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LIVER FUNCTION MARKERS AND ASSOCIATED SERUM ELECTROLYTES CHANGES IN HIV PATIENTS ATTENDING PATIENT SUPPORT CENTRE OF JARAMOGI OGINGA ODINGA TEACHING AND REFERRAL HOSPITAL, KISUMU COUNTY, KENYA

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W. O. OPIYO, A. G. M. NG'WENA and A. V. O. OFULLA

ABSTRACT

***Objective*: To determine the distribution of markers of liver function disorders and their association with co-existing fluids and electrolytes states in ambulatory HIV infected individuals.**
***Design*: A case-control study.**
***Setting*: Jaramogi Oginga Odinga Teaching and Referral Hospital's Patient Support Centre.**
***Intervention*: Biochemical analysis were performed for serum alanine-aminotranferase (ALT), aspartate-amino transferase (AST), total protein, albumin, glucose, urea, potassium, sodium, chloride, creatinine phosphate, total and direct bilirubin levels as well as CD4 lymphocyte levels.**
***Results*: Serum liver function markers were significantly altered in HIV infected individuals compared to uninfected individuals (mean serum aspartate-amino transferase (AST); 45.1U/l v/s 36.9U/l; alanine-aminotransferase (ALT), 36.5U/l v/s 30.7U/l; direct bilirubin, 4.9μmol/l v/s 4.2μmol/l; total bilirubin, 6.2μmol/l v/s 5μmol/l; albumin32.8g/l v/s 34.5g/l and protein 64g/l v/s 67.1g/l; p <0.0001). The prevalence of pathological levels of serum liver function markers was also higher in HIV-infected patients than HIV-negative participants (ALT, 4.4% v/s 0.7%, p=0.001; AST, 24.5% v/s 6.7%, p<0.0001; direct bilirubin, 43.1% v/s 36.5%, p=0.026; total bilirubin, 2.3% v/s 0%, p=0.002; serum albumin, 60.1% v/s 52.2%, p= 0.009 and serum total protein levels, 52.8% v/s 36%, p<0.0001). Gender, age and anti-retroviral treatment were not predictors of aberrations in levels of liver function markers in HIV infected patients. Marked CD4 depletion was associated with enhanced deterioration of liver function markers. Liver function anomalies did not conduce co-existing electrolyte anomalies as clinically altered ALT states only correlated and co-varied with AST states (r = 0.917) ; direct biliribun states co-varied with total bilirubin levels (r = 0.958) and serum album states correlated with protein levels (r = 0.917) and vice versa.**
***Conclusion*: Liver function disorders are not infrequent in HIV infected individuals and routine review of liver health status is essential in comprehensive care of HIV patients.**

INTRODUCTION

The population infected with Human immunodeficiency virus (HIV) has dramatically increased ever since it was described (1). Globally nearly 40 million live with HIV infection, with the greatest burden of disease in low and middle income countries (1). Africa has borne the greatest burden of HIV infection with 25 million people living with the disease yet disproportionately, scant resources have been availed for research exploring and elucidating HIV infection and its co-morbid conditions (2). In Kenya the prevalence of HIV infection remains at 5.6% (3), with regional disparities. Since inception of highly active anti-retroviral treatment (HAART), life expectancy has improved among HIV-infected persons occasioning new challenges attributed to co-morbid conditions, prolonged drugs use and chronic ailment with the virus, among other factors (1).

Besides predominantly infecting the CD4+ lymphocyte cells, studies have elucidated HIV infection of non haemopoietic cells, including liver cells (3). HIV infection of liver cells such as the primary Kupffer cells, differentiated tissue macrophages, sinusoidal cells and haepatocytes proceeds by means of several mechanisms. Kupffer cells, sinusoidal cells and haepatocytes can be infected by the HIV virus *in vivo* (3). As haepatocyte cell lines do not express CD4 cell surface markers, it is thought that their infection with the HIV virus is non CD4 dependent (4). Haepatocytes may act as transient reservoirs of the HIV virus, but in some instances undergo apoptosis leading to pro-fibrotic activity of stellate haepatic cells (5). Liver histology in HIV patients have shown a common pattern of portal vein occlusion, peri-portal or peri-sinusoidal fibrosis, low grade inflammation and steatosis (6).

Various forms of liver diseases have therefore been described among HIV infected patients including non-alcoholic fatty liver diseases (NAFLD), non-alcoholic steatohaepatitis (NASH), haepatocellular cancer (HCC) and liver decompensation. Decompensated portal hypertension in HIV patients presents with manifestations such as ascites and bleeding oesophageal varices without cirrhosis at histology (7).

In addition to direct adverse effects of the HIV virus, a variety of factors have been identified as being associated with damage to liver structure and function in HIV patients including: anti-retroviral and non-anti-retroviral drugs toxicities; tumours (lymphoma and Kaposi sarcoma); and opportunistic infections such as cytomegalovirus or mycobacterium and co-infection with Hepatitis B virus (HBV) or Hepatitis C virus (HCV) (8).

Anti-retroviral haepatotoxicity has been attributed to nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs) and non-nucleotide reverse transcriptase (NNRTIs) (9). These drugs afflict liver health and function by way of hypersensitivity, direct mitochondrial toxicity and / or disturbance of lipid or sugar metabolism (10). Anti-retroviral drug-related liver injury has prominently been one of the causes of treatment discontinuation as well as a variety of negative clinical outcomes (11). Consequent discontinuation of anti-retroviral drugs (ARVs) on the other hand hinders effective and sustained suppression of the HIV virus. As a result ARV- haepatotoxicity is a major challenge that strains medical budgets due to repeated hospital visits or admissions in order to manage the disorders resulting from it. There is need to investigate the burden, pathogenesis and determinants of liver diseases in HIV patients, as a part of comprehensive HIV management strategy in every health setting.

