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Assessment of Iron Status: Markers of Inflammatory and Immune Response in School Age Children

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Abstract. Iron deficiency is a public health problem based on the seriousness of its consequences on human health. This study assessed iron status, markers of inflammatory and immune functions of rural and urban school age children in selected Local Government Areas (LGAs) of Ogun state. A multistage sampling technique was used to select three hundred and twelve school age children from the three senatorial districts. A validated questionnaire was used to obtain information on socio-economic characteristics. Blood samples were analyzed for biochemical parameters and selected immune function markers (CD4, white blood cell differentials) were also measured using standard procedures. Data were analyzed using frequency counts, percentages, means, standard deviations, correlation. Results showed that 30.5% of the respondent families earned less than two hundred thousand naira annually. Also, 19.9% of the mothers in the rural sector had tertiary education. The study revealed the prevalence of iron deficiency to be 23.7%, aneamia was 16.3% while 13.1% of the anaemic children were due to iron deficiency aneamia. The result of the CRP showed a high risk in 13.8% and CD4 count was low in 16.7% of the children. Haemoglobin correlated positively with age (r= 0.144) and average annual income of the family(r=0.132), serum ferritin correlated positively with mothers age and household size (r= 0.159; r= 0.030). CRP positively correlated with annual income (r= 0.155). CD4 positively correlated with mothers age and education (r= 0.252; r= 0.142). Conclusively, significant relationship exists between socio economic and iron status ($p<0.05$) as well as the rate of inflammation and immune response in the children. Hence, Appropriate investigations for iron status and inflammation/infection screening, need to be integral in the evaluation of anaemia and its causes before anaemia control interventions are implemented.

Keywords: Immune, Inflammation, Anaemia, Reticulocyte

1. Introduction

Iron deficiency anaemia is one of the most widespread public health problems, especially in developing countries, and a major cause of morbidity and mortality in Africa (40). It is a worldwide problem that is highly prevalent in developing countries of the world with the highest incidence reported in Asia and Africa (31). Anaemia prevalence is high in children and its cause is frequently multifactorial. It has been estimated that about 40% of the world's population (more than 2 billion individuals) suffer from anaemia with a prevalence of 48% in school-aged children (30,37). The key nutrient deficiency observed among school-aged children is iron deficiency anaemia (23, 29).

Iron deficiency with or without anemia is associated with increased susceptibility to infection owing to impaired immune function (22). Anaemia is one of the most widespread public health problems, especially in developing countries, and has important health and welfare, social, and economic consequences. These include impaired cognitive development, reduced physical work capacity, and in severe cases increased risk of mortality (45). The

prevalence of iron deficiency anaemia had been described to be high amongst Nigerian infants, children and their mothers (2, 20).

Iron is also necessary for normal development of the immune system. Its deficiency affects the capacity to have an adequate immune response as it is necessary for immune cell proliferation and the generation of specific response to infection (3). Both experimental, and some clinical studies have emphasized the importance of iron in the integrity of the immune system especially the innate immunity (decreased bactericidal effect and respiratory burst of neutrophils) and the cellular component system of cell-mediated immunity (CMI) (decreased lymphocyte proliferation and delayed hypersensitivity responses) (3, 8).

Iron deficiency and anaemia are associated with poor growth and cognitive development, lowered immunity with increased risk to infectious diseases, and reduced work productivity. An unhealthy schoolchild is unlikely to have regular school attendance, good academic performance, and involvement in extracurricular activities. The relation of iron status and immune function in humans has been addressed primarily in studies of infants, young children, and adults (16, 36, and 41). Impaired cell-mediated immunity (CMI) and bactericidal function are generally noted in iron-deficient persons; however, some of the findings are inconsistent (16, 33, 36). The conflicting results with respect to iron deficiency and immunocompetence may be related to the different age, gender, sexual maturity, and differences in the methods of assessment of immune function across studies. Thus, this study will comprehensively determine the relationship of iron status, immune function and markers of inflammation considering the age and gender of the school age children in south west, Nigeria.

2. Research Methodology

2.1 Study Population

The study population consisted of three hundred and twelve primary school children (7-12 years old) in selected schools in both urban and rural local governments in Ogun states.

2.2 Inclusion Criteria

- School age children between 7-12 years attending the schools selected for the study.
- School children from primary two to six
- Children whose parents give permission for them to participate in the study.
- Children giving ascent participated in the study.
- Children who are present at the day of data collection
- Children not on medication that can affect the data analyses.
- Children with no known case of infection or inflammation

2.3 Procedure for Data Collection

Prior to the commencement of the research, submission of the research proposal were made at the State Hospital Ijaye Abeokuta, Nigeria and selected local governments in the three senatorial districts and also to the headmaster of each school. Ethical consent was sought from the State hospital, Ijaye, Abeokuta and also meeting with parents with the assistance of the school management for their verbal consent. Trained fieldworkers with the principal investigator engaged in data collection and Medical laboratory scientist assisted in blood sample collection and analysis.

