Original Research Article

ESTIMATION OF SERUM MINERALS (ZINC, CALCIUM, PHOSPHORUS), TOTAL PROTEIN AND LIVER ENZYMES (ALT AND AST) IN HIV PATIENTS RECEIVING HAART IN FEDERAL MEDICAL CENTRE, KEFFI, NASARAWA STATE, NIGERIA.

Abstract

Aims: This study was aimed to estimate serum minerals level(calcium, zinc, phosphorus), total protein and liver enzymes (ALT and AST) in patients taking HAART at Federal Medical Centre Keffi, Nasarawa state. Nigeria.

Study design: This study engaged a cross-sectional study design to investigate the liver enzyme alterations and the level of serum minerals in patients taking HAART when compare with treatment naïve (Pre-HAART) controls.

Place and duration of the study: The study was conducted in Federal Medical Center Keffi, Nasarawa State-Nigeria. Data collection spanned a specific duration from December, 2022 to March, 2023.

Method: A total of 143 subjects were involved in the study of which were 59 HIV positive patients on HAART,34 Pre-HAART patients and 50 healthy subjects served as controls. 5ml venous blood sample was collected after taking aseptic precaution from the study subjects into plain vacuum tubes; sample was left for 30 minutes at room temperature and centrifuged at 3000 rpm for 4 minutes. Sero-negativity of the control (healthy patients) was confirmed by HIV TRI-DOT test. Serum total calcium, serum phosphorus, serum zinc.Total proteins were estimated using spectrophotometer while AST and ALT were estimatedbycolorimetric method.

Result: The study indicated that both pre-HAART and HAART patients showed significantly higher (P<.05) AST and ALT activities compared to sero-negative patients. Pre-HAART patients had lower Zinc (11.22±4.86) and TP (43.18±22.52) levels than sero-negative patients, with Zinc (16.58±4.20) and TP (65.47±12.79). In contrast, HAART patients experienced a significant increase (P<.005) in Zinc (14.29±3.41) and TP (61.56±18.87) levels compared to pre-HAART patients (11.22±4.86) and (43.18±2.52). No significant difference in serum calcium level in all groups. Patients on HAART and sero-negative patients displayed normal Phosphorus level which is significantly higher in pre-HAART patients.

Conclusion: This study concluded that the serum level of ALT and AST were increased in HIV positive patients on HAART and pre-HAART patients. HAART improved level of serum zinc and total protein.

Keywords: Antiretroviral therapy, Serum minerals,Serum protein, Liver enzymes.

1. INTRODUCTION:

Human Immunodeficiency Virus (HIV) is a retrovirus, a linear single-stranded enveloped RNA virus of the *Retroviridae* family which attacks immune cells called CD4 cells, which are types of T cells, thereby causing acquired immunodeficiency syndrome [1]. The key factors for HIV transmission are through sex, blood or blood product transfusion prenatally, and via transgenders. Since 2010 the new HIV infections and AIDs related death have reduced by 46% and 22% respectively [2]. The duration between primary infection (virus transmission) and progression to clinical symptoms average about 10 years[3] Renal failure and hepatotoxicity are common diseases in HIV infected patients characterized by proteinuria, hypoalbuminemia, rarely hypocalcemia and altered mineral metabolism to contribute for bone disease, cardiovascular diseases and any related diseases [4,5]. HIV uses nutrients such as vitamins and minerals for its replication. This may cause metabolic disorders in future life. Zinc, calcium, and phosphorus are important major elements. Abnormal metabolism of these is responsible for metabolic disorder [6]. Hence their maintenance is important. Highly active antiretroviral therapy (HAART) had significantly improved the quality of life of patients infected with human immunodeficiency virus[7].