In a Swiss cohort of 2365 HIV –infected patients not co-infected with HBV or HCV, in whom levels of alanine aminotransferase (ALT) was monitored, 16% had chronically elevated levels of ALT (defined as >2x upper limit of normal (12). Determinants associated with these elevations were HIV RNA, prolonged exposure to anti-retroviral treatment (ART), high body mass index (BMI), alcohol and increasing age. In a study involving a Polish cohort of 182 HIV–infected individuals on HAART followed for eight years, Anita and colleagues (11) established that the most common clinical presentation was asymptomatic enzyme elevation, usually <10times the upper limit of the normal range. Lucien *et al* (13) monitored the levels of transaminases in HIV patients attending Central Hospital Yaounde, Cameroon, involving 150 participants over a three year period, and established that 54% and 24.6% had elevated levels of AST and ALT respectively.

Pathological and epidemiological studies of HIV-related liver health and disease have largely been done in resource rich, high income countries, yet it is projected the burden of HIV infection is high in resource scarce developing countries where negligible research on liver health in HIV infected individuals occurs (14).

Common patient screening strategies in long term management of HIV patients in Kenya involves the use of CD4 levels and on occasion viral load, as means of assessing progression of disease and treatment. Organ specific screening is a rare approach for assessing regular organ function partly explainable by the inhibiting cost of involved procedures. However studies have persistently reported the impact of HIV infection, ARVs and co-morbidities on various vital organs as constituting significant threats.

This study aims at determining distribution of liver function markers in HIV patients attending the Patients Support Centre (PSC) of Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) in Kisumu County, Kenya. It also explored the association of liver function markers and the pattern of concurrent alteration of other serum electrolytes. Liver enzymes AST and ALT, total bilirubin and direct bilirubin, protein and albumin levels and states were analysed as the markers of liver function.

MATERIALS AND METHODS

Subjects: Between November 2012 and April 2013, a case-control study was conducted involving 800 HIV sero-positive patients from PSC of JOOTRH and 406 HIV negative controls, over 18 years, able and willing to give verbal or written consent and of both gender. Enrolled participants in the control group were enlisted from among consenting ministry of health staff who in the period of the study were due for and underwent routine laboratory profiling at JOOTRH, as a pre-condition for vaccination against hepatitis B

and C vaccinations. Patients were excluded if they had known co-infections or co-morbid conditions such as hypertension, diabetes or alcohol abuse or history of jaundice. Control participants were excluded if they were sero-positive for HIV. Ethical approval was provided by the ethical committee of JOOTRH.

Specimen handling: Personnel in the PSC undertook routine medical review and care of HIV patient enrolled in the department and introduced them to study. Volunteers were then informed of consent provisions and consenting patients sent to the hospital's laboratory where assigned hospital laboratory personnel collected blood samples for analysis. Collected blood samples were prepared and assayed for the levels of the following analytes; total protein, albumin, random sugar, direct bilirubin, liver transaminases (alanine amino-transferase and aspartate amino-transferase), total bilirubin, sodium, potassium, chloride, urea and creatinine.

Common attributes recorded for both groups included age, gender, random blood sugar, Serum kidney and liver function parameters. Additional attributes obtained for the HIV infected individuals were ARV use and CD4 levels.

Serum total protein, albumin, creatinine, glucose, urea, total and direct bilirubin, and tranaminases were assayed using colorimetric method with wave length range of 340-670 nm (Bio systems BTS – 330 Photometer, Biosystems, SA). Sodium, potassium and chloride were measured using combined photometric-ion exchange and turbidimetry techniques (Eurolyser, Eurolyser diagnostic, and Austria). HIV status of PSC patients were confirmed from the patients' records while for the consenting control participants were confirmed by ELISA and Western Blot. CD4 + cell levels was also determined (FACSCalibur instrument, Becton Dickinson, San Jose, CA).

Comparison of means was done using student t-test while comparison of proportions was done using χ 2 and One –way analysis of variance where applicable. Linear correlation was used to explore association between quantitative variables and regression statistics was used to elucidate co-variation. SPSS version 21 was used for statistical analysis.

RESULTS

Demographics of the 800 HIV positive and 406 HIV negative participants and the distribution of twelve analytes assayed are provided in table 1 and 2 respectively.

Table 1
Characersitics of participants in the study population

	Percent	
	HIV Positive	HIV negative
Male	41.4	32
Female	58.6	68
Old (≥50yrs)	15	16.5
Young (<50yrs)	85	83.5
Using ARV	79.9	
ARV naïve	20.1	
CD4+ <500	59.1	
CD4+≥500	40.9	

Key: CD4+-lymphocyte sub-group bearing CD4 membrane markers; ARV- anti-retroviral drugs