2.4 Method of Data Collection

A pretested, structured interviewer administered questionnaire was used to collect information from the respondents. The questionnaire was used to collect information on the respondent's bio-data and socio-economic characteristics.

2.5 Blood collection (including analytical procedures)

Venous blood samples (10ml) were collected and delivered in two containers as follows: (i) 4ml blood collected in EDTA- containing tube for hemoglobin (Hb) and full blood count; (ii) 6ml blood collected in coagulant free tubes and centrifuged for the estimation of serum ferritin (SF) and C-reative protein (CRP). PCV, CD4 count, Reticulocyte count were also measured.

2.6 Iron status indices

Iron status was determined by measurement of hemoglobin, PCV and serum ferritin. Since hemoglobin, PCV and serum ferritin may be altered in the presence of infection, C- reactive protein (CRP) was used to identify individuals with inflammation and infection.

Heamoglobin: Was measured *in situ* by means of the direct cyanmet hemoglobin method (Ames Mini-Pak Hb test pack &Ames™ Minilab), using Drabkins solution and a standard photometer.
Serum ferritin (indication of iron-stores): Was determined using ELISA (Randox kits, UK)
C-reactive protein: an acute phase protein, and an indicator of acute infection, was determined using a turbidity method from Bayer Corporation (Tarrytown, NY, USA). It was spectrophotometrically measured with a Technicon RA-1000 automated system.

2.7 Immune function determination

The relevance of iron in the make-up of the immune system particularly in the innate immunity by decreasing the anti-bacterial activity as well as the respiratory burst of polymorphonuclear cell, cellular component and delay hypersensitivity have being emphasized (3).

White blood cell count and differentials were determined (neutrophil, lymphocyte, monocytes, eosiniphil and basophil) using the method of Ghai (12) and Abu syed (1)

Reticulocyte count was performed on the blood sample according to Lewis *et al.* (17) .
The level of CD4 lymphocytes count using monoclonal antibodies was verified by cytoflow method (18)

2.8 Statistical analyses

Changes in biochemical indicators were calculated. Children were defined as: (i) iron deficient if serum ferritin< 15 µg L^{-1}; (ii) anaemic if haemoglobin is (Hb) < 11.5 g dL^{-1}; and (iii) Iron deficient anaemic if serum ferritin< 15 µg L^{-1} and Hb< 11.5 g dL^{-1}. The mean value for immune parameters like CD4 count, White blood cell count and differentials, Reticulocyte count were calculated while the severity of serum ferittin, heamoglobin, PCV and reticulocyte were measured and p-value determined. SPSS was used for all statistical calculations and a p-value < 0.05 was considered significant.

3. Results

Table 1: Socio-demographic Characteristics of the Children

Characteristics	Sector			
	Urban		Rural	
	Frequency	%	Frequency	%
AGE (Yrs)				
7-8	41	25.5	36	23.8
9-10	58	36.0	51	33.8
11-12	62	38.5	64	42.4
Total	161	100.0	151	100.0
AGE (Mother) Years				
0-20	8	5.0	21	13.9
21-30	42	26.1	53	35.1
31-40	68	42.2	48	31.8
40 Above	43	26.7	29	19.2
Total	161	100.0	151	100.0
AGE (Father) Years				
0-20	2	1.2	9	6.0
21-30	44	27.3	51	33.8
31-40	57	35.4	48	31.8
40 Above	58	36.0	43	28.5
Total	161	100.0	151	100.0
Educational level of the Mothers				
No formal Education	5	3.1	18	11.9
Primary Education	22	13.7	42	27.8
Secondary Education	36	22.4	61	40.4
Tertiary Education	98	60.9	30	19.9
Total	161	100.0	151	100.0
Educational level of the Fathers				
No formal Education	3	1.9	10	6.6
Primary Education	18	11.2	33	21.9
Secondary Education	25	15.5	74	49.0
Tertiary Education	115	71.4	34	22.5
Total	161	100.0	151	100.0

Table 2: Socio-Demographic Characteristics of the Children

Characteristics	Sector			
	Urban		Rural	
	Frequency	%	Frequency	%
Family Size				
1-4	98	60.8	52	34.4
5-8	47	29.2	61	40.4
Above 8	16	10.0	38	25.2
Total	161	100.0	151	100.0
Average Annual Income (Naira)				
<100, 000	7	4.3	15	10.0
100,000-199,000	21	13.0	31	20.5
200,000-299,000	23	14.3	44	29.1
300,000-399,000	54	33.5	37	24.5
400,000-499,000	35	21.7	16	10.6
500,000 & above	21	13.0	8	5.3
Total	161	100.0	151	100.0

