

# ASSESSMENT OF SOME HAEMATOLOGICAL AND IRON STATUS PARAMETERS AMONG PRIMARY SCHOOL PUPILS IN ABIAKPO IKOT ESSIEN, AKWA IBOM STATE, NIGERIA

**Comment [JY1]:** Haematological not Haematologic...

## Abstract

Iron is an essential micronutrient which plays important roles in human health. This study aimed at evaluating some haematological and iron status parameters of learners from primary schools in Abiakpo Ikot Essien, Ikot Ekpene, Akwa Ibom State. A total of 180 primary school children, consisting of 87 male and 93 female subjects within the age bracket of 2-12 years were used for the study. Informed consent was obtained from the parents/guardians of the subjects, and a well-structured questionnaire was used to obtain relevant information about the subjects. Five (5ml) of venous blood was collected using a standard venepuncture technique from each subject for the determination of haematological parameters using Dc sheath flow method using SysmexXE-200 haematology autoanalyzer, and serum iron ferritin, Total iron binding capacity (TIBC) and Soluble transferrin receptor 1 (sTfR1) using colorimetric method. The haematological and iron status parameters of the subjects were within the standard reference ranges where the mean PCV was  $33.64 \pm 3.22\%$  with a reference range of 35-55%, the mean Hb was  $11.74 \pm 1.16$  g/dl with a reference range of 11-17 g/dl, Red blood cells (RBC) mean value was  $4.69 \pm 0.44 \times 10^6/\mu\text{l}$ . The red cell indices which are the MCV (fl) mean value was  $79.33 \pm 7.04$  with a standard reference of 80-100, MCH (pg/dl) had a mean value of  $28.11 \pm 4.98$  with a reference range of 26-34 and MCHc (g/dl) has a mean value of  $34.86 \pm 0.98$  with a reference value of 31-35 were also within the reference ranges. The Iron parameters were also within the reference ranges with sTfR1 (mg/ml) having a mean value of  $307.40 \pm 81.54$  with a reference value of 200-360, serum ferritin (ng/ml) had a mean value of  $61.68 \pm 4.67$  with a reference value of 7-140 and TIBC ( $\mu\text{g/dl}$ ) having a mean value of  $57.43 \pm 21.46$  with a reference value of 100-400. However, based on sex, male pupils had significantly higher mean values of Hb and PCV ( $11.52 \pm 1.30$  and  $32.97 \pm 3.58$  respectively) compared to the female pupils ( $11.93 \pm 0.97$  and  $34.29 \pm 2.71$  respectively). It is concluded that there were no alterations in haematological and iron status parameters in the pupils. It is recommended that all relevant bodies should make efforts to maintain good nutrition amongst school children.

**Comment [JY2]:** Haematological not haematologic

**Comment [JY3]:** Indicate full meaning of the red cell indices first before the abbreviations (MCV, MCH, and MCHC). MCHC needed to be corrected to capital letter please.

**Keywords:** haematological parameters, iron status, packed cell volume, ferritin, Akwa Ibom,

## 1. INTRODUCTION.

Iron deficiency results when ingestion or absorption of dietary iron is inadequate to meet iron losses or iron requirements imposed by growth or pregnancy. The risk of iron deficiency increases during periods of rapid growth, notably in infancy, adolescence and pregnancy [1]. Iron deficiency is one of the most prevalent nutritional disorders in the world, both in high- and low-resource countries, particularly among children and women of childbearing age [2]. It is the most common micronutrient deficiency seen in children in Nigeria and worldwide. It affects more

than two billion people including children with protein energy malnutrition (PEM) worldwide [2]. The iron status of individuals mainly influences the absorption of non-haem iron, whereas haem iron absorption is generally less affected. There is an inverse correlation between iron status and iron absorption [3].

A reduced rate of delivery of stored and absorbed iron to meet cellular iron requirements represents a more advanced stage of iron depletion, which is associated with reduced serum iron, reticulocyte haemoglobin, and percentage transferrin saturation and with higher total iron binding capacity, red cell zinc protoporphyrin, and serum transferrin receptor concentration [3].

Children who are born into the low- and middle-income families (LMICs) are usually vulnerable to physiological challenges and environmental hazards that may influence both their current and future development and as such, there is a high risk of infection and nutritional deficiencies of which iron deficiency is included [4].