Table 2
Serum electrolytes distribution by HIV status

electrolytes	Units	HIV status: HIV Positive				HIV Negative			
		Mean	Std Dev.	Min.	Max.	Mean	Std Dev.	Min	Max.
Creatinine	μmol/l	95.2	35.6	11.5	410.57	86.2	20.4	40	173.1
Potassium	mmol/l	4.2	0.7	2.8	6.4	4.2	0.7	2.9	6
Sodium	mmol/l	138.9	6	127	160	139.1	4.9	130	153
Chloride	mmol/l	100.2	3.9	90	139	99	3.2	90	108
Urea	mmol/l	4.6	2.1	1.5	36.7	4.1	1.0	1.9	8.8
Protein	g/l	64	8.4	40	89	67.1	6.9	42	93
Albumin	g/l	32.8	5.8	15.5	47.2	34.5	3.9	22.7	43.3
ALT	U/l	36.5	16	11	210	30.7	11.6	11.5	145
AST	U/l	45.1	18	11.6	301	36.9	9.6	16.3	104
Total Bilirubin	μmol/l	6.2	5.5	1.9	70	5	1.6	2.2	14.1
Direct Bilirubin	μmol/l	4.9	4	1.5	62.8	4.2	1.3	2	10.8
Glucose	mmol/l	4.2	0.9	2.1	8.6	4.1	0.9	2	6.7

Key: ALT- alanine-aminotransferase; AST- aspartate -aminotransferase

Liver Enzymes: Mean aspartate-aminotransferase (±)SD was significantly raised in HIV sero-positive individuals than in control group [45.1U/L (±18) v/s 36.9U/L(±9.6), p<0.0001) (Table 3). HIV positive individuals were also had a higher prevalence of pathologically elevated AST level (defined as >50U/L) than the HIV negative control (24.5% v/s 6.7%, x^2=56.9,p<0.0001). HIV infected participants similarly presented with elevated serum alanine-aminotransferase levels and more ALT pathology than their HIV negative counterparts (36.5 U/L v/s 30.7 U/L, p<0.0001; 4.4% v/s 0.7%, $\chi 2 = 11.7$, p=0.001 respectively). However, mean AST and ALT level in HIV infected males were 46 U/L and 37.6 U/L respectively which were not significantly different from that of females, 44.4 U/L and 35.8 U/L; p=0.226 and 0.119 respectively. Equally the proportion with deranged AST and ALT levels was not significantly different in male compared to female (27.2% v/s 22.6%, $\chi 2$=2.2, p=0.137 and 3% v/s 5.3%, p=0.116 respectively). However comparison of gender by HIV status revealed that male and female in HIV positive category had significantly raised mean AST and ALT level as well as a higher prevalence of pathologically altered AST and ALT activity than their counterparts in the HIV negative control group. At 46 U/L and 37.6 U/L mean AST and ALT in HIV sero-positive males were significantly higher than 37.2 U/L and 31.9 U/L established in males in the control group (p<0.0001 and p=0.001). The prevalence of deranged AST and ALT activity (>70U/L) in HIV-infected males was 27.2% and 3% which were higher than 8.5% and 1.5% noted in HIV negative males in the control group.

In HIV afflicted females mean AST and ALT were significantly higher than mean AST and ALT values in females in the HIV negative control (44.4 U/L v/s 36.8 U/L, p<0.0001and 35.8 U/L v/s 30.2 U/L, p<0.0001). Twenty two point six percent of females in the HIV + group had deranged AST level (defined as > 50 U/l) which was significantly higher than the prevalence of abnormal AST levels in the HIV negative control (5.8%, p<0.0001). Fiv point four percent of HIV infected females also had clinically elevated ALT levels (> 65U/l) as opposed to 0.4% of HIV negative females (p<0.001).

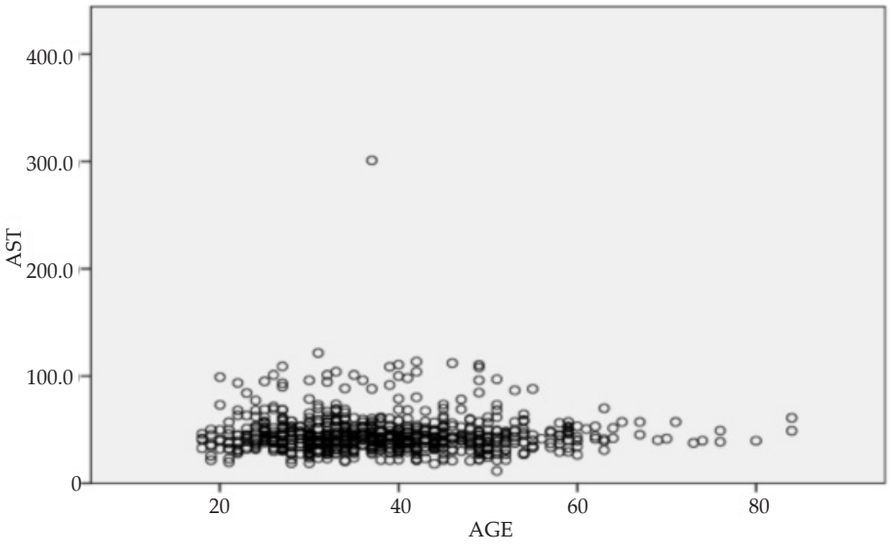
Table 3
Comparison of AST level and prevalence of abnormally elevated AST levels between various HIV population attributes

