



Calcium and Total Bilirubin Levels in Patients Co-Infected with HIV and Hepatitis C Viruses in Lautech Teaching Hospital, Osogbo, South West Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author VOM designed the study, wrote the protocol and first draft of the manuscript. Author MAM carried out the laboratory investigations. Authors CAA and RAA contributed to literature review. All authors read and approved the final manuscript.

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ABSTRACT

Aim: One third of HIV patients are co- infected with HCV. As HIV patients live longer this co-infection and its complications such as liver cirrhosis, hepatic carcinoma, metabolic syndrome are emerging as major manifestations of the disease that need to be dealt with promptly in order to avoid a reduction of the positive effects of highly active antiretroviral therapy (HAART) on HIV/AIDS introduced in 1996. Another system that could be affected by co-infection is the skeletal system. It has been shown that HIV itself and in combination with HCV could lead to a reduction in

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bone mineral density (BMD) predisposing to pathological fractures. It is thus important to determine the state of calcium metabolism among our HIV/HCV patients in order to forestall negative impacts on our patients who have been stable on HAART for several years. The majority of our patients are on combination therapy of Zidovudine, Lamivudine and Nevirapine. The hepatic complications of HIV/HCV co-infection have been well established. In our previous studies signs of hepatic inflammation have been demonstrated by raised aspartate transaminase (AST) and alanine transaminase (ALT) levels. However in this study we wish to also demonstrate liver damage through estimation of bilirubin levels.

Methodology: Antibodies to HIV were determined using Unigold and determined. immunochromatographic device was used to detect anti-HCV. Total bilirubin and calcium were analyzed using vitros DT-60 card reader.

Results: The majority of our patients were female. In group I up to %80. There was a statistically significant elevation of total bilirubin levels in HIV/HCV co-infected patients when compared to HIV mono-infected patients. There were statistically significant changes in calcium levels between the groups

Conclusion: Information on HIV/HCV co-infection and its effects on calcium metabolism in this clinical instance appears to be scarce. Intensification of research is required to firmly establish the role of HIV/HCV co-infection on calcium metabolism in our clinical instance.

Keywords: Coinfection CD4 counts HIV/HCV calcium.

1. INTRODUCTION

Since the introduction of highly active antiretroviral drugs (HAART) in 1996 the management of HIV positive patients has improved dramatically with subsequent prolongation of life of these patients. However there has been the emergence of side effects and complications hitherto unseen as a result of these patients living longer. These include side effects of the drugs themselves, drug-drug interactions, co-infection with other viruses which share similar routes of infection notably hepatitis C virus and the predisposition to hepatic fibrosis, cirrhosis and carcinoma [1,2]. Also an important complication is what is now well recognized and referred to as the metabolic syndrome (Lipodystrophy) [3,4].

Complications of co-infection may include skeletal system. It has been reported that in HIV mono-infection hypocalcaemia could occur [5] which could result in osteoporosis and pathological fractures [6,7]. The role of GH/IGF-1 in modulating the availability of calcium in mono-infected HIV patients has been observed and documented. This is thought to be responsible for the HIV associated osteopenia [6]. HIV infection has also been associated with renal disease which is characterized by nephritic-stage proteinuria (>3.5 g/dl), azotaemia, hypoalbuminaemia and occasionally hypocalcaemia. Electrolyte abnormalities and altered mineral metabolism also may occur in patients with HIV/AIDS and have been found to

contribute to bone diseases, cardiovascular disease and other clinical problems. Further studies have shown that HIV infection alone is a cause of decrease of bone mineral density (BMD). Reasons for this are said to be multifactorial, including complex interaction between HIV infection, traditional osteoporosis risk factors exacerbated by consequences of chronic HIV infection (eg, poor nutrition and low weight), high rates of smoking and alcohol consumption, low vitamin D levels, and ART-related factors [7,8,9].