The risk of iron deficiency tends to increase during the rapid growth phase significantly among school aged children in developing countries [5]. Iron deficiency is of importance because iron plays a significant role in human growth and development [6].

This study seeks to assess iron status and haematological parameters among primary school pupils in the locality.

## **2. Materials and Methods**

### **2.1. Study Design**

A cross-sectional random study design was used.

### **2.2 Study Area**

Study was conducted in Methodist primary school and St. Anne's catholic primary school, Abiakpo Ikot-Essien. Abiakpo Ikot -Essien is a village in Ikot Ekpene local government area of Akwa Ibom State, Nigeria where most of its inhabitants are mostly traders and farmers. The name Abiakpo simply means "bone doctor" and most of the inhabitants are seen to have natural fracture healing powers. The locality houses two known government owned primary schools namely Methodist Primary school (Anwanta), Abiakpo Ikot -Essien and St. Annes catholic primary school, Ikot Ebeyem and lots of other privately-owned primary schools. Ikot Ekpene Local Government Area also known as Raffia City is the historic town in the south-southern state of Akwa Ibom. It is the political and cultural capital of Annang ethnic group, located on the A342 highway that parallels the coast between Calabar to the south east and Aba to the west. Akwa Ibom is located in the coastal southern part of Nigeria, lying between latitudes 40321°N and 50331°N and longitudes 70251°E and 80251°E. The state is located in the south-south geopolitical zone and bordered on the east by Cross Rivers State, west by Rivers State and Abia State, and south by the Atlantic Ocean. It has a total population of 5,450,758 and a total land mass of 7,081km<sup>2</sup>[7].

### **2.3. Study Population**

The study population consisted of 180 primary school learners aged 2-12 years in the selected community, out of which 87 of them were males, and 93 of them were females. A well-structured Questionnaire was used to collect information on the respondent's demographic information.

## 2.4 Eligibility Criteria

### 2.4.1 Inclusion Criteria

Inclusion criteria include:

- i. Primary school learners aged between 2-12 years attending the Methodist primary school and St. Anne's catholic primary school
- ii. Learners from primary 1-6
- iii. Learners whose parents gave permission for them to participate in the study.
- iv. Learners who were present on the day of sample collection.
- v. Learners not on medication that can affect the iron status.
- vi. Learners with no known case of fever, infection or inflammation.
- vii. Learners with no history of sickle cell anaemia

**Comment [JY4]:** Please, what kind of medication tends to affect iron status? Indicate few, to be specific.

### 2.4.2 Exclusion Criteria

Exclusion criteria include:

- i. Learners above the required age and not attending the selected schools.
- ii. Learners whose parents/guardians did not give consent for them to participate in the study.
- iii. Learners that were not present on the days of sample collection.

**Comment [JY5]:** You can remove this, there is no need for it.

- iv. Learners with any known fever, inflammation or congenital disorders like sickle cell anaemia or any other disease.
- v. Learners on any form of medication.

## **2.5. Sample Size**

Sample size was calculated using Gpower and it was 128 (at power of 0.8 and error of 0.5). Gpower version 3.1.9.2 but for this research work, the sample size was increased to 180.

## **2.6 Ethical Approval/Informed Consent**

Ethical approval was obtained from the Ministry of Health, and also from State Universal Primary Education Board (SUPEB) in Akwa Ibom State, as shown in the appendices. Verbal informed consent was obtained from the parents or guardians of the subjects. Oral consent was also obtained from the learners.

## **2.7 Sample Collection, Processing and Transportation**

Five millilitres (5ml) of venous whole blood was drawn using a sterile hypodermic syringe and needles by standard venepuncture technique as described by Brooks (2014)[8]. Two millilitres (2mls) was dispensed into EDTA tube for the determination of haemoglobin concentration, packed cell volume and red cell indices. The remaining 3ml of the venous blood was dispensed into plain tubes without anticoagulant for the determination of serum iron, ferritin, TIBC and sTfR. Blood samples were kept in ice packs during transport to the laboratory. The haematological parameters were analysed almost immediately, while the samples in the plain bottles were spun, and separated at the University of Uyo Teaching Hospital, Uyo, Akwa Ibom State, Nigeria, and was stored in the freezing compartment of the refrigerator until the time for

analysis, which was later performed at the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State.

## **2.8. Determination of Haematological Parameters**

### **2.8.1 Method**

The Dc sheath flow method using system XE-2100 as described by Nkoane (1999) was used [9].