3.1 Biochemical Indices of Children

Table 3 shows the mean biochemical indices of the children. The mean values for males were: haemoglobin concentration for boys (12.14±1.39 g/dl), serum ferritin (21.52±20.38µg/l), CD4 (741.71±347.20 cells/µl), C-reactive protein (3.32±5.06 mg/l), PCV (36.73±3.60), reticulocyte (0.83±0.37), WBC(7.02±2.21(100/µl) and neutrophils, lymphocytes, monocytes, Eosinophils, Basophils were (3.51±1.63), (3.85±4.45), (0.57±0.15), (0.36±0.31), and (0.04±0.05), respectively. The mean values for females were: haemoglobin level for girls (11.73±0.97 g/dl), serum ferritin (19.64±16.50µg/l), CD4 (695.13±345.12cells/µl), C-reactive protein (3.92±5.62 mg/l), PCV (36.10±3.92), reticulocyte (0.89±0.41), WBC (7.49±2.99 (100/µl) and neutrophils, lymphocytes, monocytes, Eosinophils, Basophils were (3.69±3.29), (3.51±1.52), (0.55±0.19), (0.34±0.35), and (0.04±0.03) respectively.

Table 3: Biochemical Indices of Respondents according to gender (N=312)

Variable	Male (n=155)	Female (n=157)	Total (N=312)	Cut-off Range	p-Value
Hb(mg/dl)	12.14±1.39	11.73±0.97	11.93±1.20	11.5-13.0	0.000*
SF (µg/l)	21.52±20.38	19.64±16.50	20.57±18.52	12-150	NS
CD4 (cells/µl)	741.71±347.20	695.13±345.12	718.27±346.39	365-1571	NS
CRP(mg/l)	3.32±5.06	3.92±5.62	3.62±5.34	3-10	0.002*
PCV(%)	36.73±3.60	36.10±3.92	36.42±3.76	>34	NS
Reticulocyte	0.89±0.41	0.83±0.37	0.86±0.39	0.5-1.5	NS
WBC(100/ µl)	7.02±2.21	7.49±2.99	7.26±2.64	4.0-11.0	NS
Neutrophils (1000/µl)	3.51±1.63	3.69±3.29	3.60±2.59	2.0-7.5	NS
Lymphocytes (1000/µl)	3.85±4.45	3.51±1.52	3.68±3.32	1.5-4.0	0.003*
Monocytes (1000/µl)	0.57±0.15	0.55±0.19	0.56±0.17	0.1-1.5	0.04*
Eosinophils (1000/µl)	0.36±0.31	0.34±0.35	0.35±0.33	0.04-0.4	NS
Basophils (1000/µl)	0.04±0.05	0.04±0.03	0.04±0.04	0.0-0.1	NS

Hb- Haemoglobin, SF- Serum Ferritin, CD4 – Cluster of differentiation 4, CRP- C-reactive protein, PCV – Packed cell volume, WBC- White blood cells

Table 4: Biochemical Indices of Respondents according to sector (N=312)

Variable	Urban(n=155)	Rural (n=157)	Total (n=312)	Cut-off Range	p-Value
Hb(mg/dl)	12.15±1.23	11.71±1.15	11.93±1.21	11.5-13.0	0.004*
SF (µg/l)	20.51±20.59	20.63±16.08	20.57±18.52	12-150	NS
CD4(cells/µl)	727.11±338.59	708.85±355.39	718.27±346.39	365-1571	0.002*
CRP(mg/l)	3.24±5.09	4.05±5.71	3.63±5.40	3-10	0.001*
PCV(%)	36.65±3.50	36.04±3.86	36.36±3.68	>34	NS
Reticulocyte	0.87±0.38	0.85±0.41	0.86±0.39	0.5-1.5	NS
WBC(100/µl)	7.28±2.19	7.24±3.05	7.26±2.64	4.0-11.0	NS
Neutrophils (1000/µl)	3.88±2.94	3.31±2.14	3.60±2.59	2.0-7.5	NS
Lymphocytes (1000/µl)	3.03±0.97	4.38±4.57	3.68±3.32	1.5-4.0	0.002*
Monocytes (1000/µl)	0.59±0.15	0.53±0.18	0.56±0.17	0.1-1.5	NS
Eosinophils (1000/µl)	0.33±0.25	0.38±0.39	0.35±0.33	0.04-0.4	NS
Basophils (1000/µl)	0.04±0.03	0.04±0.05	0.04±0.04	0.0-0.1	NS

Hb- Haemoglobin, SF- Serum Ferritin, CD4 – Cluster of differentiation 4, CRP- C-reactive protein, PCV – Packed cell volume, WBC- White blood cells

3.2 Iron status of the children

Figure 1 showed the Iron status of the respondents, it shows that 23.7 were iron deficient, 16.3% were anaemic while 13.1% had iron deficiency anaemia and the remaning 46.7% are iron sufficient. Iron status of the respondents is classified based on their sector and gender in Table 5, it shows that 20.6% of the male were iron deficient and 12.9% of iron deficient respondents were anaemic while 11.0% of the anaemia among the male is as a result of iron deficiency (IDA). Females that are iron deficient were 26.8%, while 19.7% of them were anaemic and 15.3% of the anaemia among the female is as a result of iron deficiency (IDA). In urban LGAs, 18.6% of the children were iron deficient and 7.5% of iron deficient respondents were anaemic while 13.7% of the anaemia among the children is as a result of iron deficiency (IDA). In rural LGAs, the percentages of children that are iron deficient were 29.1%, while 25.8% of them were anaemic and 12.6% of the anaemia among the rural children is as a result of iron deficiency (IDA).