Various studies have also shown that HIV/HCV co-infection is associated with decrease in bone mineral density and increased incidence of fractures [10]. Calcium is the predominant mineral in bone, and crystals of calcium compounds give bone its hardness and strength. Individuals that are at high risk or that have been diagnosed with osteoporosis may need to consume up to 1,200 mg/day. In an observational study carried out in the veterans affairs clinic in the USA, findings revealed an increased incidence of fractures in HIV/HCV co-infected patients [11]. This study also related the degree of liver dysfunction to the tendency to develop osteoporosis and hence pathological fractures in HIV/HCV co-infected patient [11]. This makes the study further interesting, and could be described as vicious circle because it has been well established as previously mentioned that HIV/HCV co-infection has a particularly lethal effect on the hepatic system, citing liver complications as the most common

cause of death in co-infected patients [12,13] Thus far this article has stressed the harmful impact of HIV/HCV co-infection on the skeletal and hepatic systems. Markers usually used as indicators of liver damage include aspartate transaminase (AST), alanine transaminase (ALT) and total bilirubin [14,15,16]. In addition to determining calcium levels it was also decided to determine total bilirubin levels in these co-infected patients and this decision was reached due to the relationship between liver damage and skeletal abnormalities. In similar studies it was found that bilirubin levels were raised suggesting some degree of liver damage [14].

2. MATERIALS AND METHODS

Blood samples were collected from the 50 patients co-infected with hepatitis C and HIV, based on prevalence of 3.3% [17]. Fifty subjects living with HIV/AIDs who tested negative for hepatitis C virus were included concurrently. Finally 50 subjects sero-negative for hepatitis C and HIV were included as negative control subjects. Total number of participants n=150. Informed consent was sought from all patients. Also all those who consumed alcohol, intravenous drug users (IVDU) and on drugs other than HAART as prescribed by the physicians were not included in this study. None were cigarette smokers. Neither were any of the patients on anti HCV therapy and all are of African descent. By CDC criteria all patients are in stage A. All patients with tuberculosis were excluded. The significant clinical manifestations of our patients were dermatitis and thrush (oral and vaginal) and were otherwise stable.

For all subjects, information on prescribed medications and laboratory parameters was obtained from clinical and laboratory databases. Data on patient demographics, social practices, clinical and laboratory parameters, and prescribed antiretroviral and other medications were abstracted from charts by trained and authorized personnel. The study was approved by the ethical research committee of the Lautech teaching hospital, Osogbo, Osun state, Nigeria.

Setting: The study was carried out in Osogbo the capital of Osun state located in South West Nigeria. It is an urban setting with a population of 3,416,959. The residents are majorly Yoruba however there are other tribes including Hausas, Igbos and those of Edo state origin. The weather is typically tropical with periods of heavy rain fall alternating with the dry season.

Standard laboratory assessments were performed by licensed clinical laboratories, including, serum chemistry panels, alanine transaminase (ALT) levels, aspartate transaminase levels (AST), bilirubin levels, CD4 cell counts and calcium levels.

2.1 Inclusion Criteria

Group I Inclusion criteria: 1) HIV positive patients 2) HCV positive patients 3) Patients who give consent 4) Only patients managed in our facility 5) Patients on HAART

Exclusion criteria: 1) HCV negative patients 2) Patients who do not give consent 3) Patients not on HAART

Group II Inclusion criteria: 1) HIV positive patients 2) HCV negative patients 3) patients who gave consent 4) patients on HAART

Exclusion criteria: 1) HCV positive patients 2) Patients who did not give consent 3) Patients not on HAART

Group III Inclusion criteria: 1) HIV negative patients 2) HCV negative patients 3) Patients who give consent

Exclusion criteria: 1) HIV positive patients 2) HCV positive patients 3) Patients who did not give consent

3. RESULTS

The total number of participants was 150 (n=150). There were three groups of patients, those who were HIV positive and HCV positive (Group I), the second group in which they were HIV positive and HCV negative (Group II) and the third group which served as a control group where the patients were both HIV and HCV negative (Group III). For group I the median age was 40.64±9.39, while for group II 39.22±8.62 and for group III 39.84±10.00 years. In all groups the majority were female the highest percentage of them being in group I at 80%, group II 68% and 64% in group III respectively. Using the CDC criteria for staging and from the results of CD 4 counts we obtained all our patients were in stage A. The majority of the patients are on a combination therapy of Zidovudine, Lamivudine (3TC) and Nevirapine.