### **2.8.2 Principle**

It uses the technology of fluorescence flow cytometry to determine the standard five -part differential, juvenile granulocytes, nucleated red blood cells, reticulocyte count, immature reticulocyte fraction and optical fluorescence platelet count. The side scatter, forward scatter and fluorescence intensity of nucleated cells combines to give a precise image of each detected cell in the ~~peripheral~~ blood sample.

**Comment [JY6]:** Remove peripheral, I added sample to the blood in blue.

### **2.8.3 Procedure**

With the aid of a hydrodynamic focusing, the cells are passed through an aperture one cell at a time. A laser is directed at the cells where the scattered light is measured at multiple angles and absorbance is been recorded. The cells are identified based on the intensity of the scattered light and also their level of absorbance.

## **2.9 Determination of Total Iron Binding Capacity (TIBC)**

### **2.9.1 Method.**

This was done using Colorimetric method as described by Kasvosve and Delanghe(2002)[10].

### **2.9.2 Principle**

Iron in excess is added to serum helping it bind all the ferritin in iron absorbed. The iron bound with the ferritin is separated from the protein by the action of acid solution and reductant.  $Fe^{3+}$  in the serum is reduced to  $Fe^{2+}$  which binds with bipyridine to form a pink complex. The amount of TIBC present is positively correlated with the colour intensity. The absorbance of the solution is measured using a wavelength of 520nm as serum in a filter colorimeter.

### **2.9.3 Procedure**

80 $\mu$ l of sample or standard was added to the calibrator test tube .100 $\mu$ l of acid reagent (R1) was added to both the blank and calibrator test tube, mixed and incubated for five minutes at 37<sup>0</sup>c.300  $\mu$ l of the neutral buffer(R2) was added to both the blank and calibrator test tube each. It was mixed and read as A1 at 660nm.The mixture was incubated again at 37<sup>0</sup>c for 7.5minutes and read as A2 at the same 660nm.The final absorbance was read as A2-A1.

## **2.10 Determination of Soluble transferrin receptor 1 (STfR 1)**

### **2.10.1 Method**

This was done using sandwich Enzyme linked Immunosorbent Assay (ELISA) technique as described byKasvosve and Delanghe(2002)[10].

### **2.10.2 Principle**

Samples or standards are added to a micro -ELISA plate wells to combined with specific antibody. A biotinylated detection antibody specific for human sTfR1 and Avidin Horseradish Peroxidase (HRP) conjugate are added to each micro-plate well and incubated. The free components are washed away and the substrate solution added to each well. Wells containing

human sTfR1, biotinylated detection antibody and avidin -HRP will appear blue in colour. The enzyme -substrate reaction is stopped by the addition of a stop solution changing the colour to yellow. It is measured using a spectrophotometer at a wavelength of  $450 \pm 2$  nm and OD value is proportional to the concentration of human sTfR1.

### **2.10.3 Procedure**

100ml of standard or sample was added to the wells and incubated at  $37^{\circ}\text{C}$  for 90 minutes, after which 100ml of biotinylated antibody was added and incubated at  $37^{\circ}\text{C}$  for 60 minutes. The mixture was aspirated and the plate washed for three times and 100ml of HRP conjugate working solution was added and incubated at  $37^{\circ}\text{C}$  for thirty minutes. It was aspirated and the plate washed for five times, after which 90ml of substrate was added and incubated at  $37^{\circ}\text{C}$  for fifteen minutes. Lastly, 50ml of stop solution was added and the plate read at 450nm.

## **2.11 Determination of Serum Ferritin**

### **2.11.1 Method**

This was done using Sandwich Enzyme Linked Immunosorbent Assay (ELISA) technique as described by Kasvosve and Delanghe (2002) [10].

### **2.11.2 Principle**

It makes use of a solid phase sandwich assay method which is based on streptavidin-biotin principle. The standard, samples and anti-ferritin antibody reagents are added into designated wells coated with streptavidin. Endogenous ferritin from the patient's serum binds to the

antigenic site of the biotinylated anti-ferritin antibody. At the same time, the biotinylated antibody is immobilized onto wells through the high affinity streptavidin- biotin interaction. Unbound protein as well as excess biotin conjugated antibody are washed off by a wash buffer. Peroxidase (HRP) conjugated anti-ferritin antibody reagent is added and a sandwich complex is formed with the analyte of interest been in between the two highly specific antibodies and unbound proteins are washed off using wash buffer. When substrate is added, the colour intensity developed is directly proportional to the concentration of ferritin in the samples. A standard curve is prepared relating colour intensity to the concentration of the ferritin.