Despite this positive effect, HAART give rise to adverse effects that may lead to discontinuation of the treatment [8]. Adverse drug reactions might be asymptomatic. Symptomatic adverse effects may result in treatment failure, drug resistance and regiment change [7,8]. Different drugs had distinct adverse effects. Liver enzymes elevation and low level of macronutrients are common problem that are encountered in patients taking HAART. Antiretroviral (ARV) drugs damage the liver cells by direct toxicity of the drug from its active metabolites. Duration of therapy and onset of liver disease provided a clue to the cause of liver injury. Liver injury may be predictable or unpredictable. In case of predictable liver injury, toxicity might be related with the dose of the drug[9]. From different studies the incidence of liver injury was different among different populations and different drug combination[10]. A study conducted in Cameroon the incidence of hepatotoxicity was variable [11].This indicated that ARV drugs have effects on the liver which might damage the liver due to the toxic effects of the drugs.

Although, many studies were conducted in different countries, they lack homogeneity in the magnitude and incidence of adverse effects of antiretroviral drugs. This might be due to

geographical and individual difference [12]. Therefore, in this study we aimed at investigating the liver enzyme alterations and the level of serum minerals in patients taking HAART when compare with treatment naïve (Pre-HAART) control at Federal Medical Centre Keffi, Nasarawa state, Nigeria. The findings on these parameters will be a yardstick in assessing the efficacy of HAART on these patients.

2. METHODS

2.1 Study area

This study was conducted at Federal Medical Centre Keffi. Keffi is one of the 13 Local Government Area in Nasarawa State, North Central Nigeria located about 50km from the Federal Capital Territory (FCT) Abuja it is located between latitude 8^{II} 85^{I} North of the equator and longitude 7^{II} 87^{I} East of the meridian with an altitude of 850 meters above sea level [13].

2.2Study Design and study populations

This study engaged a cross-sectional study design to investigatethe liver enzyme alterations and the level of serum minerals in patients taking HAART when compare with treatment naïve (Pre-HAART) controls.HIV sero-positive patients attending ART Clinic at Federal Medical Center Keffi were included in this research, and any patient that did not meet with the inclusion criteria was excluded from this research. Written informed consent was obtained from the clients, in which their confidentiality was assured.

2.3 Sample size calculation and sampling:

The sample size for the study was determined using Cochran's formula.

$$N = \frac{1.96^{2} \text{ X } P(1-P)}{e^{2}}$$

Where 1.96 = confidence level

P = prevalence of HIV Globally at 2 % [14].

N = Sample size

e = error of margin at 5% (0.05)

Therefore $N = \frac{(1.96)^2 \text{ X } 0.02(1-0.02)}{0.05^2}$

$$= \frac{(1.96)^2 \text{ X } 0.02(0.98)}{0.05^2}$$

$$= \frac{3.18416\text{X } 0.0196}{0.0025} = 30.1$$

n= 30.1

Attrition Rate

Attrition rate at 10% $= \frac{10}{100} \text{ x } 30 = 3$

The sample size = 30+3=33

Therefore, the overall minimum sample size = 93(33 on HAART, 33 pre HARRT and 33 zero negative)

2.4 Sample collection

8mls of blood samples were collected using vacutainers into a plain container. They were allowed to clot and retract. They were then centrifuged at 3,000 revolution per second. The supernatant was aspirated using pastures pipette into sterile plain containers, and then labelled appropriately. These were then stored frozen until analyzed. They were used for laboratory analysis of zinc, calcium, phosphorus, Total protein and liver enzymes.

2.5 Sample analysis

2.5.1 HIV Testing

HIV testing for the entire patient was performed using the current National Algorithm for HIV sero diagnosis. This involves the use of 3 rapid diagnostic kits, following the manufacturer instruction. The patient's serum was screened for the presence of HIV antibodies using Alere Determine and Uni-Gold Recombigen. When both show positivity, they were regarded as positive for HIV infection and vise versal.

A STAT-PAK Rapid test kit is used as a Tie breaker to confirm the result of the first two test kits when the results were discordant.

2.5.2 Calcium, phosphorus and zinc estimation

The analysis was carried out using commercially prepared spectrum diagnostic kits. 0-cresolphthalein complexone method for Calcium, Fiske and Subbarow method for Phosphorus, and 5-Bromo-PAPS method was used for Zinc the assay were performed spectrophotometrically usingGenrui-semiautomated chemistry analyzer [15].

2.5.3 Analysis of total protein

Total protein (TP) was analyst by colorimetry method (Biuret reagent) the concentration was estimated spectrophotometrically.

