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Full Length Research Paper

Correlation of hepatobiliary ultrasonographic findings with cd4cell count and liver enzymes in adult hiv/aids patients in Jos

A. Angbalaga[1], C. C. Ani[1], D. Atsukwei[2], E. D. Eze[3*], A. M. Afodun[4], E. O. Igoh[1], S. C. Owoeye[1] and C. B. Ukaonu[1]

[1]Department of Radiology, Faculty of Medical Sciences, Jos University Teaching Hospital, Jos, Nigeria.
[2]Department of Radiology, Federal Medical Centre, Keffi, Nasarawa State, Nigeria.
[3]Department of Physiology, Faculty of Biomedical Sciences, Kampala International University, Western Campus, Ishaka-Bushenyi, Uganda.
[4]Department of Anatomy, Faculty of Biomedical Sciences, Kampala International University, Western Campus, Ishaka-Bushenyi, Uganda.

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Hepatobiliary diseases occur commonly in patients with Human immunodeficiency virus (HIV) infection and are now the commonest cause of death in HIV positive patients on antiretroviral therapy (ART) in western countries. The hepatobiliary manifestations of AIDS are related to the levels of CD_4^+ count as well as liver enzymes. Abdominal ultrasound examination is easy to perform, non-invasive, inexpensive, readily available and provides valuable information about hepatobiliary findings in AIDS. The study was to evaluate hepatobiliary sonographic findings in HIV/AIDS adult patients and correlate with their CD_4^+ count and abnormal liver enzymes (ALP, ALT and AST) in Jos, Plateau State, Nigeria. A cross-sectional study of hepatobiliary ultrasound findings of 410 adult patients with HIV/AIDS was carried out over a period of six months. Two hundred and sixteen (52.7%) of the patients had normal hepatobiliary ultrasound findings while 194 (47.3%) patients had various abnormalities. The common abnormalities seen include enlarged gallbladder in 33.5%, increased liver parenchymal echogenicity in 26.3%, hepatomegaly in 23.7%, and thickened gallbladder wall in 7.8% patients. Other findings included gallbladder sludge and gall stone in 6.6 and 2.1% respectively. These findings correlated significantly with the patients' CD_4^+ count and liver enzymes (ALT, AST and ALP) with p value < 0.05 in both. Ultrasonography as a baseline imaging modality in HIV/AIDS patients, together with CD_4^+ count and liver enzymes are useful in the assessment of the disease state, monitoring of therapy and management of these patients.

Key words: Hepatobiliary ultrasonography, adult patients, CD4 cell count, HIV/AIDS, liver enzymes.

INTRODUCTION

Human immune deficiency virus (HIV) is a retrovirus that stores its genetic material as ribonucleic acid (RNA). Two strains of HIV are known to infect humans; HIV-1 and HIV-2. HIV-1 is by far the most common pathogen worldwide (Sebastian, 2002; Obajimi et al., 2008). In 2013, it was estimated that the number of people living