The overall iron status of the respondents shows that 74(23.7%) of the respondents were deficient in iron and 51(16.3%) were anaemic while iron deficiency anaemia was noticed in 41(13.1%) of them. One hundred and forty six (46.7%) are sufficient in iron.

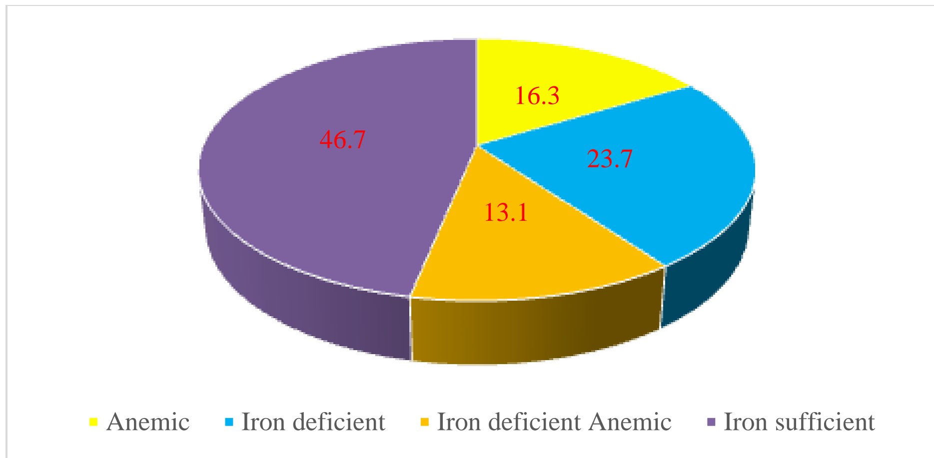

Figure 1: Iron Status of the Children

Table 5: Classification of school children according to iron status(N=312).

Variables	Sector			p-value	Gender			p-value
	Urban	Rural	Total		Male	Female	Total	
Anaemia (Hb<11.5g/dl)	12(7.5)	39(25.8)	51(16.3)	0.003*	20(12.9)	31(19.7)	51(16.3)	0.032*
Iron Deficient (Hb≥11.5g/dl &SF <12µg/l)	30(18.6)	44(29.1)	74(23.7)	0.022*	32(20.6)	42(26.8)	74(23.7)	0.041*
Iron Deficiency Anaemia (Hb<11.5g/dl &SF <12µg/l)	22(13.7)	19(12.6)	41(13.1)	0.013*	17(11.0)	24(15.3)	41(13.1)	0.022*
Iron Sufficient (Hb≥ 11.5g/dl & SF≥12µg/l)	97(60.2)	49(32.5)	146(46.7)	0.012*	85(55.5)	60(38.2)	146(46.7)	0.013*
Total	161	151	312		155	157	312	

3.3 Severity of some biochemical indices among the children

Table 6 shows the level of some of the indicators of iron in both urban and rural LGAs and this classification is necessary to show the actual status of the indicators. It shows that 80.0% of the male had a normal haemoglobin level and 12.3% are mildly deficient while 5.8% and 1.9% are moderately and severely deficient in haemoglobin respectively. As for the females, 71.3% had normal level of haemoglobin and 19.1% are mildly deficient while 5.7% and 3.8% are moderately and severely deficient in haemoglobin respectively. In urban LGAs, 83.2% had a normal haemoglobin level and 10.6% are mildly deficient while 4.3% and 1.9% are moderately and severely deficient in haemoglobin respectively. As for the rural LGAs, 67.5% had normal level of haemoglobin and 21.2% are mildly deficient while 7.3% and 4.0% are moderately and severely deficient in haemoglobin respectively. The overall haemoglobin status shows that 75.6% had a normal level and 15.7% are mildly deficient while 5.8% and 2.9% are moderately and severely deficient in haemoglobin respectively. There is a significant difference in heamoglobin status across the sector (p=0.032) and gender (p=0.040).

Parked cell volume (PCV) was at low level in 19.4% of the males and 34.4% females while 21.1% and 33.1% had a low level of PCV in urban and rural LGAs respectively, with a significant difference in both the sector (0.024) and gender (0.032). Serum ferittin was deficient in 25.2% of the males and 34.4% females while in the LGAs, deficiency of ferittin was noticed in 22.4% of the children in urban and 37.7% in rural LGAs. Reticulocyte which is an immature red blood cell was low in 18.7% and 24.2% of male and female respondents and noticed to be high in 14.2% and 17.2% of male and female respondents respectively. It also shows that 16.1% and 27.2% have a low level of Reticulocyte while it was high in 14.3% and 17.2% in urban and rural LGAs.