2.5.4 Analysis of Liver enzyme activity(ALT and AST)

Liver enzyme Aspartate Aminotransferase (AST/GOT) and Alanine aminotransferase (ALT/GPT) activities were assay by Colorimetric method using Genrui semi-automated chemistry analyzer

2.6 Statistical analysis:

The data was analyzed using statistical package for social science (SPSS) version 23. One way analysis of variance was used to determine mean group comparison. T-test was used to tell the level of significant of association between any two variables compared. P-value <0.05 was used to assess the level of significant of the assumed hypothesis.

2.7 Ethical clearance

Ethical clearance was obtained from the constitutional review board ethics and committee of Federal Medical Centre Keffi, Nasarawa State. Confidentiality and privacy were ensured through Federal Medical Centre Keffi.

3. Results

The study indicated that both pre-HAART and HAART patients showed significantly higher ($p<0.05$) AST and ALT activities compared to seronegative patients. Pre-HAART patients had lower Zinc (11.22±4.86) and TP (43.18±22.52) levels than seronegative patients, with Zinc (16.58±4.20) and TP (65.47±12.79). In contrast, HAART patients experienced a significant increase ($p<0.005$) in Zinc (14.29±3.41) and TP (61.56±18.87) levels compared to pre-HAART patients (11.22±4.86) and (43.18±2.52). No significant difference in serum calcium level in all groups. Patients on HAART and seronegative patients displayed normal Phosphorus level which is significantly higher in pre-HAART patients.

Table 1: Socio-Demographic Characteristics of the Study Participant

Parameters	Sero Negative (n = 50)	HIV Patients (n = 93)
Gender		
Male	24 (48%)	41 (44%)
Female	26 (52%)	52 (56%)
Age Group		
< 20	1 (2%)	1 (1%)
20 – 29	11 (22%)	8 (9%)
30 – 39	15 (30%)	23 (25%)
40 – 49	19 (38%)	31 (33%)
50 and above	4 (8%)	30 (32%)

Data available on table1, shown that majority of the patients are females which represent the 52% of the study populations.Moreover, most of the patients fall within the 40-49 age groups.

Table 2: Comparison of Measured Parameters of Pre- HAART-Patients with Controls

Parameters	Sero Negative (50) Mean ± SD	Pre-HAART (N=34) Mean ± SD	t-Value	P-Value
Zinc (µmol/L)	16.58 ± 4.20	11.22 ± 4.86	11.262	0.003
Calcium (mmol/L)	2.12 ± 0.37	2.09 ± 0.40	0.238	0.789

Phosphorus (mmol/L)	1.09 ± 0.30	1.59 ± 1.04	12.607	<0.001
TP(g/L)	65.47 ± 12.79	43.18 ± 21.52	17.473	<0.001
AST(IU/L)	20.17 ± 6.70	46.75 ± 40.08	11.850	<0.001
ALT(IU/L)	15.90 ± 7.10	29.65 ± 28.44	8.837	<0.001

P values<0.05 areconsidered statistically significant.
Values with the same superscript are not statistically significant
Key
TP = Total Protein
AST = Aspartate aminotransferase (AST/GOT)
ALT = Alanine aminotransferase (ALT/GPT)

From table 2 above, there were significant reduction in the levels of Zinc and TP. No significant change in Calcium, Phosphorus, AST and ALT were significantly elevated.

Table 3: Comparison of Measured Parameters of HAART-Patients with Controls

Parameters	**Sero Negative (50) Mean ± S.D**	**HAART (N=59) Mean ± S.D**	**t-Value**	***P*-Value**
Zinc (µmol/L)	16.58 ± 4.20	14.29 ± 3.41	5.262	0.606
Calcium (mmol/L)	2.12 ± 0.37	2.14 ± 0.43	0.238	0.789
Phosphorus (mmol/L)	1.09 ± 0.30	1.00 ± 0.27	0.617	0.245
TP(g/L)	65.47 ± 12.79	61.56 ± 18.87	0.438	0.532
AST(IU/L)	20.17 ± 6.70	36.91 ± 25.03	6.170	0.005 *
ALT(IU/L)	15.90 ± 7.10	32.20 ± 23.88	8.837	0.001*

P values<.05 areconsidered statistically significant.