For group I the median calcium was 8.90 mg/dl, for group II where the clients were only HIV positive the median calcium was 7.10 mg/dl and in group III where they were negative for both viruses the mean calcium was 8.75 mg/dl. When

group I was statistically compared to group III there was no statistical significance. However when group II was compared with group III and group I was compared to group II there was statistical significance ($P=0.000$) (Table 3B).

For total bilirubin (B1) the median value for group I was 11.90 $\mu\text{mol/l}$, the median for group II was 7.10 $\mu\text{mol/l}$ and for group III 9.00 $\mu\text{mol/l}$. For conjugated bilirubin the median in group I is 6 $\mu\text{mol/l}$, in group II 5.90 $\mu\text{mol/l}$ and in group III 3 $\mu\text{mol/l}$. On closer analysis it was discovered that certain parameters were skewed (except calcium and age) hence the use of median and inter-quartile range was employed as a measure of central tendencies and spread respectively. As a result the Kruskal-Wallis method was used in place of analysis of variance (ANOVA) to examine the median differences in the groups in some instances (Table 4).

4. DISCUSSION

The majority of our patients are on HAART combination of lamivudine, Zidovudine and

Nevirapine. Initiation of HAART itself is thought to reduce the bone mineral density (BMD) especially within the first two years of therapy and has even been compared with the effects of early years of menopause in females on BMD. This is somewhat similar to the age range of our study groups in which the majority were female between 30 and 49 years of age (Table 2). The majority of our patients have been on HAART for at least three years. However this effect of HAART on BMD is thought to reach a plateau or even become insignificant after a period of time. HAARTs implicated in this include Zidovudine, Lamivudine, Lopinavir and Ritonavir. Zidovudine stimulates osteoclastic activity, and tenofovir has been shown to inhibit mineralization of bone [18,19]. Some studies suggested that this HAART related reduction in BMD is more pronounced in cases with lower CD4 + counts. Our groups (I and II) of patients had lower CD4 counts than control group III (Table 1). Also protease inhibitors (PIs) are thought to be a group of drugs with a propensity to reduced BMD [20,21,22].

Table 1. Summary statistics of measured parameters

Groups statistics	AST	ALT	CA	B1	B2	CD4
Group I						
25 Percentiles	7.00	4.7500	7.78	9.00	3.9750	2.8100E2
Median	10.00	6.0000	8.90	11.90	6.00	4.4850E2
75 Percentiles	23.50	12.25	9.20	20.70	15.00	6.7825E2
IR	16.5	7.5	1.40	11.70	11.03	3.97E2
Group II						
25 Percentiles	9.00	5.00	6.38	7.25	3.90	1.8750E2
Median	11.00	6.50	7.10	9.10	5.90	4.3550E2
75 Percentiles	14.00	10.25	7.63	11.00	6.30	8.0500E2
IR	5.00	5.25	1.26	3.75	2.40	6.18E2
Group III						
25 Percentiles	5.00	3.00	7.95	7.00	2.00	8.1550E2
Median	6.50	4.00	8.75	9.00	3.00	9.2150E2
75 Percentiles	9.25	7.00	9.17	12.00	4.00	1.0655E3
IR	4.25	4.00	1.22	5.00	2.00	2.50E2

Table 2. Showing the age range and gender

Variable	Group 1	Group 2	Group 3
Age	n=50	n=50	n=50
≤20 – 29 yrs	7	3	6
30 -39 yrs	16	26	21
40-49 yrs	16	16	16
50-59 yrs	11	3	4
≥60 yrs	0	2	3
Sex			
Male	10	16	18
Female	40	34	32

It has been suggested that middle aged Caucasian (non black) men are more predisposed to the factors that could lead to reduced bone mineral density than black men are [23]. Unfortunately we were not in a position to test this factor in our study as all our patients are Negroid and of African descent. On the issue of age and gender, majority of our patients were female mostly between 30-49 years (Table 2). There could be an influencing factor of effects of early menopause on the skeletal system (osteopenia and osteoporosis) in females contributing to the higher incidence of the female sex within our statistically significant study group (Table 2) but the contribution of menopause is likely to be only part of the story because of the age range of our study group in our clinical instance which could in this case be compared to what obtains in the west [24,25]. This is taking lower calcium levels contributing to osteoporosis.