with HIV worldwide were more than 35 million; 70% of these individuals were in sub-Saharan Africa were in sub-Saharan Africa and the majority (61%) were women. Southern Africa is the worst affected region with the adult HIV prevalence exceeding 17.3% in eight southern African countries in 2011 (Igbinedion et al., 2009; Global Fact sheet, 2012; Worldwide AIDS and HIV statistics, 2011). The individuals who require antiretroviral therapy (ART) may not have access to it in sub-Saharan Africa thus worsening the morbidity and mortality. The prevalence of HIV infection in Plateau state is 4.9% while North Central Nigeria has the highest prevalence of 6.1%, the National prevalence is 4.4% (World Bank Nigeria Report, 2008; Federal Ministry of Health, 2005). Liver enzymes [alanine/aspartate transaminases (ALT/AST), and alkaline phosphatase (ALP)] abnormalities have been shown to be common in HIV/AIDS patients, probably due to direct inflammation induced by HIV virus on the liver cells and gall bladder or by other opportunistic agents (Ejilemele et al., 2007). Hepatobiliary disease is an increasingly important cause of morbidity and mortality in patients with HIV/AIDS (Gore et al., 1998). With the availability of highly active anti-retroviral therapy (HAART), there has been an observable change in the pattern of liver disease in these patients (Goldin et al., 2002). Although opportunistic infections and neoplasm are still seen, co-infection with hepatitis viruses, especially HCV, is now emerging as the most significant cause of liver disease in this group of patients (Guardaat el., 1983). In addition, drug-induced liver damage is becoming more prevalent due to the increased complexity and toxicity of the HAART regimens used (Goldin et al., 2002). Biliary disorders seen in AIDS patients can be classified into AIDS cholangiopathy (AC), acalculous cholecystitis (ACC) and non-HIV associated disease such as gallstones (Flum at el., 1997). Gallstone disease is the most commonly observed cause of acute cholecystitis in this population (Flum at el., 1997). While HIV related biliary disorders are rare, they are associated with significant morbidity when they occur. AC and ACC were first reported in 1983 by Guarda and Pitlik respectively (Guarda et al., 1983; Pitlik et al., 1983). The two diseases can occur concurrently with opportunistic infections. Bile ducts in AIDS patients appear to be uniquely susceptible to opportunistic infections (Enns et al., 2003). Since the advent of highly active antiretroviral therapy (HAART), the incidence of AIDS associated biliary disorders have been steeply declining (O'Hara et al., 2009; Ko et al., 2003). Distinct hepatobiliary sonographic findings have been reported in these patients and include hepatomegaly, altered liver echotexture, gall bladder sludge, gall bladder wall thickening, gallstones, AIDS Cholangitis and periportal lymphadenopathy (Pawar et al., 2013). CD_4 cell count is one of the baseline measurements and an important parameter used in the assessment of AIDS progression and follow-up of AIDS patients. CD_4 cell depletion in HIV-infected patients results from ongoing viral replication. Hepatobiliary diseases were initially thought to be associated with advanced immunosuppression (CD_4 cell counts < 200 cells/microlitre), but it was later recognized that the lesions associated with the disease can occur at any stage of HIV-1 infection, even before antibody seroconversion (Herman et al., 2003). The relationship of hepatobiliary sonographic findings with CD_4 cell count and liver enzymes has not been well exploited in this environment.

METHODOLOGY

Study design, period and setting

This was a hospital based cross-sectional study that spanned over the period of six months from November 2015 to April 2016 in the Department of Radiology, Jos University Teaching Hospital (JUTH), a tertiary health institution situated in the central part of Jos, Nigeria.

JUTH is one of the teaching hospitals in the North-Central Zone of Nigeria. The hospital provides medical and surgical care, gynaecology and obstetrics services along with radiology, emergency services and an outpatient clinic to the population of the city and neighboring states of Bauchi, Gombe, Benue, Kogi, Nasarawa, Taraba, Adamawa and parts of Kaduna State. The hospital has an outpatient daily clinic (Clinic II) for HIV/AIDS management.

Ethical approval and consent to participate

Ethical clearance was obtained from the Research and Ethical Committee of Jos University Teaching Hospital, Jos, Nigeria dated 27th March 2015 with authorization: JUTH/DCS/ADM/127/XIX/6074. In addition, informed consent was obtained from each enrolled participant.

Target population/study participants

The study population comprised patients aged 18 years and above confirmed with HIV who were referred for abdominal ultrasound scan from Clinic II located within the Jos University Teaching Hospital complex. Clinic II is a specialized clinic exclusively for the management of HIV/AIDS patient.

Study inclusion criteria

Participants in this study were those patients aged 18 years and above confirmed with HIV infection. Participants were selected

*Corresponding author. E-mail: daneze4@gmail.com.

Table 1. Demographic characteristics of HIV/AIDS patients in Jos.