	Mean AST level (U/L)	t	p-value	Deranged AST level (percent)	$\chi 2$	p-value
Hiv+	45.1			24.5		
		10.3	<0.0001		56.9	<0.0001
Hiv-	36.9			6.7		
Male HIV+	46			27.2		
		5.7	<0.0001		19.1	<0.0001
Male HIV-	37.2			8.5		
Female HIV+	44.4			22.6		
		8.7	<0.0001		35.8	<0.0001
Female HIV–	36.8			5.8		
Male HIV+	46			27.2		
Female HIV+	44.4	1.212	0.226		2.2	0.137
Arv-yes	45.3			24.9		
Arv-no	44.3	0.615	0.538	23	0.25	0.616
≥50yrs HIV+	43.6			24.2		
		3.6	<0.0001		9.8	0.002
≥50yrs HIV-	37.1			6		
<50yrs HIV+	45.3			24.6		
		9.6	<0.0001		47.1	<0.0001
<50yrs HIV-	36.9			6.8		
≥50yrs HIV+	43.6			24.2		
		0.967	0.334		0.008	0.927
<50yrs HIV+	45.3			24.6		

ARV yes- using anti-retro viral drugs; ARV no: Not on anti-retro viral treatment

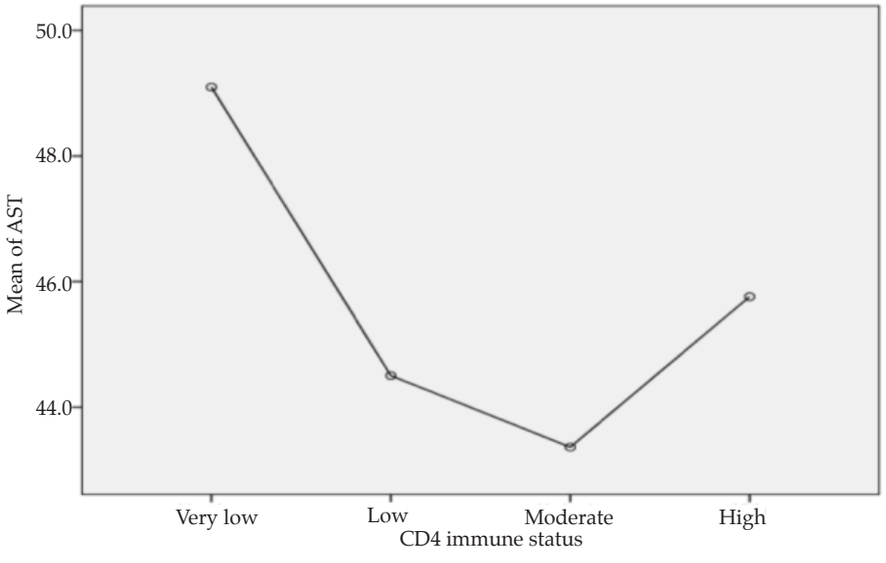
Age difference among HIV infected participants was not related with differential outcomes in aberrations in AST and ALT levels. Thus in patients over 50 years old with HIV infection, the mean AST (43.6 U/L) and ALT (36.1 U/L) were not significantly higher than 45.3 U/L and 36.6 U/L recorded in HIV infected participants below 50 years. Indeed there was no correlation between age and AST level or ALT (r=0.018, p=0.613 and r=0.003, p=0.924) and virtually no variation in AST or ALT level could be attributed to age change (r2 <0.0001) (Figure 1). The rate of occurrence of elevated AST states was distributed evenly between the two age categories of HIV positive patients (24.2% v/s 24.6%, p=0.927). The prevalence of pathological ALT levels was also not significantly different between the two age groups.

Figure 1
Correlation of age and AST in HIV patients
r = 0.018



Even though changes in CD4 levels were not accompanied with similar alteration in liver transaminases, extreme CD4 depletion (<200cell/mm3) was associated with highest mean serum AST (Figure 2) and ALT levels. Nonetheless the prevalence of clinically elevated AST and ALT states was not related to changes in CD4 lymphocytes count ($\chi 2 = 3.3$, p=0.342 and $\chi 2 = 1.4$, p=0.711 respectively). Liver enzyme levels and anomalies in patients using ARVs remained virtually unchanged in comparison to those not using ARVs (4.4% v/s 4.3% and 24.9% v/s 23% respectively).

Figure 2
Mean serum aspartate-aminotransferase level against CD4 cell count



Key; Very low-CD4 <200; Low-201-500; Moderate-501-800; High->800cells/μl

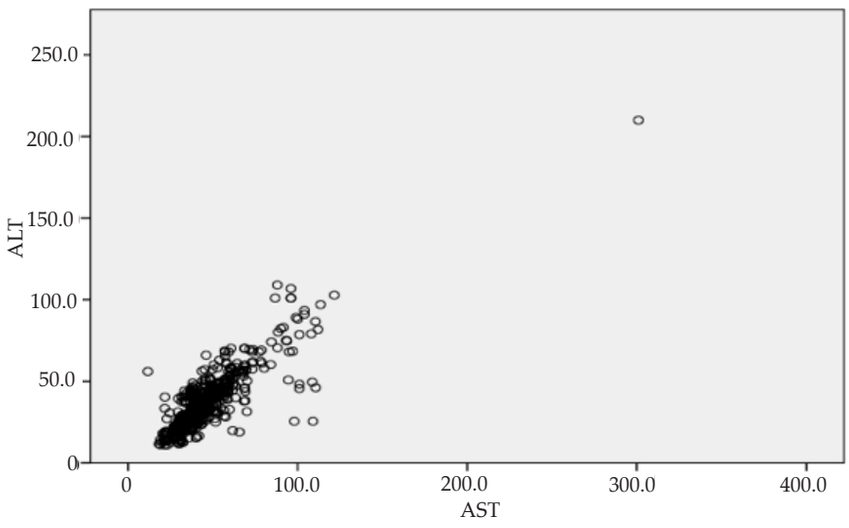
Correlation of changes in liver enzymes to changes in other serum electrolytes: In HIV negative controls, AST showed strong correlation to ALT (r=0.721) with 52% of variations that occurred in ALT associated with variations occurring in AST (r2 =0.520) (Figure 3). Such correlation was mirrored by HIV+ patients with normal AST levels. In this group, AST levels showed a strong correlation with ALT (r=0.701) with 49.2% co-variation noticed between ALT and AST. However in HIV positive participants with elevated AST