### **2.11.3 Reagent Preparation**

A 1x wash buffer was prepared by adding contents of the bottle to 475ml of distilled water and stored at room temperature prior to use.

### **2.11.4 Procedure**

25µl of ferritin standards, controls and samples were added to the appropriate wells. 100 µl of biotin reagent was added to each well and the plate rocked for 10 seconds after which it was covered and incubated at room temperature for 30 minutes. After incubation, the liquid was removed from the well and the wells washed three times with 300µl of X1 wash buffer and blotted using absorbance paper. 100µl of enzyme reagent was added to each well and the plate covered again and incubated for 30 minutes at room temperature. After that, the lid was removed and the liquid was drained from the wells and the wells were washed three times with 300µl X1 wash buffer and blotted with an absorbance paper. 100µl of 3,3',5,5' tetramethylbenzidine (TMB) substrate was added to all the wells and 50µl of stop solution was added to all the wells. The plates were shaken for 10 seconds for proper mixing and read on ELISA reader at 450nm

## **2.12. Statistical Analysis**

Data were analyzed using the statistical package for social sciences(SPSS), version 23. Results were expressed as mean  $\pm$ SD, with  $p \leq 0.05$  being considered statistically significant.

UNDER PEER REVIEW

### **3 RESULTS**

#### **3.1 Demographic Characteristics of Study Subjects**

A total of 180 learners was used for the study; 87 males and 93 females whose guardians gave consent from the selected government primary schools, and were apparently healthy were used. Recruited subjects ranging from ages 0-5 years were 30, while those ranging from 6 years and above were 150. Learners in which their fathers were either publicly or privately employed were 64 in number, those that their fathers were self-employed were 106 and those that were not working were 10 in number. Those whose mothers were either publicly or privately employed were 43 in number, those their mothers were self-employed were 127 in number while those their mothers were unemployed were 10 in number. The details of the family size were also included. Learners with family size of 1-5 were 128 while those with family size of 6-10 were 49 and those of 11 and above were 3. Their daily feeding schedule showed only 1 learner to be eating once a day, 12 eats twice a day and 167 eat more than twice a day.

### **3.2 Haematological Parameters of the Study Subjects with Established reference**

#### **Ranges**

The haematological parameters of the study subjects which included packed cell volume (PCV), haemoglobin concentration (Hb), mean cell haemoglobin (MCH), mean cell volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW) and their red blood cell volume (RDWSD) was compared with reference values as described by Scott et al. (2008), [11] details are shown in Table 4.4. The mean PCV was  $33.64 \pm 3.22\%$  with a reference range of 35-55%, the mean Hb was  $11.74 \pm 1.16\text{g/dl}$  with a reference range of 11-17g/dl, the mean MCV was  $79.33 \pm 7.04\text{fl}$  with a reference range of 80-11fl, the mean MCH was  $28.11 \pm 4.98\text{pg/dl}$  with a reference range of 26-34pg/dl, the mean MCHC was  $34.86 \pm 0.98\text{g/dl}$  with a reference range was 31-35g/dl, the mean RDWCV was  $11.41 \pm 1.26\text{fl}$  with a reference range of 10-16fl, the mean RDWSD was  $34.18 \pm 5.30\text{fl}$  with a reference range of 37-46fl, and the RBC was  $4.69 \pm 0.44 \times 10^6 \mu\text{l}$  with a reference range of  $4.0\text{-}6.2 \times 10^6 \mu\text{l}$ . All the haematological parameters were within the limits of the reference ranges.

### 3.3 Iron Parameters of the Study Subjects with Established Reference Ranges

Table 3.3 shows comparison of the values of soluble transferrin receptors, serum ferritin, and total iron-binding capacity with the reference ranges as described by Bishop *et al.* (2005)[12]. The values of sTfR, serum ferritin and TIBC were  $307.4 \pm 81.54$  mg/ml,  $61.68 \pm 4.67$  ng/ml and  $57.43 \pm 21.46$   $\mu$ g/dl in the same order. The value of the sTfR was higher than the reference range, the value of ferritin was within the reference range, while the TIBC value was lower than the reference range.