Variables	Gender			χ^2/t-test	P
	Male (%)	Female (%)	Total (%)		
Age group					
≤30	26(18.6)	82(30.4)	108(26.3)	29.96	0.001
31-40	29(20.7)	100(37.0)	129(31.5)		
41-50	51(36.4)	53(19.6)	104(25.4)		
>50	34(24.3)	35(13.0)	69(16.8)		
Total	140(100)	270(100)	410(100)		
Mean age± SD	42.84±11.29	37.59±10.57	39.38±11.09	4.659	0.001

being informed of the nature of the study, the potential benefits and safety of the procedure, as well as the voluntary nature of the participation.

Study exclusion criteria

Participants who were pregnant HIV positive patients, HIV positive adults with confirmed co-infection with Hepatitis A, B or C viruses and HIV positive adults with co-existing medical conditions such as Diabetes mellitus, sickle cell disease and hypertension were excluded from the study.

Data collection procedure and analyses

Patients were prepared by asking them to fast for 6 to 8 h to reduce bowel gas and ensure gallbladder distension. Blood pressures were measured using a standard mercury sphygmomanometer to exclude hypertensive patients from the study. Western Blot and CD_4^+ results were obtained from patients' case files. All the patients were on Highly Active Antiretroviral Therapy (HAART). The time between CD_4^+ count and ultrasound findings were less or equal to six months. Five millilitres (5 ml) of blood sample was collected from each patient using a new syringe into a clean dry glass test tube and centrifuged to obtain the serum. This serum was analysed for liver enzymes (ALT, AST and ALP) by enzymatic method using spectrophotometer. The ultrasound scan examination was performed using a high resolution real time ultrasound scanner (ALOKA SSD-3500, Co. Ltd., Tokyo, Japan, 2007) equipped with 3.5 MHz curvilinear transducer and Doppler facility. Ultrasound scan is operator dependent; therefore, all scanning and measurements were done only by the researcher in order to minimize inter-observer error. All measurements were taken twice to reduce intra observer variability. The liver, gallbladder and biliary ducts were then examined sonographically using standard ultrasound scanning procedures.

Data preparation, management and analysis

At the end of the examinations, the hepatobiliary ultrasonographic findings and the data obtained were entered into a computer and processed using SPSS for window version 20.0 (Microsoft® Inc. Chicago, Illinois, USA. 2011). Mean ± standard deviation was used to summarize variables. The variables were tested for correlation via the Chi- square test, students' test and cross tabulations. P value of 0.05 or less was considered statistically significant.

RESULTS

Sex and age variables

A total of 410 HIV positive patients, who met the inclusion criteria were studied. This comprised 140 males (34.15%) and 270 females (65.85%) with male to female ratio of approximately 1:2. Majority of the male patients were within age range 41-50 years while most female patients presented at an earlier age range of 31-40 years. The mean age was significantly higher in males than females (Table 1).

CD4 count and liver enzymes analyses

The frequency of patients reduced with increasing severity of disease and decreasing CD_4^+ count from 47.6% for 'not significant' (≥500 cell/microlitre) to 9.3% for 'severe' (<200 cell/microlitre) CD_4^+count (Table 2). The result showed 69.8% of patients had normal serum ALT; while 30.2% had various degrees of elevated ALT. The highest abnormal frequency for ALT was seen in Grade 1 (19.8%) and the lowest (1.5%) in grade 3 (Table 2). Most patients (51.7%) had normal serum AST while 48.3% had abnormal levels of serum AST. The highest abnormal frequency for AST was also seen in Grade 1 (22.2%) and the lowest (1.5%) in grade 3 out of this 22.2% was seen in grade 1 (Table 2). Majority of patients (60.5%) had various grades of abnormal ALP with the highest abnormality seen in Grade 0 (32.4%) and the lowest in Grade 2 (2.5%) (Table 2). No patient had Grade 3 ALP abnormality.