From table 3 above, the values with the same superscript are not statistically significant. HAART restored the values of Zinc, Phosphorus and TP to normal (*P*>.05) when compared with Table 2.

Table 4: Comparison of Measured Parameter of HAART Patients with Pre- HAART

Parameters	Pre-HAART (N=34) Mean ± SD	HAART (N=59) Mean ± SD	t-Value	*P*-Value
Zinc (µmol/L)	11.22 ± 4.86	14.29 ± 3.41	9.252	0.006*
Calcium (mmol/L)	2.09 ± 0.40	2.14 ± 0.43	0.238	0.789
Phosphorus (mmol/L)	1.59 ± 1.04	1.00 ± 0.27	12.607	<0.001*
TP(g/L)	43.18 ± 21.52	61.56 ± 18.87	17.473	<0.001*
AST(IU/L)	46.75 ± 40.08	36.91 ± 25.03	11.850	<0.001*
ALT(IU/L)	29.65 ± 28.44	32.20 ± 23.88	2.612	0.743

P values<.05 areconsidered statistically significant.

As shown in table 4, the values with the same superscript are not statistically significant. There is significant increase in the serum level of zinc and TP of patients on HAART when compared with Pre-HAART patients. There is also significant reduction in the values of AST and phosphorus when compared with Pre-HAART. The value of ALT was not statistically altered but rather showed an increase from that of Pre- HAART.

Table 5: Gender Distribution of Measured Parameters of Pre-HAART HIV Positive Patients

Parameters	Pre-HAART HIV Positive Males (N=17)	Pre-HAART HIV Positive Females (N=17)	t-value	*P*-value
Zinc (µmol/L)	13.11 ± 3.51	15.31 ± 5.81	4.854	0.035*
Calcium (mmol/L)	2.15 ± 0.46	2.02 ± 0.33	0.327	0.571
Phosphorus (mmol/L)	1.44 ± 0.76	1.74 ± 1.27	1.384	0.240
TP(g/L)	81.54 ± 18.59	78.17 ± 20.77	0.018	0.895
AST(IU/L)	31.71 ± 37.77	26.52 ± 17.88	0.453	0.107
ALT(IU/L)	57.56 ± 53.72	37.51 ± 20.97	9.027	0.006*

P values<.05 areconsidered statistically significant.

The data available on table 5, shown that level of serum zinc in female Pre- HAART patients is significantly higher (*P*> .05) than that of the male counterpart while ALT is significantly lower (*P*<.05) in females when compared with the males.

Table 6: Gender Distribution of Measured Parameters of HAART Receiving Patients

Parameters	HAART HIV Positive Males (N=23)	HAART HIV Positive Females (N=36)	t-value	*P*-value
Zinc (µmol/L)	14.32 ± 3.66	13.87 ± 3.43	0.473	0.647
Calcium (mmol/L)	2.14 ± 0.51	2.15 ± 0.39	1.201	0.278
Phosphorus (mmol/L)	1.07 ± 0.30	0.96 ± 0.23	2.417	0.126
TP(g/L)	58.71 ± 16.41	51.83 ± 19.57	1.152	0.288
AST(IU/L)	53.16 ±49.77	40.50 ± 35.81	4.381	0.041*
ALT(IU/L)	35.93 ± 26.07	31.03 ± 25.69	0.055	0.816

P values<.05 areconsidered statistically significant.

Table 6, shown that the level of zinc, phosphorus, TP, AST and ALT in the males HAART patients were higher than that of the females' counterparts but not significant, while the AST is significantly higher (*P*<.05) in males than that of their females' counterparts

Table 7: Correlation of Measured Parameters of HIV Patients on Drug (HAART) with Control.