### **3.4 Comparison of Mean $\pm$ SD of Haematological Parameters of Study Subjects according to Sex**

This table shows the comparison of mean  $\pm$  SD of haematological parameters of study subjects according to sex. The PCV for male and female subjects were  $32.97 \pm 3.58\%$  and  $34.29 \pm 2.71\%$  respectively, with the value in female subjects being significantly higher ( $p < 0.05$ ) than that in male subjects. The Hb concentration for male and female subjects were  $11.52 \pm 1.30 \text{g/dl}$  and  $11.93 \pm 0.97 \text{g/dl}$  respectively, with the concentration in female subjects being significantly higher ( $p < 0.05$ ) than that in male subjects. The MCV for male and female subjects were  $79.85 \pm 5.34 \text{fl}$  and  $78.85 \pm 8.31 \text{fl}$  respectively, with no significant difference between them. The MCH for male and female subjects were  $28.09 \pm 2.47 \text{pg/dl}$  and  $28.12 \pm 6.52 \text{pg/dl}$  respectively, with no significant difference between them. The MCHC for male and female subjects were  $34.84 \pm 1.06 \text{g/dl}$  and  $34.88 \pm 0.91 \text{g/dl}$  respectively, with no significant difference between them. The RDW-CV for male and female subjects were  $11.59 \pm 1.59$  and  $11.23 \pm 0.82$  respectively, with no significant difference between them. The RDW-SD for male and female subjects were  $34.95 \pm 7.00 \text{fl}$  and  $33.48 \pm 2.83 \text{fl}$  respectively, with no significant difference between them. Similarly, the RBC for male and female subjects were  $4.14 \pm 0.50 \times 10^6 \mu\text{l}$  and  $5.20 \pm 0.86 \times 10^6 \mu\text{l}$  respectively, with no significant difference between them.

### 3.5 Comparison of Iron Parameters of Study Subjects according to Sex

Table 3.5 depicts comparison of iron parameters of study subjects according to sex. The mean value of sTfR for male and female subjects were  $302.90 \pm 78.77$  ng/ml and  $301.61 \pm 84.25$  ng/ml respectively, with no significant difference between them. The value of ferritin for male and female subjects were  $61.99 \pm 7.20$  ng/ml and  $61.39 \pm 6.06$  ng/ml respectively, with no significant difference between them. Similarly, the value of TIBC for male and female subjects were  $55.51 \pm 19.05$   $\mu$ g/dl and  $59.23 \pm 23.44$   $\mu$ g/dl respectively, with no significant difference between them.

### **3.6 Correlation between the Haematological and Iron Parameters of the Study Subjects**

Table 3.6 depicts the correlation between haematological and iron parameters of the study subjects. It shows a non-significant negative correlation between PCV and sTfR with a p-value of 0.596, a significant negative correlation between PCV and ferritin with a p-value of 0.000, a non-significant negative correlation between PCV and TIBC with a p-value of 0.780, and a non-significant negative correlation between PCV and family size with a p-value of 0.339. It shows a non-significant negative correlation between Hb and sTfR with a p-value of 0.677, a significant negative correlation between Hb and ferritin with a p-value of 0.000, a non-significant negative correlation between Hb and TIBC with a p-value of 0.566, and a non-significant negative correlation between Hb and family size with a p-value of 0.288. It shows a non-significant negative correlation between MCV and sTfR with a p-value of 0.328, a non-significant negative correlation between MCV and ferritin with a p-value of 0.637, a non-significant positive correlation between MCV and TIBC with a p-value of 0.384, and a non-significant positive correlation between MCV and family with a p-value of 0.154. It shows a non-significant positive correlation between MCHC and sTfR with a p-value of 0.688, a non-significant negative correlation between MCHC and ferritin with a p-value of 0.497, a non-significant negative correlation between MCHC and TIBC with a p-value of 0.314, and a non-significant negative correlation between MCHC and family size with a p-value of 0.716. It shows a non-significant

negative correlation between RDW-CV and sTfR with a p-value of 0.560, a significant positive correlation between RDW-CV and ferritin with a p-value of 0.07, a non-significant negative correlation between RDW-CV and TIBC with a p-value of 0.772, and a non-significant positive correlation between RDW-CV and family size with a p-value of 0.230. It shows a non-significant negative correlation between RDW-SD and sTfR with a p-value of 0.626, a non-significant positive correlation between RDW-SD and ferritin with a p-value of 0.077, a non-significant positive correlation between RDW-SD and TIBC with a p-value of 0.978, and a non-significant positive correlation between RDW-SD and family size with a p-value of 0.565. Similarly, it shows a non-significant positive correlation between RBC and sTfR with a p-value of 0.792, a non-significant negative correlation between RBC and ferritin with a p-value of 0.530, a non-significant negative correlation between RBC and TIBC with a p-value of 0.339, and a non-significant negative correlation between RBC and family size with a p-value of 0.887.