activity (>50U/L), an even stronger correlation was exemplified (r=0.784) with a higher proportion (61.3%) of ALT changes associated with changes in AST levels (r2 =0.613). Conversely HIV+ patients with elevated ALT activity (females>65U/L;males>70U/L), also showed a strong correlation between ALT and AST levels (r=0.917, p<0.0001) with 84% co-variation noted between liver enzymes in this group. Thus strong co-directional shift in levels of ALT and AST was noted where AST was regressed against clinically deranged ALT cases or where ALT was regressed against cases with elevated AST activity. Where the strength of correlation was considerably large, it was noticed that shifts in the AST levels that predominantly resulted in abnormal levels of AST (>50U/L), were accompanied by similar shifts in the proportion of the co-variant electrolytes towards abnormal level. Thus, elevated AST states were accompanied with a higher proportion of abnormal ALT states than normal AST states (17.3% v/s 0.2%, $\chi 2 = 104.4$, p<0.0001). In the HIV negative control AST displayed an additional mild correlation with glucose only (r=0.102) and only a dismal 1% of their values shared common variation trends. Similarly in the HIV positive group with normal AST levels chloride was the only other electrolyte that was correlated with AST (r=0.084, p=0.039), with 3.9% of their values noted to have a common variation trend. In contrast AST in the HIV positive group with abnormal altered AST levels (>50U/L), was further correlated with seven other electrolytes namely; creatinine, urea, direct bilirubin, total bilirubin, sodium, chloride and albumin (Table 4, for r-values).The proportion of alteration in these seven electrolytes that portrayed common variation trend with AST equally diminished proportionately from creatinine to albumin in this group. However with regard to the seven electrolytes, correlation was not necessarily accompanied with co-directional shift towards anomalous level. Indeed out of the seven electrolytes, only total bilirubin shifted significantly towards abnormal levels in relation to similar shift of AST levels towards such anomalous states (abnormal AST v/s normal AST states = 4.1% v/s 1.7%, $\chi 2 =3.96$, p=0.047). In the remaining six (creatinine, urea, directbilirubin, sodium, chloride and albumin) the proportion of abnormal cases were fairly evenly distributed between HIV infected patients with normal AST levels and abnormal AST states. This implies that though correlated to changes in AST, changes in these six electrolytes are not necessarily precipitated from the same source as those that resulted in AST anomalous shift.

Table 4
Correlation of serum AST levels with other serum electrolytes

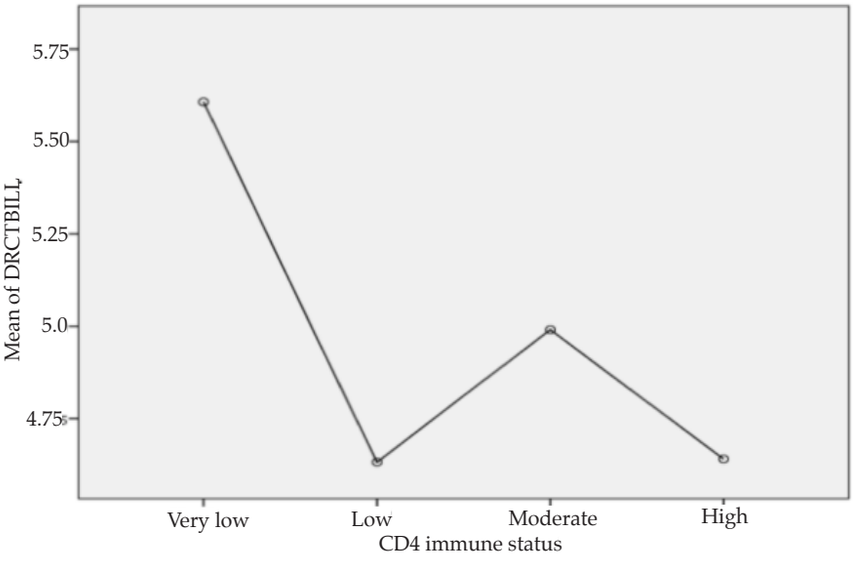
	AST in HIV negative			HIV positive AST normal (< 50U/L)			HIV positive AST elevated (AST>50U/L)		
	R	p-value	r2	r	P-value	r2	R	P-value	r2
ALT	0.721	<0.0001	0.520	0.701	<0.0001	0.492	0.784	<0.0001	0.613
Creatinine	0.057	0.254	0.003	0.054	0.189	0.003	0.470	<0.0001	0.221
Chloride	0.021	0.668	<0.0001	0.084	0.039	0.007	0.172	0.016	0.030
Sodium	0.008	0.880	<0.0001	0.031	0.441	0.001	0.222	0.002	0.049
Potassium	0.057	0.250	0.003	0.060	0.141	0.004	0.051	0.474	0.003
Urea	0.039	0.433	0.002	0.028	0.496	0.001	0.304	<0.0001	0.092
Glucose	0.102	0.039	0.010	0.076	0.063	0.006	0.127	0.075	0.016
Total bilirubin	0.035	0.484	0.001	0.003	0.950	<0.0001	0.227	0.001	0.051
Direct bilirubin	0.064	0.195	0.004	0.001`	0.981	<0.0001	0.268	<0.0001	0.072
Protein	0.050	0.311	0.003	0.008	0.845	<0.0001	0.120	0.094	0.014
Albumin	0.046	0.630	0.002	0.020	0.627	<0.0001	0.156	0.029	0.024