Table 6: Severity of some biochemical indices among the children

Variables	Sector			p-value	Gender			p-value
Heamoglobin (mg/dl)	Urban	Rural	Total	0.03*	Male	Female	Total	0.040*
Severe	3(1.9)	6(4.0)	9(2.9)		3(1.9)	6(3.8)	9(2.9)	
Moderate	7(4.3)	11(7.3)	18(5.8)		9(5.8)	9(5.7)	18(5.8)	
Mild	17(10.6)	32(21.2)	49(15.7)		19(12.3)	30(19.1)	49(15.7)	
Normal	134(83.2)	102(67.5)	236(75.6)		124(80.0)	112(71.3)	236(75.6)	
PCV(%)				0.024*				0.032*
Low	34(21.1)	50(33.1)	84(26.9)		30(19.4)	54(34.4)	84(26.9)	
Normal	127(78.9)	101(66.9)	228(73.1)		123(80.6)	103(65.6)	228(73.1)	
	161	151	312		155	157	312	
Ferittin(ng/ml)				0.021*				0.051
Deficient	36(22.4)	57(37.7)	93(29.8)		39(25.2)	54(34.4)	93(29.8)	
Normal	125(77.6)	94(62.3)	219(70.2)		116(74.8)	103(65.6)	219(70.2)	
Reticulocyte				0.001*				0.122
Low	26(16.1)	41(27.2)	67(21.5)		29(18.7)	38(24.2)	67(21.5)	
Normal	112(69.6)	84(55.6)	196(62.8)		104(67.1)	92(58.6)	196(62.8)	
High	23(14.3)	26(17.2)	49(15.7)		22(14.2)	27(17.2)	49(15.7)	
	161	151	312		155	157	312	

[a] WHO, 2001; [b] Tatala et al, 2004; [C] Thurnham et al, 2010 and [d]Thurnham et al, 2010

3.4 Severity of Markers of inflammation and immune function of the subjects

Table 7 shows the maker of inflammation and immune function of the respondents. In the LGAs, the CRP was at low risk in 72.7% of urban and 68.2% of the rural respondents while mild risk in 13.7% urban and 17.9% of the rural but at high risk in 13.7% of urban and 13.9% of the rural children. As for the gender, it shows that 71.0% males and 70.1% females were at low risk while mild risk was noticed in 17.4% males and 14.0% females but 11.6% males and 15.9% females were at high risk without any significant difference in both gender and sector. The CD4 count which is a determinant of immune status appeared to be low in 17.4% and 15.9% in urban and rural local government area while the respondents with low CD4 are 16.8% males' and16.6% females.

Table 7: Severity of Markers of inflammation and immune function of the subjects

Variables	Sector			p-value	Gender			p-value
CRP	Urban	Rural	Total	0.211	Male	Female	Total	0.121
Low risk	117(72.7)	103(68.2)	220(70.5)		170(71.0)	110(70.1)	220(70.5)	
Mild risk	22(13.7)	27(17.9)	49(15.7)		27(17.4)	22(14.0)	49(15.7)	
High risk	22(13.7)	21(13.9)	43(13.8)		18(11.6)	25(15.9)	43(13.8)	
	161	151	312		155	157	312	
CD4				0.113				0.342
Low	28(17.4)	24(15.9)	52(16.7)		26(16.8)	26(16.6)	52(16.7)	
Normal	133(82.6)	127(84.1)	260(83.3)		129(83.2)	131(83.4)	260(83.3)	
	161	151	312		155	157	312	

Table 8 shows the correlations between socio economic status, markers of iron status, inflammation and infection. Haemoglobin correlated positively with childs age (r= 0.144, p= 0.05) and average annual income of the family(r=0.132, p=0.05), serum ferritin correlated positively with mothers age and household size (r= 0.159, p= 0.05; r= 0.030, p= 0.05). CRP positively correlated with annual income(r= 0.155, p= 0.05), PCV correlated positively with mothers education (r= 0.180, p= 0.01). CD4 positively correlated with mothers age and education (r= 0.252, p= 0.05; r= 0.142, p= 0.01). There was no significant correlation between reticulocyte counts and socio demographic parameters.

There were significant relationships between white blood differentials and socio demographic parameters. Neutrophills related positively with childs age, mothers education and water source (r= 0.101, p= 0.05; r= 0.175, p= 0.01; r= 0.588, p= 0.01), lymphocyte correlated positively with mothers age (r= 0.202, p= 0.01) and fathers occupation (r= 0.511, p= 0.01) while monocyte related positively to water source (r=0.103, p= 0.01) but eosinophills and basophills has no significant correlation with the socio demographic parameters.