**Table 1: Demographic Characteristics of Subjects**

<b>Variable</b>	<b>Frequency (%)</b>
<b>Sex</b>	
Male	87 (48.3)
Female	93 (51.7)
<b>Age (Yrs)</b>	
0-5	30 (16.7)
6yrs and Above	150 (83.3)
<b>Occupation of Father</b>	
Employed (Public and private)	
Self-employed	64 (35.6)
None	106 (58.9)
<b>Occupation of Mother</b>	
Employed (Public and private)	10 (5.5)
Self-employed	43 (23.9)
None	127 (70.6)
<b>Family Size</b>	
A (1-5 persons)	128 (71.1)
B (6-10 persons)	49 (27.2)
C (11 and above)	3(1.7)
<b>Frequency of daily feeding</b>	
Once	1 (0.6)
Twice	12 (6.7)
More than twice	167 (92.7)

**Table2: Haematological Parameters of the Study Subjects with Established reference Ranges**

Variable	Value (Mean $\pm$ SD)	Reference Range**
PCV (%)	33.64 $\pm$ 3.22	35-55
Hb (g/dl)	11.74 $\pm$ 1.16	11-17
MCV (fl)	79.33 $\pm$ 7.04	80-11
MCH (pg/dl)	28.11 $\pm$ 4.98	26-34
MCHC (g/dl)	34.86 $\pm$ 0.98	31-35
RDWCV	11.41 $\pm$ 1.26	10-16
RDWSD (fl)	34.18 $\pm$ 5.30	37-46
RBC ( $\times 10^6/\mu\text{l}$ )	4.69 $\pm$ 0.44	4.0-6.2

**KEY:** \*\*Scott *et al.* (2008)

PCV = Packed cell volume

Hb = Haemoglobin

MCV = Mean cell volume

MCH = Mean cell haemoglobin

MCHC = Mean cell haemoglobin concentration

RDW = Red cell distribution width

RBC = Red blood cell

RDWCV=Red cell distribution width cumulative frequency RDWSD=Red cell distribution weight standard deviation

**Table 3: Iron Parameters of the Study Subjects with Established Reference Ranges**

<b>Variable</b>	<b>Value (Mean <math>\pm</math> SD)</b>	<b>Reference Range**</b>
sTfR (mg/ml)	307.40 $\pm$ 81.54	200-360
Ferritin (ng/ml)	61.68 $\pm$ 4.67	7-140
TIBC ( $\mu$ g/dl)	57.43 $\pm$ 21.46	100-400

Key: sTfR- soluble transferrin receptor, TIBC-total iron binding capacity

\*\*Bishop *et al*, 2005

**Table4: Comparison of Mean  $\pm$  SD of Haematological Parameters of the Study Subjects According to Sex**

	PCV (%)	Hb (g/dl)	MCV (fl)	MCH (pg/dl)	MCHC (g/dl)	RDWCV	RDWSD (fl)	RBC ( $\times 10^6/\mu\text{l}$ )
Male	34.29 $\pm$ 2.	11.93 $\pm$ 0.	79.85 $\pm$ 5.	28.09 $\pm$ 2.47	34.84 $\pm$ 1.	11.59 $\pm$ 1.	34.95 $\pm$ 7.	4.14 $\pm$ 0.5
(n=87)	71	97	34		06	59	00	0
Female	32.97 $\pm$ 3.	11.52 $\pm$ 1.	78.85 $\pm$ 8.	28.12 $\pm$ 6.52	34.88 $\pm$ 0.	11.23 $\pm$ 0.	33.48 $\pm$ 2.	5.20 $\pm$ 0.8
(n=93)	58	30	31		91	82	83	6
p-value	0.006	0.017	0.339	0.971	0.813	0.057	0.060	0.238
t-value	2.781	2.408	0.958	0.036	0.237	1.915	1.890	1.184
Remark	S	S	NS	NS	NS	NS	NS	NS

s

S=Significant, NS=Non-significant, PCV=Packed cell volume, Hb-Haemoglobin concentration, MCV=mean cell volume, MCH=mean cell haemoglobin concentration, MCHC=mean corpuscular haemoglobin concentration, RDWSD=red cell distribution width specific density, RDWCV=red cell distribution width cumulative frequency, RBC=red blood cell concentration.