Age and CD4 count analysis

The CD_4^+ count decreased with increasing age in all CD_4 classification groups, except for age range 31-40 years and >50years which showed no specific trend with CD_4^+count. There was significant negative correlation between age and CD_4^+ count (P=0.001) (Table 3).

Table 2. Distribution of patients in relation to CD_4^+ count and liver enzymes.

CD4+ count and liver enzymes	Frequency	Percentage
CD4+ classification		
Not significant(≥500)	195	47.6
Mild(350-499)	106	25.8
Advanced (200-349)	71	17.3
Severe(<200)	38	9.3
Total	410	100
ALT grade (IU/L)		
Normal (≤40IU/L)	286	69.8
0(<1.25 times ULN)	30	7.2
1(1.25-2.25 times ULN)	81	19.8
2(>2.25-5 times ULN)	7	1.7
3(>5.0-10 times ULN)	6	1.5
Total	410	100
AST grade (IU/L)		
Normal (≤40IU/L)	212	51.7
0(<1.25 times ULN)	80	19.5
1(1.25-2.25 times ULN)	91	22.2
2(>2.25-5 times ULN)	21	5.1
3(>5.0-10 times ULN)	6	1.5
Total	410	100
ALP grade (IU/L)		
Normal (21-92IU/L)	162	39.5
0(<1.25 times ULN)	133	32.4
1(1.25-2.25 times ULN)	105	25.6
2(>2.25-5 times ULN)	10	2.5
3(>5.0-10 times ULN)	-	-
Total	410	100

ULN = Upper Limit of Normal; ALT = Alanine Transaminase; AST = Aspartate Transaminase; ALP = Alkaline Phosphatase.

Table 3. Relationship between age and CD_4^+ count of HIV/AIDS patients frequency (%).

Age group (years)	CD_4^+ classification				Total
	Not significant	Mild	Advanced	Severe	
≤30	67(62.0)	18(16.7)	15(13.9)	8(7.4)	108(100)
31-40	43(33.3)	52(40.3)	23(17.8)	11(8.6)	129(100)
41-50	53(51.0)	24(23.2)	13(22.1)	4(3.8)	104(100)
>50	32(46.3)	2(17.4)	10(14.5)	15(21.7)	69(100)
Total	195(47.6)	106(25.9)	71(17.3)	38(9.3)	410(100)

$\chi^2 = 44.507$, df = 9, P = 0.001.

Sex and CD4 count analysis

For both genders, the frequency of patients reduced with increasing severity of disease and decreasing CD_4^+ count. Most of the patients (47.6%) were in the 'not significant' disease group (Table 4).

Table 4. Relationship between gender and CD_4^+ count of HIV/AIDS patients frequency (%).

CD_4^+ classification (cell/microlitre)	Gender		Total
	Male	Female	
Not significant(≥500)	54(38.6)	141(52.2)	195(47.6)
Mild(350-499)	38(27.1)	68(25.2)	106(25.8)
Advanced (200-349)	37(26.4)	34(12.6)	71(17.3)
Severe(<200)	11(7.9)	27(10.0)	38(9.3)
Total	140(100)	270(100)	410(100)

Table 5. Relationship between age and Liver enzymes of HIV/AIDS patients.