Table 8: Pearson's Correlations between socio economic status, markers of iron status, white blood differentials and makers of infection.

Variable	Childs age	Mothers age	Mothers Education	Fathers Occupation	Annual Income	HH Size
Hb	0.144*	-0.059	0.055	-0.167	0.132*	0.039
SF	-0.120	0.159*	-0.110	0.215	0.062	0.030*
CRP	0.107	0.105	0.070	-0.040	0.155*	0.103
PCV	-0.068	-0.005	0.180*	-0.041	0.001	-0.015
CD4	-0.120	0.252*	0.142**	0.005	0.144	0.131
RETICS	0.012	-0.156	0.027	0.116	0.103	-0.088
WBC	0.127*	0.030	0.110*	0.109	0.163*	0.067
NEUT	0.101*	-0.011	0.175**	-0.014	0.173	0.124
LYMP	-0.078	0.202**	-0.043	0.511**	-0.049	-0.109
MONO	-0.026	0.071	0.002	-0.020	0.008	-0.010
EOSINO	0.171	0.080	-0.027	-0.007	0.101	0.039
BASO	-0.008	0.080	0.030	-0.007	0.048	0.144

**Correlation coefficient is significant at the 0.01 level (2- tailed

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

4. Discussion

The purpose of the study was to assess iron status, marker of inflammatory and immune functions of school age children in selected schools in Ogun states, Nigeria. Malnourished children have increased risk of dying, with most deaths caused by infectious diseases and one mechanism behind this may be impaired immune function (26) and that is the more reason why it is necessary to assess the immune status of the children in relation to iron and markers of inflammation, so that it can be easily established from the outcome of the study if all these variables are related or not.

Iron deficiency has been reported to be the most prevalent nutritional deficiency in the world (4). A considerable number of children are anaemic in this study but it is not surprising to note that more than one third is as a result of iron deficiency (Of all the children in the study, 16.3% were anaemic (Hb < 11.5 g/dL), 23.7% iron deficient (Hb ≥ 11.5 g/dL and SF < 12 μg/L) and 13.1% had iron deficiency anaemia (Hb < 11.5 g/dL and SF < 12 μg/L)). This high rate of anaemia and iron deficiency anaemia in the present study may be indicative of the fact that the diet of the school age most especially in the rural LGAs are not adequate for their iron needs. Observations and brief interviews of the people living in this rural areas during the data collection revealed that "garri" (cassava flakes) is their most common staple food with no form of animal protein. Farming is the major occupation and only crops that are likely to yield some income are planted. The level of income of family breadwinners is also low, judging by their houses and the yield from their farms. Their financial access to meat and other good animal sources of iron is therefore very limited. Considering other biochemical parameters under study, it can be easily concluded that though anaemia affect both urban and rural LGAs but the majority of those affected in urban may likely not be as a result of diet but other factor like infections and all these factors come into play in the rural LGAs, that is both diet and infections contributed to the level of anaemia in rural LGAs. It is worthy to note that the public health effects of iron deficiency and anemia include reduced work capacity and mental performance, poor growth development, impaired regulation of body temperature, impairments in behavior and intellectual performance, and decreased resistance to infections (42). However, the values of aneamia and iron deficiency in this study are closer to that reported by Achouri *et al.* (43). Other studies (22), have reported higher prevalence of anemia and iron deficiency among children. Another study by Ughasoro *et al.* (35) report the prevalence of anaemia and iron deficiency anaemia (IDA) to be 49.2% and42.3%. Also, Onyemaobi *et al*. (44) reported 48.8% iron deficiency among school age while Anumdu *et al.* (46) reported 63% aneamia among children. Poor nutrition especially iron deficiency in school-aged children is associated with retardation of growth and poor cognitive development (28). According to other study, school age children are at risk of iron because of an expanding red cell and muscle mass (53). In other to show the severity of the iron deficiency in the subjects, the heamoglobin concentration was further classified into severe, moderate, mild and normal according to World Health Organization classification (38). The result indicated that a good number of the children were severely and moderately anaemic with more in rural areas and female gender. The value reported in this study was lower than what was reported on the severity of anaemia among schoolchildren (6–15 years) in rural Nigeria by Rufina *et al.*(25) but consistence with the study of Onimawo and colleagues where the overall prevalence of anaemia was 82.6% with rates of mild, moderate and severe anaemia being 9.6%, 71.6% and