Figure 3
Correlation of serum AST to serum ALT level
r = 0.847



Bilirubin: Serum total bilirubin and direct bilirubin were significantly elevated in HIV positive participants than in HIV negative controls (6.2μmol/l v/s 5μmol/l, p<0.0001 and 4.9μmol/l v/s 4.2μmol/l, p<0.0001 respectively). Consequently it was noted that HIV infected participants presented with a higher prevalence of deranged total bilirubin (>19μmol/l) (2.3% v/s 0%, $\chi 2 = 9.3$, p =0.002) and direct bilirubin levels (4.2μmol/l) (43.1% v/s 36.5%, $\chi 2 = 4.96$, p = 0.026). Among the HIV infected patients there was no association between gender, age and anti-retro viral treatment and changes in serum direct or total bilirubin. Even though changes in CD4 levels were not accompanied with similar alteration in serum bilirubin, extreme CD4 depletion (<200cell/mm3) was associated with highest mean serum direct bilirubin (Figure 4) and total bilirubin.

Figure 4
Mean serum direct bilirubin level against CD4 cell count



Key; Very low-CD4 <200; Low-201-500; Moderate-501-800; High->800cells/μl

In HIV negative controls direct bilirubin was strongly correlated with total bilirubin levels (r=0.925) with 92.5% of variations in levels of either electrolyte tied to variation in level of the other. On the other hand direct bilirubin showed a weak correlation to creatinine (r = 0.103) with only 1.4% of alterations in levels of either electrolytes associated with changes in levels of the other. Total bilirubin in this

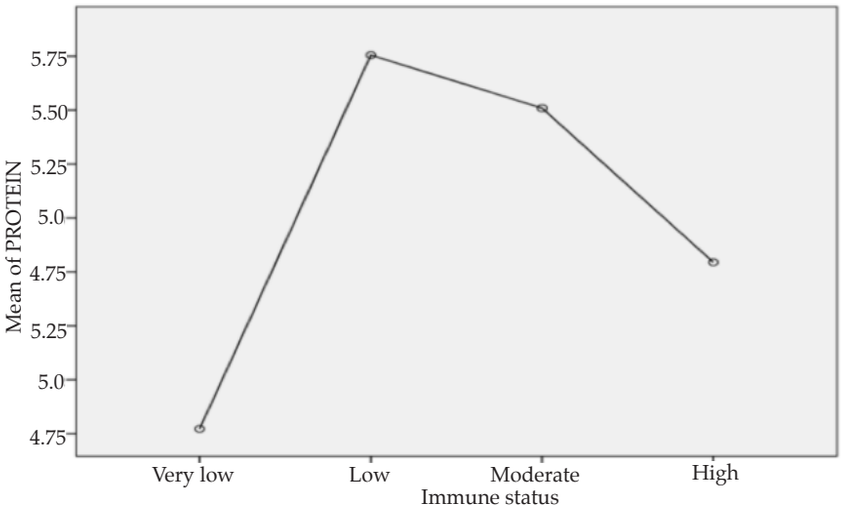
group had a weak correlation with creatinine and urea (r=0.116 and r= 0.102 respectively). In HIV infected participants with normal direct bilirubin levels, correlation was only noted between direct bilirubin and total bilirubin (r = 0.752, p<0.0001) with 56.5% co-variations existing between them (r2 = 0.565). However, HIV infected patients with normal levels of total bilirubin, a stronger correlation was noted between total bilirubin and direct bilirubin (r = 0.955, p<0.0001) with 91.3%co-vriation between the two electrolytes (r2 = 0.913). In contrast in HIV infected participants with clinically elevated direct bilirubin states (>4.2μmol/l), four other electrolytes showed increased correlation with direct bilirubin in addition to its co-variation with total bilirubin (r= 0.958). These electrolytes included creatinine (r = o.412, p<0.0001), urea (r= 0.362, p<0.0001), AST (r= 0.212, p<0.0001) and glucose (r= 0.139, p=0.010). On the other hand in HIV infected patients with raised total bilirubin (>19μmol/l), correlation existed between total bilirubin and direct bilirubin only (r=0.875, p<0.0001) with 76.6% co-variation between them. Pathological shift in levels of bilirubin (direct and total) were also in tandem, with all the patients with abnormal levels of total bilirubin also having abnormal levels of direct bilirubin while none of the patients with normal levels of direct bilirubin had abnormal levels of total bilirubin. On the other hand the strength of correlation between direct bilirubin and creatinine in HIV infected patients with abnormal levels of direct bilirubin was a pointer to co-existence rather than co-directional shift in magnitude of pathological alterations of both. Thus even though there was 17% co-variation between direct bilirubin and creatinine in HIV patients with abnormal direct bilirubin levels, the prevalence of abnormal creatinine in them (27.5%) was not significantly different from the proportion with abnormal creatinine level in HIV infected patients with normal direct bilirubin levels (25.1%, P=0.429). Urea levels also portrayed similar outcomes with a weak correlation not sufficient to engender co-directional pathological shift.