		Zinc(µmol/L) control	Calcium(mmol/L) control	$PO4^{3-}$ (mmol/L) control	TP(g/L) control	AST(IU/L) control	ALT(IU/L) control
Zinc(µmol/L)	*r value*	-0.183	0.187	0.213	-0.22	-0.235	-0.227
	p value	0.102	0.970	0.069	0.441	0.050	0.056
Calcium (mmol/L)	*r value*	0.042	0.172	-0.013	-0.091	-0.101	-0.180
	p value	0.387	0.116	0.464	0.265	0.242	0.105
$PO4^{3-}$ (mmol/L)	*r value*	-0.214	0.015	-0.085	0.026	0.042	0.006
	p value	0.067	0.460	0.279	0.428	0.386	0.484
TP(g/L)	*r value*	-0.198	-0.162	0.146	0.140	0.023	-0.087
	p value	0.084	0.130	0.156	0.166	0.436	0.274
AST(IU/L)	*r value*	0.102	-0.174	-0.007	0.023	0.089	-0.031

	p value	0.242	0.116	0.480	0.436	0.254	0.415
ALT(IU/L)	*r value*	0.120	0.165	-0.102	0.008	-0.031	-0.160
	p value	0.204	0.127	0.221	0.478	0.415	0.134

* Indicate significant difference at .05

Data available on table 7 indicates that there are no significant differences in the Correlation of the measured parameters of HIV patients on drug (HAART) with control. This shows that there is improvement among patients on drug.

Table 8: Correlation of Measured Parameters of HAART with Pre - HAART.

		Zn(µg/dl) Pre-HAART	**Ca^{2+} (mmol/L) Pre-HAART**	**$PO4^{3-}$ (mmol/L) Pre-HAART**	**TP(g/L) Pre-HAART**	**AST(IU/L) Pre-HAART**	**ALT(IU/L) Pre-HAART**
Zn(µg/dl) HAART	*r value*	0.201	-0.077	0.165	-0.087	-0.079	0.079
	p value	0.108	0.665	0.352	0.623	0.658	0.657
Ca^{2+}(mmol/L) HAART	*r value*	-0.042	0.018	0.405*	-0.107	-0.019	0.128
	p value	0.544	0.919	0.018	0.546	0.914	0.472
$PO4^{3-}$ (mmol/L) HAART	*r value*	0.054	0.048	0.208	0.080	-0.009	0.030
	p value	0.762	0.786	0.237	0.653	0.961	0.865
TP(g/L) HAART	*r value*	0.134	0.363*	0.134	0.039	0.046	0.258
	p value	0.450	0.035	0.450	0.828	0.798	0.140
AST(IU/L) HAART	*r value*	0.005	-0.242	-0.005	-0.238	0.087	-0.191
	p value	0.977	0.168	0.980	0.175	0.624	0.279
ALT(IU/L) HAART	*r value*	-0.070	-0.005	0.052	0.142	-0.003	-0.104
	p value	0.694	0.976	0.770	0.424	0.987	0.560

* Indicate significant difference at 0.05

Data available in table 8 shows the correlation between the parameters of patients on drug (HAART) with those before taking drug (pre-HAART) shown that there is significant different in calcium recorded among patients on drug (HAART) correlated with phosphorus recorded in patients not on drug (pre-HAART). Also, the total protein recorded among patients on drug is significantly difference to the value of calcium recorded among pre – HAART patients.

4. Discussion

The use of Highly Active Antiretroviral Therapy (HAART) is a significant breakthrough in managing patients with HIV. It has been proven to reduce early mortality rates caused by opportunistic infections and other effects of the disease. However, concerns have recently been raised about the impact of HAART on liver function markers such as Total Protein (TP), Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT), as well as other biochemical molecules like Zinc, Calcium, and Phosphorus. Therefore, our study was aimed at thoroughly investigating the therapeutic effects of HAART on these parameters in HIV-positive patients from Nasarawa State, Nigeria.

This study unequivocally demonstrates that HIV infection significantly reduces the level of Zinc (11.22±4.86) as compared to HAART (14.29± 3.41) and seronegative controls (16.58 ± 4.20). This finding is in line with previous research conducted by [10,16] which discovered that HAART therapy improves the level of Zinc within the first three months. It is noteworthy that Zinc levels in female patients before HAART were significantly (P<.05) higher than in male patients, which could be attributed to hormonal differences [16] reported that the monthly cycle affects the Zinc level, with lower levels observed during the luteal phase than the follicular phase. However, there were no significant differences in the serum level of calcium among HAART, pre-HAART, and seronegative patients. This contradicts the findings of [12], who observed a decrease in calcium levels in patients, and [17], which conducted a study in America and discovered an elevation in calcium levels in women on antiretroviral therapy.