1.4%, respectively and all the subjects that had severe anaemia were females while none of the males had severe anaemia (23). Thando *et al.* (32) also reported 51.2% and 41.9% for mild and moderate forms of anaemia respectively, while severe anaemia was 2.3%. A jointly sponsored study in 2001 by WHO/UNICEF/UNN reported varying degrees of anaemia with 38.0% mild anaemia, 31.8% were moderately anaemic and 0.8% was severely anaemic (39). Another study on 15450 children attending the Korle Bu Teaching Hospital, Accra showed that 71.1% of the children had haemoglobin (HB) levels below 11.0 Gm/dl while 27.7% of anaemic patients had Hb levels below 7.0 gm/dl (48). Indeed, 71.1% of children with severe anaemia had Hb levels below 5.0 gm/dl, thus requiring urgent blood transfusion (48). Alongside, is the study of Ahumareze et al, in which the prevalence of anaemia in the study was 54.2%; where severe anaemia, mild and moderate anaemia was 1.2% and 53.0% respectively (49). However, low haemoglobin may not be totally a specific indicator for anaemia because it is also influenced by blood depleting parasites, chronic infections and haematological conditions (8, 55) and that is the reason for considering other parameter like PCV. It is however, interesting to note that majority of the children that are severely anaemic were females. This suggests that anaemia may not only have occurred as a result of low haemoglobin but may have been influenced by other factors like monthly menstrual period.

Serum ferritin (SF) concentration has been identified as the most specific biochemical test that correlates with relative total body iron store, hence is a precondition for iron deficiency in the absence of infection (30). Low levels of SF were used to indicate iron depletion (27). The result of the study shows that almost one third of the children had a low level of ferittin indicative of low iron stores in the body. There was significant difference in serum ferritin values in rural LGAs and urban LGAs but more female as compared to male were anemic. This results obtained in this study is at variance with the studies of Thando *et al*.(32) and that of Onabanjo *et al*. (22) that assessed the anthropometric and iron status of Adolescents in Ogun State. The serum ferritin values reported by Onimawo *et al*. (23) are lower than the present study.

C-reactive protein (CRP) is a marker of infection or inflammation in the body. It is released into the blood by the liver shortly after the start of an infection or inflammations as an early indicator of these problems and its levels can rise quickly. CRP and Serum ferritin when used in combination showed the best agreement with body iron stores (32). In the present study, result shows that CRP was elevated in a very close number of the subjects both in urban and rural LGAs which could be due to inflammation or infection and also more female gender than the males were affected. Elevated level of CRP (above 20mg/l) in this study is a pointer to the fact that malaria is still endermic in most of the areas featured in the study and other parasites like hookworm still affects good number of subjects in both urban and rural settlements.

The overall percentage of the subjects with elevated CRP in this study is close to the value reported by John et al. (15) in which raised CRP levels was identified in 16% of the children. The results obtained in this study is similar to works of others studies, (7, 10,34) that found CRP to be higher in girls than in boys. Conversely, Colantonio *et al.* (6) reported no gender differences in CRP values of children.

Reticulocyte are young, anucleate erythrocytes, which are released from bone marrow into the blood in increased numbers as a response to anemia caused by hemolysis (destruction) or loss (hemorrhage) of erythrocytes. Detection and identification of immature anucleate RBCs verifies whether the bone marrow is responding to the anemia by increasing RBC production in a regenerative response. Reticulocyte hemoglobin content is a reliable and early indicator of bone marrow iron status and may detect functional iron deficiency with more sensitivity than biochemical parameters (59). Low values indicate a low production of red cells possibly due to nutrient deficiency, whereas high values indicate a high production of reticulocytes to replace lost blood and healthy hematopoiesis. The result shows that more subjects in the rural LGAs have low level of Reticulocyte as compared to urban subjects and this may likely be as a result of nutrient deficiency and this result is in line with the study of Foy *et al*. (11) that reported reticulocyte hemoglobin content to be significantly lower in the blood donor group in their study, but it is not surprising that there are also subjects from urban LGAs that are anaemic because it reflected from the result that good number of subjects from both urban and rural LGAs had higher number of Reticulocyte than normal range which is very likely to be a result of other factors like infections apart from diet. The result of Reticulocyte heamoglobin content is a pointer to the fact that the rate of anaemia recorded in this study is not solely as a result of inadequate diet but other factors also come into play, more subjects had a low level of Reticulocyte in rural which is suggestive of inadequate diet but also an increase in the level of

Reticulocyte was noticed in almost the same number of children in both urban and rural LGAs which is indicative of other parasitic infections and this is supported by the result of CRP in this study that reflects almost the same number of children in both urban and rural with elevated level of CRP. The implication of this is that the high level of anaemia in this study is not only as a result of diet but also infection plays a major role.

It also show from the study that high family size increases the risk of infection, the odds of infection for high family sized were 2-fold higher than children with low family size and it is in line with the study of Berhanu (58). Personal hygiene greatly reduces the burden of infections. This finding agrees with finding from different parts of the world (60). This is due to the reason that proper personal hygiene breaks the chain of parasite transmission. The presence of infections may have multiple effects among children including physical and mental developments. The presence of chronic and heavy parasitic infection may cause intestinal bleeding, malabsorption of nutrients, nutritional deficiency, destruction of cells and tissues and other associated effects. The overall effect of these results in growth retardation reduced mental development, school absenteeism, low academic performance, susceptible to malnutrition and infection (5).