Total Protein and Albumin: Mean total protein (64g/l) and albumin (32.8g/l) in the HIV infected individuals declined substantially below those of HIV negative controls (67.1g/l., p<0.0001 and 34.5g/l, p<0.001 respectively). The proportion with deranged protein levels (defined as <65g/l or >80g/l) and albumin levels (<35g/l or >50g/l), was higher in HIV infected patients than their HIV negative counterparts (52.8% v/s 36%, χ2 =30.5, p<0.0001 and 60.1% v/s 52.2%, χ2 = 6.9, p=0.009 respectively). Within the HIV infected category, gender and anti-retroviral treatment were not associated with differential alterations in levels and prevalence of serum protein and albumin disorders. HIV sero-positive patients over 50 years and below 50 years had almost equal mean protein and albumin levels. However younger patients (<50 years) tended to have a higher prevalence of serum protein and albumin disorders than older patients (53.4% v/s 31%, χ 2 = 7.7, p=0.005 and 59.7% v/s 40.5%, χ 2 = 5.8, p=0.016 respectively).

CD4 depletion (<200cell/mm3) was associated with lowest mean serum total protein and albumin (Figure 5 and 6). However at every CD4 level, the proportion with clinically deranged protein and albumin level was over 50%.

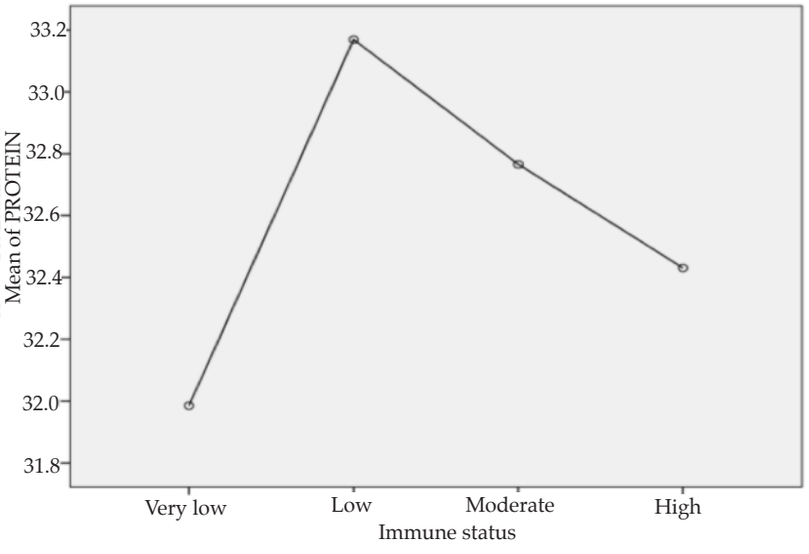
Figure 5

Mean serum total protein level against CD4 cell count



Key; Very low-CD4 <200; Low-201-500; Moderate-501-800; High->800cells/μl

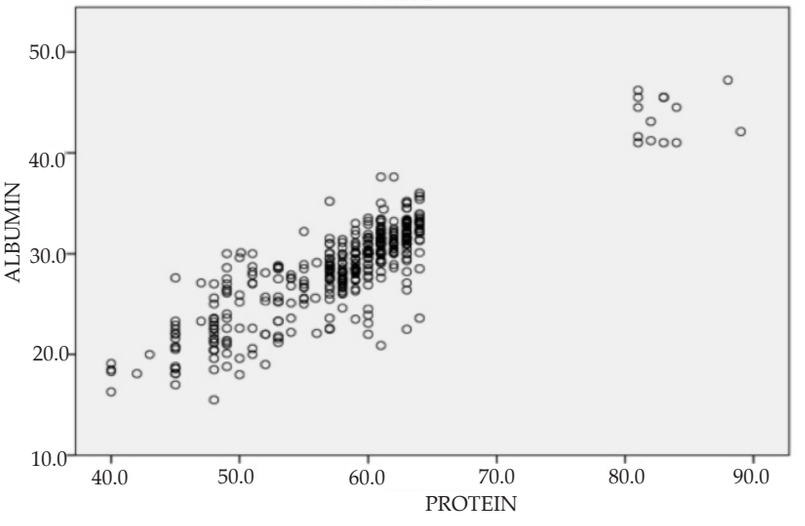
Figure 6
Mean serum albumin level against CD4 cell count