Liver enzymes	Age (years)					χ^2	Df	P
	≤30	31-40	41-50	>50	Total			
ALT grade								
Normal	69(63.9)	96(74.4)	71(68.3)	50(72.5)	286(69.8)	20.248	12	0.063
0	4(3.6)	12(9.3)	8(7.7)	6(8.7)	30(7.3)			
1	29(26.9)	21(16.3)	18(17.3)	13(18.8)	81(19.8)			
2	3(2.8)	0(0.0)	4(3.8)	0(0.0)	7(1.7)			
3	3(2.8)	0(0.0)	3(2.9)	0(0.0)	6(1.5)			
Total	108(100)	129(100)	104(100)	69(100)	410(100)			
AST grade								
Normal	66(61.1)	64(49.6)	51(49.0)	31(44.9)	212(51.7)	21.324	12	0.018
0	17(15.7)	19(14.7)	21(20.2)	23(33.3)	80(19.5)			
1	19(17.6)	38(29.5)	22(21.2)	12(17.4)	91(22.2)			
2	3(2.8)	8(6.2)	7(6.7)	3(4.4)	21(5.1)			
3	3(2.8)	0(0.0)	3(2.9)	0(0.0)	6(1.5)			
Total	108(100)	129(100)	104(100)	69(100)	410(100)			
ALP grade								
Normal	46(42.6)	52(40.3)	42(40.4)	22(31.9)	162(39.6)			
0	36(33.3)	39(30.2)	35(33.7)	23(33.3)	133(32.4)	19.785	9	0.019
1	23(21.3)	38(29.5)	20(19.2)	24(34.8)	105(25.6)			
2	3(2.8)	0(0.0)	7(6.7)	0(0.0)	10(2.4)			
Total	108(100)	129(100)	104(100)	69(100)	410(100)			

Age, sex and liver enzymes analysis

Across all the age groups, ALT was normal in 69.8% of patients. Of these, the ALT abnormality was mostly Grade 1 seen in 19.8% and least frequent abnormality (1.5%) was grade 3. There was no significant association between patients age and serum ALT (P=0.063) (Table 5). About half (51.7%) of patients had normal AST and the proportion of patients with normal values decreased with increasing age group, this finding was statistically significant (P=0.018, Table 5). The most frequent AST abnormality was grade 1 and least grade 3 seen in 1.5% of subjects. Only about 40% of subjects had normal ALP and the proportion of patients with normal values decreased with increasing age group, this finding was statistically significant (P=0.019, Table 5). The most frequent ALP abnormality was grade 0 seen in 32.4% and least was grade 2 seen in 2.4% of subjects. In both genders and for all the enzymes, the frequency of patients generally reduced with increasing grade of liver enzymes abnormalities. This finding was not statistically significant (P=0.995, 0.97 and 0.970 respectively (Table 6).

Ultrasound findings

Two hundred and sixteen (52.7%) patients out of the 410

Table 6. Relationship between gender and Liver enzymes of HIV/AIDS Patients.

Liver enzymes	Gender			χ^2	Df	P
	Male (%)	Female (%)	Total (%)			
ALT grade						
Normal	112(70.0)	174(69.6)	286(69.8)	0.205	4	0.995
0	11(6.9)	19(7.6)	30(7.3)			
1	32(20.0)	49(19.6)	81(19.8)			
2	3(1.9)	4(1.6)	7(1.7)			
3	2(1.4)	4(1.6)	6(1.4)			
Total	160(100)	250(100)	410(100)			
AST grade						
Normal	80(50.0)	132(52.8)	212(51.7)	0.493	4	0.974
0	32(20.0)	48(19.2)	80(19.5)			
1	37(23.1)	54(21.6)	91(22.2)			
2	9(5.6)	12(4.8)	21(5.1)			
3	2(1.3)	4(1.6)	6(1.5)			
Total	160(100)	250(100)	410(100)			
ALP grade						
Normal	65(40.6)	97(38.8)	162(39.6)	0.243	3	0.97
0	52(32.5)	81(32.4)	133(32.4)			
1	39(24.4)	66(26.4)	105(25.6)			
2	4(2.5)	6(2.4)	10(2.4)			
Total	160(100)	250(100)	410(100)			

Table 7. Distribution of abnormal hepatobiliary ultrasound findings in HIV/AIDS patients.

Ultrasound findings	Frequency	Percentage
Enlarged GB	116	33.5
Increased liver Echogenicity	91	26.3
Hepatomegaly	82	23.7
Thickened GB wall	27	7.8
Gall bladder sludge	23	6.6
Gall stones	7	2.1
Total	*346	100.