Packed Cell Volume is the proportion of the blood volume occupied by RBCs and is determined by cell number and size. Concentrations below the reference range may indicate abnormal cell development. In this study, about one third of the children had values below the reference range for PCV using WHO criteria but more of the children with low PCV are from rural LGAs and also more female subjects were affected. The results obtained in this study are similar to the finding of other study that reported 87.1% of the subjects been anaemic using PCV and also went further to categorized PCV values into mild anaemia, moderate anaemia and lastly severe anaemia (23) The result showed that PCV is positively correlated to CD4 (r= 0.05) and the implication is that when the immune system is been compromised there is every likelihood that the PCV level becomes low.

The CD4 count is like a snapshot of how well the immune system is functioning. CD4 cells (also known as CD4+ T cells, T-lymphocytes, or helper cells) are white blood cells that fight infection. It gives an indication of the healthy immune system and is the body natural defense system against pathogens, infections and illnesses. In this study, about one quarter of the children had a low level of CD4 with close values for male and female. This observation is similar to the report of Olga et al (2010) that assessed immune status and enzymes activity in blood lymphocytes in adult patients where $CD4^{+}$-lymphocytes content was lower with increased Ig M and Ig G concentration. It is interesting to note that several studies have stated the link between low level of CD4 and anaemia may not necessarily be diet but majorly due to infection. Some of the studies include that of Hughes *et al.* (41) which reported that total white blood cell and lymphocyte counts in peripheral blood are not decreased in malnourished children, and granulocytes are frequently elevated. Likewise, T-lymphocytes and CD4 counts appear normal in malnourished children. Their levels seem to be determined more by infections than by nutritional state, and do not reflect the degree of malnutrition-related immune deficiency, as high infectious mortality is seen in malnourished children, despite unaffected white blood cell counts (41). Other studies in support of this finding is the studies by Keusch *et al*, (62) which associated a decline in CD4 to result from high pathogen load rather than nutrient deficiencies, and thus primarily a cause of malnutrition, particularly of stunting. The result of the study clearly shows a weak correlation between immune status (CD4) and iron deficiency anaemia which implies that most of the subject that are anaemic in this study may not necessarily have a compromised immune system due to inadequate diet but because certain infection might have set in due to other factors. However, when data for anaemic participants were analyzed, there was a weak positive correlation between Hb concentration and CD4 count (r = 0.249). Results from this present study therefore suggest that Hb concentration may not be a suitable predictor of immune status (based on CD4 count). Results of more recent studies have supported the non-suitability of Hb concentration for predicting CD4 count (63, 64, 65). Some other studies have however reported that Hb concentration can increase the sensitivity of total lymphocyte count in predicting CD4 count (66), but this was not explored in the study. Nevertheless, food availability is still of vital importance in the study of nutritional status and their relationship with low CD4 cell outcomes since hunger is often a barrier (14); perhaps, the association between the nutritional status and immune status should be uniquely observed in populations where extreme malnutrition is persistent and thus stronger correlations can be deduced.

5. Conclusion

The following can be concluded from this study:

- The socio-economic status was very poor among the children who participated in the study, particularly in rural areas.
- The study revealed high prevalence of iron deficiency (23.7%) and aneamia (16.3%) with 13.1% of the prevalence of aneamia caused by iron deficiency among the children. This is also significant ($p<0.05$) for gender and sector differences in the iron status.
- Data on immune markers (CD4 and white blood differentials) showed that more females had lower values than males. About 16.7% of the children had a low level of CD4 though more in rural with no significant difference among male and female ($p = 0.015$).
- The result of Reticulocyte (21.5%), ferittin (29.8%), C-reactive protein (13.8%) and PCV (26.9%) also buttress the fact that some of the children health status has been affected which could in turn leads to a compromised immunity.
- The study revealed significant relationship between markers of iron status, and makers of inflammation and infection in the children that participated in the study.

6. Recommendations

Based on the findings of the study, the following recommendations were made:

- Utmost care and attention must be focused on these socioeconomically and disadvantaged children living most especially in the rural areas. Sincere efforts must be undertaken to make a significant impact on child's nutrition with multipronged approach such as giving priority to education especially for women, creating awareness regarding benefits of factors like limiting family size, proper storage of drinking water, and so forth, and providing toilet facility in the household.
- Appropriate investigations for iron status and inflammation/infection screening, need to be integral in the evaluation of anaemia and its causes before anaemia control interventions are implemented. Interventions that target the multifactorial nature of anaemia in school-aged children need to be strengthened. Additionally, regular screening of anaemia in school-aged children from disadvantaged communities is recommended.
- Advocacy for fortification of food with the essential micronutrients should be stepped up to assist in meeting the children requirements of these micronutrients in their diet for healthy development.

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