A lot of emphasis has been placed on shared routes of infection [26], and the effect of HIV/HCV co-infection on the hepatic system. It has been shown in other reports that liver enzymes (AST, ALT) and their elevation are serious markers of hepatic inflammation which may result in fibrosis, cirrhosis and carcinoma [16]. Our patients had raised levels of liver

enzymes (Table 1) compared to the control and as such are candidates for these stages of liver pathology. The majority of patients co-infected in this study (Fig. 1) were between 30 and 49 years (Table 2). In a study on epidemiology and co-infection carried out by Victoria et al in 2010 the majority of patients fell between 25 and 40 years of age [26]. This similarity could be due to the sustained sexual activity within this reproductive age group of the population. The majority of our patients were women (Fig. 2), in contrast to a study carried out in Brazil in which the majority of patients were male [26]. However in another study it was discovered that HIV positive women who were infected with hepatitis C and on therapy were more likely to experience adverse events (AE) than men [27] this may include skeletal pathology.

Extensive research employing popular medical search engines including Pub Med revealed that there is a paucity of information on calcium metabolism in HIV/HCV co-infected patients in this clinical instance. However analyzing our findings ,the calcium levels did not seem to differ much between group I and III (Tables 3A and 3B) and was not statistically significant (P=1.00). Though for the other groups, I and II and II and III the reverse was found to be the case.

Table 3A. Analysis of variance for levels of calcium and age by groups

Variables	$\bar{x} \pm SD$	F	P value
Calcium			
Gp I	8.50±1.44	32.6	0.000
Gp II	7.04±0.81		
Gp III	8.66±.96		
Age			
Gp I	40.64±9.40	0.289	0.749
Gp II	39.22±8.62		
Gp III	39.84±10.00		

Table 3B. Pairwise comparison of mean levels of calcium and age

Variable	Mean difference	P value
Calcium		
Gp I VsGp II	1.45800	.000
Gp I VsGp III	-.16400	1.000
Gp II VsGp III	-1.62200	.000
Age		
Gp I VsGp II	1.42000	1.000
Gp I VsGp III	.80000	1.000
Gp II VsGp III	-.62000	1.000

Group I (HIV and HCV positive); Group II (HIV positive and HCV negative); Group III (HIV and HCV negative)