Key; Very low-CD4 <200; Low-201-500; Moderate-501-800; High->800cells/μl

In HIV negative controls, protein levels were associated only with albumin levels (r = 0.867). In HIV infected participants with normal protein levels, protein was associated with albumin levels (r = 0.705) while in HIV infected participants with normal albumin states, albumin was correlated only to protein levels (r = 0.738). However in HIV infected patients with deranged albumin states (<35g/l or >50g/l), albumin level alterations correlated with changes in creatinine (r = 0.130), AST (r = 0.097), protein (r = 0.841), total bilirubin (r = 0.171), and direct bilirubin (r = 0.161). Thus conditions associated with albumin anomalies conduced alterations of five other electrolytes. On the other hand in HIV infected patients with abnormal protein states (<65g/l or >80g/l), alterations in levels of protein were correlated only with alterations in creatinine (r = 0.108) and albumin (r = 0.872) see Figure 7. In these two categories, strong co-directional variation existed between protein and albumin accounting for over 70% of each other's alterations while the rest registered less than 2% co-variations with either protein or albumin. Most of protein anomalies were due to depletion states (defined as < 65g/l) with only 18 HIV infected patients with protein retention states (defined as > 80g/l). However all albumin anomalies were due to depletion states (defined as < 50g/l). All protein retention states were accompanied with albumin depletion disorders while 97.6% of protein depletion states had concomitant albumin depletion disorders. Only 24.5% of patients with normal protein levels had albumin depletion disorders. Protein and albumin levels had a common shift towards depletion states. 27.8% of patients with protein retention disorders had creatinine retention disorders while 5.6% had creatinine depletion states. Conversely in protein depletion disorders, 22% had creatinine retention disorders while 1.3% had creatinine depletion disorders. In patients with normal protein states, 17.4% had creatinine retention disorders while 1.9% had creatinine retention disorders. The distribution of pathological creatinine states between patients with various protein states indicates that there was virtually no co-variation (r2 = 0.012) between the two electrolytes.

Figure 7
Cotrrelation of albumin to proteins in HIV infection with abnormal protein levels



DISCUSSION

The aim of this study was to determine the distribution of liver function markers, possible risk factors to which anomalous states of liver function markers can be attributed and changes in other electrolytes that are influenced by or associated with anomalous changes in these liver function markers in HIV patients in Kisumu County. The characteristics of distribution of liver transaminases (AST/ALT), Bilirubin and albumin that were assessed were mean and proportions. Their correlation with the distribution of anomalies of seven serum electrolytes were assessed to detect any likely influence of liver malfunction on mechanisms responsible for regulating these electrolytes in HIV infection.

In the current study mean serum transaminase levels were higher in HIV infected individuals than in the sero-negative individuals. This concurred with findings by Ignatius *et al* (4) that mean liver transaminases' activity levels were higher in HIV patients compared HIV negative. While most studies reported that ARV treatment were predictors of changes in liver transaminases, this was unexpectedly not found to be the case in our study. However, Sulkowski (15) alluded that etiopathogenesis of drug-induced liver injury, are difficult to assess because of the presence of many complicating factors, such as drug-drug interactions, the clinical condition of the patient, and the haepatic effects of various co-morbid diseases. Indeed gender, age and CD4 levels did not also explain the various liver enzyme states observed in the current.

Liver tranaminase anomalous states were predominant in HIV than in HIV negative population. Kovari e*t al* (12), reported elevated liver transaminases affected 16% of HIV patients. The current study on the other hand found that 24.6% of HIV infected participants had transaminitis of any grade. Among the HIV infected patients, ARV use, gender, and age, did not explain elevation of transaminases. However ,Ingiliz *et al* (16) reported that though several studies have observed association between ARV use, co-infection with hepatitis C (HCV) or hepatitis B virus (HBV) as well as alcohol consumption with elevation of liver transaminases, in a significant proportion of the HIV population the underlying explanation remains unknown. In HIV infected individuals abnormal levels of serum alanine –aminotranferase established levels of enzyme activity correlated only levels of aspartete –amino transferase (r = 0.784) but not with levels of other concomitantly anomalously elevated electrolytes. Therefore, though other electrolytes anomalies co-existed with elevated liver enzyme states in HIV infection, the later could not be explained by underlying determinants of transaminitis in this population. Patients with depleted immunity (<200) had the more prominent states of liver function anomalies compared with those with more robust immunity.

Most studies have explored the distribution of bilirubin in HIV infection with greater emphasis on the underlying determinants contributing to the various states of bilirubin. The current study established that average bilirubin levels were elevated in HIV patients compared to their counter parts without HIV. It also established that the prevalence with anomalous levels of direct and total bilirubin was higher in the HIV group. Findings that gender, age, ART and CD4 levels were not explanatory of these anomalous levels in HIV infection, contrasted reports from other studies. In clinical trials, significant increases in the total bilirubin level (>12.5 times the upper limit of normal (UNL) were observed in 22%–47% of patients treated with atazanavir (15).

Mean albumin in HIV patients was lower than that in the HIV un-infected group in our study. Similarly HIV patients were more likely to have hypoalbuminemia than HIV uninfected group. Among the HIV infected group, gender and ARV use were not associated with abnormal albumin. Younger patients had a higher prevalence of hypoalbumin states than older patients. In patients with advanced immune depletion albumin levels were markedly lower than in patients with more robust immunity. Though other anomalies of other electrolytes co-existed with HIV with abnormal albumin levels these were not attributable to the abnormal albumin states per se. This finding concurs with Mehta *et al* (17) who denoted that low albumin levels are a consequence of HIV infection and that albumin <35 g/liter after HIV sero-conversion is associated with faster HIV disease progression and increase mortality rates.

Mean total protein in HIV patients was lower than that in the HIV un-infected group and HIV patients were more likely to have hypoproteinemia than HIV uninfected group in our study. Younger patients had a higher prevalence of hypoprotein states than older patients. In patients with advanced immune depletion protein levels were markedly lower than those with more robust immunity. Protein anomalous states correlated with albumin states but not with other co-existing anomalous electrolytes levels.

CONCLUSION

Markers of liver function are altered in a significant proportion of HIV infected individuals. It was also noticed that markers indicative of liver function anomalies were considerably prominent in extensive immune depletion states . Therefore additional studies need to be done to establish underlying determinants and the impact that liver malfunction has on prognosis of HIV patients in general in our local health set up.

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