The study found a significant difference in TP values between Pre-HAART patients (43.18±21.52), HAART patients (61.56±18.87), and seronegative patients (65.47±12.79) with a p-value of less than 0.05. This contradicts [18] findings but aligns with [8] Akinola *et al.*'s 2012 and [19]. The decrease in TP levels of Pre-HAART patients may be due to factors such as malnutrition, malabsorption, chronic infections, and opportunistic infections like tuberculosis, leading to significant weight loss and muscle wasting, as noted by [7].

Phosphorus is an indispensable structural component in DNA and RNA membranes, vital for metabolism and energy storage. Researchers have taken a keen interest in phosphate levels in HIV patients undergoing antiretroviral treatment. The study reveals that serum phosphate levels were higher in patients who had not yet commenced HAART (1.59± 1.04) as compared to those already on HAART (1.00 ± 0.27). This finding corroborates the research of [17] while

contradicting [20] who noted a decrease in serum phosphate levels in pre-HAART patients. The heightened levels of phosphorus in pre-HAART patients corrected by HAART could be attributed to co-infections such as hepatitis B and C or dehydration.

ALT and AST activities are crucial indicators of liver cell injury, which makes them highly valuable in diagnosing acute hepatocellular conditions. The study conducted by [4] found that the ALT activity in HAART patients was significantly higher (291±2849) compared to pre-HAART patients (32.20±23.88) and seronegative individuals ($P<.05$). It is important to note that ALT is mainly produced in liver cells, while AST is produced in several other tissues such as the heart, skeletal muscles, kidneys, brain, pancreas, lungs, and erythrocytes, as [11] highlighted in 2006. Whenever liver cell membranes are damaged, the activities of both AST and ALT increase in the plasma. The claims made by [4, 21] are supported by this discovery, demonstrating that patients taking HAART may indeed experience higher liver enzyme activities.

The AST activities in patients taking HAART are significantly lower (36.91±25.03) compared to those in the Pre-HAART group (46.75±40.08), as observed in our study. This finding is consistent with a previous study conducted by [22] which also reported a decrease in AST activity in women taking HAART. However, it contradicts a study by [8], which reported an increase in AST activity in patients receiving HAART.

It is essential to note that our study did not observe any severe forms of liver enzyme elevation. Nevertheless, other studies have reported varying degrees of liver enzyme elevation or hepatotoxicity resulting from ARV drugs, ranging from mild to moderate or severe. Numerous factors may potentially contribute to this issue, such as co-infection with the hepatitis virus, alcohol consumption, the particular drug regimen, the duration of treatment, and even the geographic location.

5. Conclusion

Antiretroviral drugs are effective treatments for HIV, organization worldwide recommend that everyone tested positive for the virus begin antiretroviral therapy (ART) as soon as possible. HAART can reduce the risk of HIV related complication, stop virus from progressing and prevent further transmission. It can also increase a person's quality of life and expectancy.

However, this positive impact of antiretroviral therapy also carries negative side effects. Prolong used of HAART can cause renal toxicity, hepatoxicity, weak bones or osteoporosis.

HAART improve levels of zinc and total protein in HIV patients, the phosphorus level that was high was corrected with HAART. This further strengthened earlier works that posited that HAART can improve life expectancy through repletion of micronutrients. This decrease in micronutrients and total protein that accompanies HIV infection suggest a potentially important role of nutritional supplementation and good nutrition in proper management of HIV/AIDs.

This study also confirmed an increased risk of Hepatotoxicity and the depletion of micro nutrients in HIV infected patients, a situation that could result to early deaths if HAART were not used.

Regular monitoring of transaminases is therefore recommended when HIV patients are being treated with HAART. This will help to decide on discontinuation of treatment if toxicity levels become too high.

Consent

All authors unanimously declare that written informed consent was obtained from the participants for publication of this study finding.

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