METHOD

Method for HIV screening was as previously described by Onyenekwe et al [7]. The Bromocresol Green (BCG) method was used to determine serum Albumin concentration, while the Haemoglobin concentration was determined by Cyanomethaemoglobin method.

Detection of UIBC and TIBC: The UIBC and TIBC were determined using the ferrozine method. The procedure for the detection of UIBC and TIBC was as described by the manufacturer of the iron/TIBC reagent (TECO Diagnostic, Anaheim, USA). In brief, the test tubes for the detection were pre-treated with HCL and rinsed several times with de-ionised water after which the test tubes were appropriately labelled as participants, standard and blank tubes. Into the respective tubes was added 2.0ml of unsaturated iron binding capacity buffer reagent. While 1.0ml of iron free water was added to the blank tube, 0.5ml of iron standard solution plus 0.5ml of iron free water were added to standard tube and 0.5ml of the respective participants samples plus 0.5ml iron standard solution were added to the appropriately labelled participant's tubes. The reagent blank was used to zero the spectrophometer at 560nm wavelength. Then the absorbance of the participant's test samples was read and recorded as A_1 reading. Then 0.5ml of iron colour reagent was added to the content of all the tubes respectively; and the tubes were placed in a heating bath at 37°C for 10 minutes. The reagent blank was again used to zero the spectrophometer at 560nm before a second reading was taken as A_2 for the respective participants tests and standard. The under listed formula was used to calculate UIBC.

UIBC (ug/dl) = **Conc. of STD - (A_2 test - A_1 test)/ (A_2 S-A_1 S) x Conc. of STD/ 1**

TIBC ug/dl = serum iron concentration + UIBC

Detection of serum iron: The serum iron was determined photometrically using Iron/TIBC reagent (TECO diagnostics, Anaheim, USA). The principle of the test is based on the dissociation of iron from the transferrin complex by the addition of acidic buffer containing hydroxylamine. The procedure was as described by the manufacturer of the kit. In brief; the test tubes were pre-treated with HCL and rinsed severally with de-ionised water. Into the appropriately labelled participant's tubes, standard tube and blank tube were added 2.5ml iron buffer reagents. This was followed by addition of 500ul of participant's sample to respective tubes. Similarly, 500ul of iron free water was added to the blank tube while 500ul of iron standard was added to the standard tube. The blank solution was used to zero the spectrophotomer at 560nm. The initial absorbance of the standard and respective participants tests was read A_1. Thereafter, 0.55ml of iron colour reagent was added to all the tubes. The contents of the tubes were mixed properly and then placed in the water-bath at 37°C for 10 minutes after which the blank solution was used to zero the spectrophotometer 560nm and the absorbance of all the tubes was read as A_2. Serum iron concentration was calculated from the formula:

Serum iron (ug/dl): $[A_2T - A_1T / A_2S - A_1S]$ *x conc. of STD*

STATISTICAL ANALYSIS

The variables were expressed as mean ±SD. The student-t test was used to determine significant difference in mean. Significant level was considered as $P<0.05$.

RESULTS

The mean ±SD serum iron concentration (ug/dl), TIBC (ug/dl) and Albumin (g/l) were significantly lower in Asymptomatic HIV subjects compared with control participants ($P<0.01$ in each case). However, the mean ±SD UIBC (ug/dl) and Haemoglobin (g/dl) were not significantly different from both groups. See table 1.

Sex differentiation was only observed in both control and HIV participants with haemoglobin. No other parameter showed sex differentiation. See table 2 and table 3.

DISCUSSION

A significant drop in serum concentrations of iron in HIV infected subjects was observed in the present study. This could be an indication that they may be susceptibility to anaemia. Studies have reported high prevalence of anaemia in sub-Saharan Africa that was linked to under-nutrition [1,2]. Anaemia has serious impact on the quality of lives of HIV/AIDS patients.

The reduced serum iron and TIBC in HIV infected subjects in the present study, suggest anaemia of chronic disease. In most Health Institutions in Nigeria, Haemoglobin level and the packed cell volume are the most commonly used makers' for detection of iron status and iron deficiency anaemia. However, In the present study we did not observe any significant difference in Haemoglobin level between the HIV infected subjects and control subjects. If in the present study we had relied on Haemoglobin level alone to determine iron status in HIV infected subjects, we would have missed the diagnosis of anaemia.

Studies have reported that two or more serum iron makers should be used to determine the presence of anaemia [8,9,10]. The use of Haemoglobin with other parameters as TIBC or UIBC and percent transferrin saturation has been suggested. The use of two or more serum iron makers could also help to identify the presence of excess free iron and iron overload. The mean serum albumin concentration was lower in HIV positive subjects. The reduced serum albumin level in HIV infected participants could be due to suppression of its synthesis in the liver. It could also be due to the impact of HIV infection on their host ability to utilize nutrients intake. Serum albumin levels have been shown to be useful in predicting mortality due to HIV infection [7,11,12,13].

The study concludes that HIV infection predisposes to anaemia of chronic disease. Therefore, we suggest that blood iron status be monitored in HIV infected subjects for early detection and treatment of anaemia in order to reduce the morbidity and mortality associated with anaemia. Furthermore, the strength of Haemoglobin as an indicator of anaemia is weak and may lead to missed diagnosis of iron status.

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TABLE 1 : Mean ± SD Serum Concentrations of Iron ((µg/dl) UIBC (µg/dl), TIBC (µg/dl) Albumin (g/l) and haemoglobin (µg/dl) in HIV seropositive group and control group.

Parameters	HIV (n=76)	Control (n=30)	P-value
Serum iron (µg/dl)	88.51± 45.62	210.38± 78.94	(P<0.01)
UBIC (µg/dl)	204.59± 84.57	243.89± 112.53	(P>0.05)
TIBC (µg/dl)	293.36±76.91	444.26 ±122.46	(P<0.01)
Albumin (g/l)	32.86 ± 4.98	37.60± 3.32	(P<0.01)
Hb (g/dl)	11.3±2.0	11.5±1.1	(P>0.1)

Key: Hb = Haemoglobin
HIV = asymptomatic HIV with and without malaria co-infection

Table 2: Sex Distribution of Mean ±SD Serum Concentrations of Iron ((µg/dl) UIBC (µg/dl), TIBC (µg/dl), Albumin (g/l) and haemoglobin (µg/dl) in HIV Seropositive Group

Sex	Male (n=36)	Female (n=40)	P-value
Serum iron	96.17±49.83	81.61±40.87	(P>0.1)
UIBC	199.29±79.40	209.36±89.71	(P>0.1)
TIBC	296.00±68.75	290.00±84.39	(P>0.1)
Albumin	33.88±3.71	31.94±5.78	(P>0.1)
Hb	11.8±1.7	10.8±1.9	(P<0.05)

Table 3: Sex Distribution of Mean ±SD Serum Concentrations of Iron ((µg/dl) UIBC (µg/dl), TIBC (µg/dl), Albumin (g/l) and haemoglobin (µg/dl) in Control Group

Sex	Male (n=15)	Female (n=15)	P-value
Serum iron	233.05±83.16	187.71±69.95	(P>0.1)
UIBC	220.22±110.22	267.55±113.49	(P>0.1)
TIBC	433.27±137.88	455.26±108.89	(P>0.1)
Albumin	37.65±4.18	37.54±2.187	(P>0.1)
Hb	12.1±1.0	10.9±0.9	(P<010)