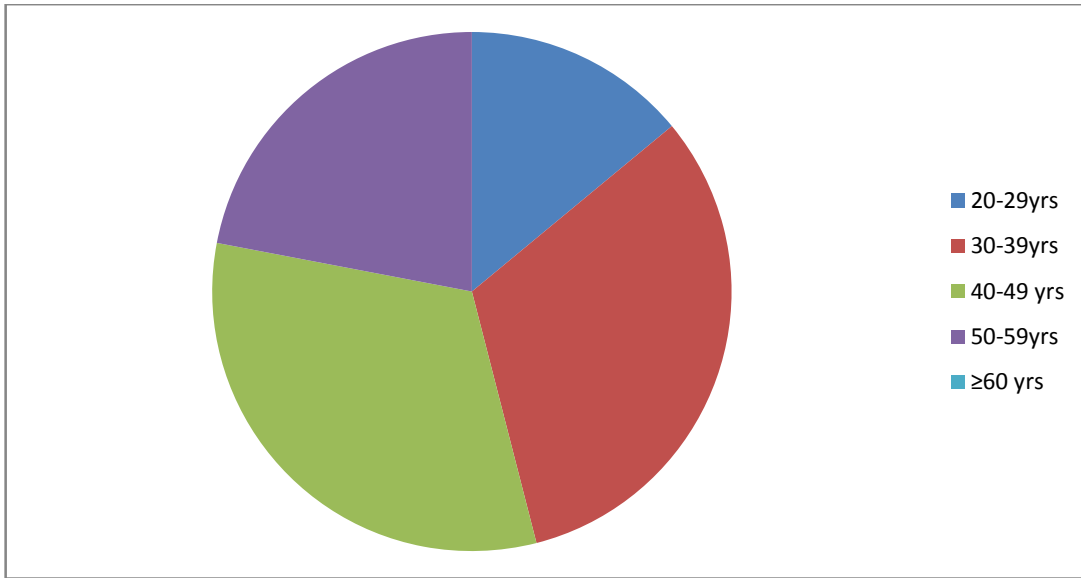


Fig. 1. Pie chart showing age distribution in among study population

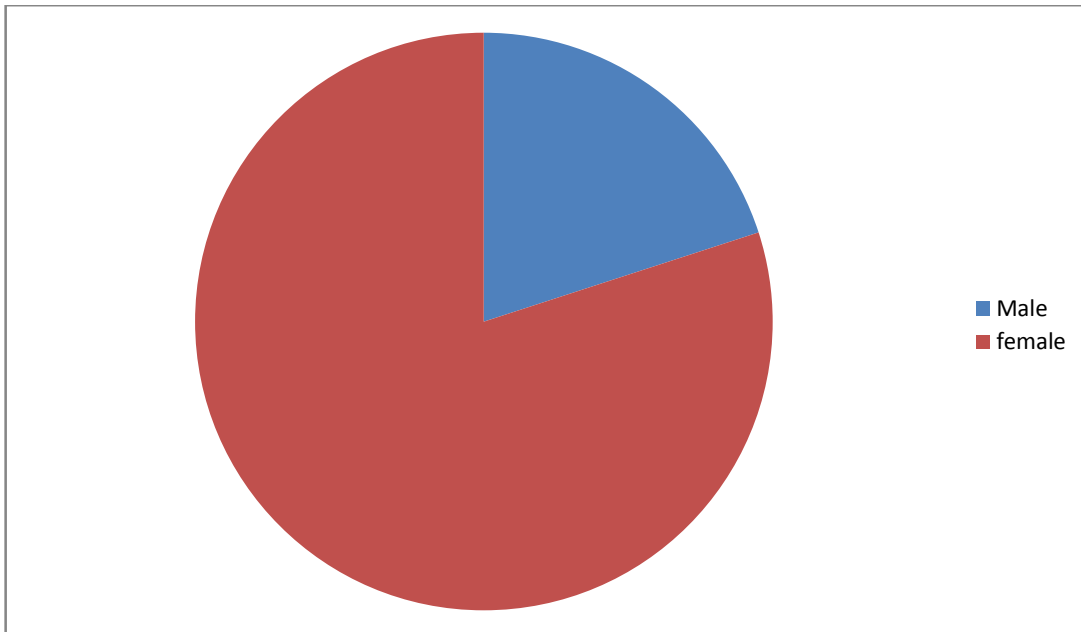


Fig. 2. Pie chart showing gender difference among study population

In previously cited references from studies carried out in other environments, co-infection with HIV/HCV produced various degrees of osteoporosis. It appears though that this may not be a feature in our group of patients. However it would have been desirable to perform investigations such as dual x-ray absorptiometry (DXA) and or quantitative computed tomography but for the resource limitations of our clinical

instance. It may appear that this paper is one of the earliest attempts to investigate calcium metabolism in HIV/HCV co-infected patients in Nigeria, ironically a prominent member of resource limited countries (RLC) despite vast oil reserves. More pending issues such as hunger poverty pushing such esoteric but vital research such as this into the back ground.

It is not in dispute that HIV/HCV co-infection leads to a reduced BMD and has even been described as an independent risk factor for low energy impact trauma associated with pathological fractures. What might not be quite clear is the mechanisms involved [28]. These are suggested to be multifactorial including infection, traditional osteoporotic risk factors and antiretroviral therapy (ART) as described above [28].