**Table 5: Comparison of Mean  $\pm$  SD of Iron Parameters of Study Subjects according to Sex**

	<b>sTfR (ng/ml)</b>	<b>Ferritin (ng/ml)</b>	<b>TIBC (<math>\mu</math>g/dl)</b>
Male	302.90 $\pm$ 78.77	61.99 $\pm$ 7.20	55.51 $\pm$ 19.05
Female	301.61 $\pm$ 84.25	61.39 $\pm$ 6.06	59.23 $\pm$ 23.44
p-value	0.475	0.949	0.245
t-value	0.716	0.063	1.167
Remarks	NS	NS	NS

NS=non-significant, sTfR=Soluble transferrin receptor, TIBC=Total iron binding capacity.

**Table 6: Correlation between Haematological and Iron Parameters of the Study Subjects**

		STfR(ng/ml)	Ferritin (ng/ml)	TIBC (µg/dl)	Family Size
PCV	Correlation	-.040	-.285	-.021	-.072
	p-value	.596	.000	.780	.339
Hb(g/dl)	Correlation	-.031	-.295	-.043	-.080
	p-value	.677	.000	.566	.288
MCVfL	Correlation	-.073	-.035	.065	.025
	p-value	.328	.637	.384	.734
MCHpg	Correlation	.073	-.050	-.031	-.107
	p-value	.333	.504	.680	.154
MCHCgdl	Correlation	.030	-.051	-.075	-.027
	p-value	.688	.497	.314	.716
RDWCV	Correlation	-.044	.201	-.022	.090
	p-value	.560	.007	.772	.230
RDWSDfl	Correlation	-.037	.132	.002	.043
	p-value	.626	.077	.978	.565
RBCx106µl	Correlation	.020	-.047	-.072	-.011
	p-value	.792	.530	.339	.887

Correlation is significant at the 0.05 level (2-tailed).

**Comment [JY7]:** The tables are good and self explanatory, but, transfer each table to the proper positions. ie beneath each explanation of the parameters!!!

#### 4 Discussion

This study evaluated the iron status of learners from primary schools in Abiakpo Ikot -Essien, Ikot Ekpene, Akwa Ibom State, Nigeria. Results from the study indicated that there were 93 (51.7%) female pupils and 87 (48.3%) male pupils. This high number of female pupils may be attributed to the increased awareness about education of the female child.

The haematological and the iron status parameters of the subjects were within the reference ranges. This indicates that there were no alterations in the haematological parameters. These findings may be due to the exposure of the subjects to some local vegetables and foods which are known to be rich in vitamins and trace elements [13].

The haemoglobin level as well as the packed cell volume were significantly higher in male subjects compared to the female subjects. This agrees with the report of another study [14] which reported that male children generally have higher haemoglobin levels than their female counterparts. The other haematological parameters did not differ significantly based on sex. This is in agreement with the work of another study [13], which reported a similar finding among children in Cote d'Ivoire. Our finding could be due to the fact that the subjects were exposed to similar diets, being residents of the same locality with the same diet pattern.

There were no statistically significant differences ( $p>0.05$ ) in the iron parameters between the male and female subjects. This may be because the female subjects were exposed to similar dietary and environmental factors as the males; this study agrees with that of another author [15].

The results from this study indicate that there was a significant negative correlation between ferritin and PCV ( $r= -0.285$ ,  $p\leq 0.001$ ) and haemoglobin levels ( $r= -0.295$ ,  $p\leq 0.001$ ) of the subjects. Since ferritin reflects iron stores in the body [16], this negative correlation may indicate active physiological utilization of iron in the subjects.

**Comment [JY8]:** You need more discussion to justify this work.

## 5 Conclusion

The results from this study indicate that there were no alterations in haematological parameters and iron status parameters among pupils used in this study. However, based on sex, male subjects had significantly higher packed cell volume (PCV) and haemoglobin levels compared to the female subjects. Also, there was a significant negative correlation of PCV and haemoglobin levels with ferritin levels. The results indicate that the subjects have within range levels of haematological and iron status parameters.

**Comment [JY9]:** There are no page numbers!!

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