value. The high value of serum ferritin in Abeokuta may be a reflection of adequate iron stores. On the contrary, similar finding was obtained by Ramezani *et al.* [37] which showed that serum ferritin are elevated in alcohol excess. The serum ferritin level in females in this study is in agreement with the standardized values [19]. However, ferritin levels differ according to age, with level rising till it peaked at 48-57 years age group with median of 140.95ng/ml compared to standardized value of 118.00ng/ml before it started to reduce. This is at variance with a work by Gibson [38] who submitted that serum ferritin values among men peak between 30-39 years of age and then tend to remain constant until about 70 years of age. Among women, serum ferritin remains relatively low until this level abruptly increases in women after menopause [39]. According to Zacharski *et al.* [40], this study equally agreed that serum ferritin level differs according to age, gender, ethnicity and lifestyles.

On the contrary, serum iron concentration value in our study area was significantly lower than the standardized reference value documented by Dacie and Lewis [19]. When comparing serum iron concentration of the subjects used according to age groups, the median serum iron concentration trended with a progressive drop from 18-47 years age group. This value elevated at the 48-57 years age group and finally declined at the age group of 68-77 years. According to gender,

however, there were significantly higher levels of serum iron in males compared to females; this is due to child bearing and loss of blood during menstruation. This finding is in line with the study conducted by Dosoo *et al.* [15] which found significant higher level of serum in males than that of the females.

The values of serum iron concentrations obtained for both genders in this study were higher than values obtained from Caucasians using 97.5<sup>th</sup> percentile of the current study. Similar observations have been made by Anupama *et al.* [41] which reported that increase in serum iron level was because of the complex biological response of the body tissues to harmful stimuli. In a study conducted by Ahlan *et al.* [42], mean values obtained for serum iron among healthy Saudis population of both genders were higher than those in our study but the values obtained in this study are also lower than the established reference values.

In this study, the mean values of TIBC in both genders were found to have relatively low values than the standardized values. The study observed elevated level of TIBC value in males than females. This report agrees with the finding of Tietz *et al.* [43] who reported higher total iron binding capacity mean values in males than in females. However, Oluboyede *et al.* [44] reported contrary findings with regard to TIBC levels in males and females. Dacie and Lewis [19] reported a single range values for serum iron and total iron binding capacity reference values in both genders. However, our TIBC and serum iron reference values in this study were higher at the 97.5<sup>th</sup> percentile than the standardized reference values.

In comparison of total iron binding capacity (TIBC) concentration among Subjects of various age groups, TIBC showed a final and sharper decline at 68 to 77 years age group (Figure 4.3). However, the highest median values for TIBC were observed in the 38 to

47 years group. The outliers were prominent in age group 28-37 years.

The study has shown that the reference values for iron ferritin, serum iron and total iron binding capacity varies by age; as they are lower in women than men in most parameters and these have been confirmed by other studies [40, 7, 9]. The lower reference values in females residing in Abeokuta is probably due to low level of dietary iron intake and this is made worse by monthly blood losses. As well, parasitic infection such as hookworm infestations were not excluded. However, the results show that all the determined reference values were significantly different from that of the standardized reference values. This is in accordance with the study carried out by Olawumi *et al.* [10].

## CONCLUSION

The study has shown significantly lower values of haemoglobin, red cell count, MCH, MCHC, MCV, haematocrit, serum iron and TIBC, and significantly higher values of RDW and ferritin compared to standardized reference values of Caucasians. The study further showed influences of age and gender on most of the indices. These findings in this study will serve as reference values for apparently healthy individuals in Abeokuta, South Western Nigeria and therefore improve the diagnoses of disorders of iron metabolism in our locality

Based on the findings in this study, the following recommendations are made:

- i. Further research is needed to establish iron reference values for adult individuals in African population to compare with those derived from developed.
- ii. Further study should be carried out on C-reactive protein and similar markers which might throw light on

the reasons for higher values of serum ferritin in our reference population.

- iii. It is appropriate to use this established reference values in Abeokuta rather than the Caucasians values to prevent misclassification, under-diagnosis and possible over-diagnosis iron disorders.

#### LIMITATION OF THE STUDY

A fundamental limitation is that the study participants were selected on the basis of their willingness to participate in the study. These factors might create selection bias which may limit the possibility of generalizing the results obtained in this study to the entire adult population based on age and gender population in Abeokuta.

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