It is estimated that up to 9% of HIV mono-infected patients will develop a reduction in BMD due to the negative effects of the virus on osteoclastic activity, alterations in vitamin levels, low body weight, hypogonadism, chronic inflammation and smoking [28]. None of our patients are smokers. HIV viral proteins, Vpr and gp120 are thought to stimulate osteoclast activity assisting in reduction of BMD. Tumour necrosis factor and IL6 have also been implicated in stimulating osteoclastic activity [20,29,30].

There was a marked increase in bilirubin levels when comparing the co-infected group I with the non-infected group III which was statistically significant (Table 4). This is in keeping with findings in other major HIV care delivery centers in the world. There was also a marked increase in markers of hepatic activity such as AST and

ALT (Table 1) in these patients when compared to the control group and this could lead to reduction in bone mineral density within the concept of hepatic osteodystrophy.

Studies have shown that co-infection (HCV/HIV) has contributed more to reduced bone mineral density (BMD) and osteoporosis than in patients uninfected with either virus or those mono-infected with just HIV. More recent studies have revealed that HCV mono-infection with hepatic decompensation are more likely to develop low BMD than in those with HCV monoinfection but with normal hepatic function [31-33]. This further emphasizes the link between calcium level determination and investigation of hepatic activity using bilirubin levels in addition to liver enzymes. It may be interesting at this point to mention the concept of hepatic osteodystrophy (HO) [28] which is thought to be implicated in the reduced BMD associated with HIV/HCV co-infected patients. HO refers to the distabilisation in bone mineral density found in patients with chronic liver disease (CLD). The alterations in BMD usually includes osteopenia and or osteoporosis. It has already been mentioned that the hepatitis C component of HIV/HCV co-infection is an important cause of varying degrees of hepatopathy including fibrosis, cirrhosis and hepatic carcinoma.

Table 4. Kruskal-Wallis Test showing mean rank difference and P values in selected parameters

	Group	N	Mean rank	P value
AST	Gp I	50	85.74	0.000
	Gp II	50	91.33	
	Gp III	50	49.43	
	Total	150		
ALT	GP I	50	83.63	0.000
	Gp II	50	88.32	
	GpIII	50	54.55	
	Total	150		
B1	Gp I	49	93.95	0.001
	Gp II	50	64.22	
	Gp III	50	67.21	
	Total	149		
B2	Gp I	50	96.65	0.000
	Gp II	50	87.97	
	Gp III	50	41.88	
	Total	150		
CD4	Gp I	50	57.12	0.000
	Gp II	50	59.15	
	Gp III	50	110.23	
	Total	150		

In a study carried out at the Johns' Hopkins HIV clinic it was discovered that patients who had lower levels of hepatic markers including AST, albumin and bilirubin were less likely to progress to fibrosis [34]. Our patients had raised levels of AST and ALT and bilirubin. Together with the statistically significant levels of calcium among the groups it would be necessary to maintain monitoring of these parameters (Table 3). It should be mentioned that the treatment of hepatitis C involves the combination of Pegylated interferon and Rabavarin [35]. However none of our patients are on this due to adverse drug drug interactions that are known to occur in co-infected patients and most importantly lack of access to these drugs due to financial reasons. It has been suggested though, that removal of the interferon component would remove adverse drug reactions to a significant degree [36] thereby improving treatment outcomes. It is our hope to commence treatment of HIV/HCV coinfected patients despite the various obstacles highlighted using the HAART and introducing Rabavrin, as laboratory investigations and clinical findings dictate. It is important to note that not all the variables revealed normal Gaussian curve on statistical analysis. Only calcium and age showed normal distribution. Therefore the Kruskal-Wallis test was employed to analyse those parameters which did not show a normal distribution and they were found to be statistically significant across all groups (Table 4).

5. CONCLUSION

This study revealed that our patients had statistically significant levels of calcium across the coinfected and monoinfected groups and elevated liver markers and could therefore be potential candidates for hepatic osteodystrophy. It is essential to maintain regular monitoring of these patients. Follow up studies would be needed to include liver histology preferably with non-invasive methods (e.g. Elastometry) to enable improved interpretation of results. Patients should be informed of this potential complication at point of entry into the health facility.

ETHICAL CLEARANCE

This was obtained from the Lautech research ethics committee (LTH/EC/2014/08/0178).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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