GB=Gallbladder. *Some patients presented with more than one ultrasound findings.

studied had normal hepatobiliary ultrasound scan while the remaining 194 patients had various sonographic abnormalities (Table 7). The commonest hepatobiliary abnormality seen was enlarged gall bladder followed by increased liver Echogenicity and hepatomegaly (Figure 1) while the least frequent abnormal finding was gall stones (Figure 2) seen only in female patients. No significant association was seen between sonographic abnormalities and gender (P = 0.507) (Table 8).

Age and abnormal ultrasound findings

Hepatomegaly and increased liver echogenicity were the commonest abnormalities seen in majority of patients over >50 years while thickened gallbladder wall and gallbladder sludge were more frequent in age group 41-50 years. Enlarged gallbladder was seen commonly in younger age group of ≤30 years. There was statistically significant association between the abnormal ultrasound

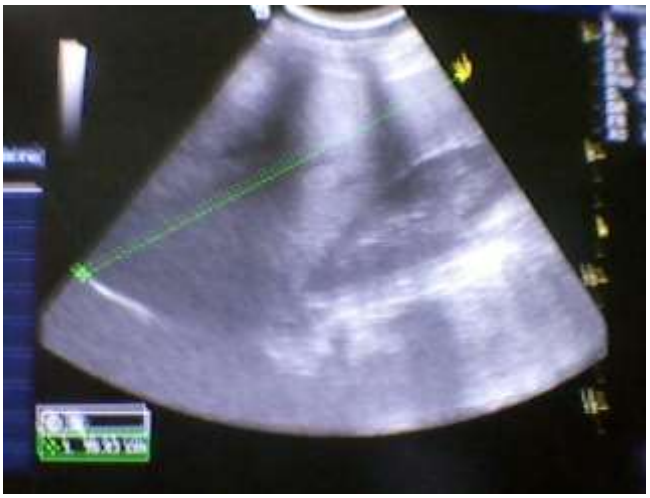

Figure 1. Longitudinal ultrasound image of the liver demonstrating increased liver echogenicity and hepatomegaly in a 43 year old female HIV positive patient.

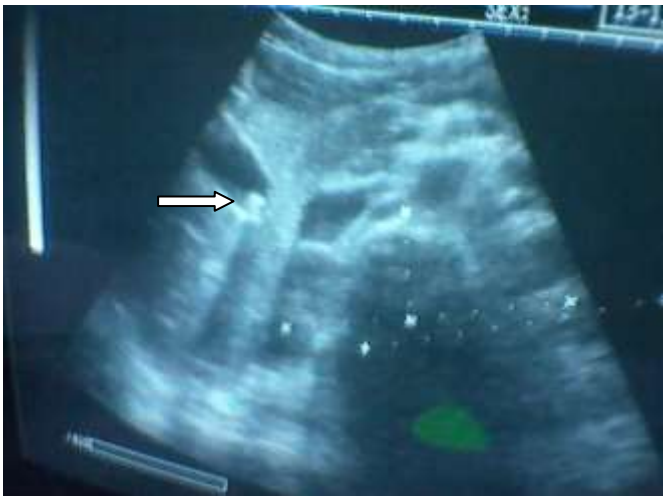

Figure 2. Longitudinal ultrasound image of the gallbladder (GB) showing a gallstone (White arrow) in a 39 year old HIV female positive.

Table 8. Distribution of Ultrasound findings in relation to gender.

Ultrasound findings	Gender		
	Male	Female	Total
Enlarged GB	25(32.1)	91(34.0)	116(33.5)
Increased liver Echogenicity	21(26.9)	70(26.1)	91(26.3)
Hepatomegaly	21(26.9)	61(22.8)	82(23.7)
Thickened GB wall	4(5.1)	23(8.6)	27(7.8)
Sludge	7(9.0)	16(6.0)	23(6.6)
Gall stone	0(0.0)	7(2.6)	7(2.1)
Total	78(100.0)	268(100.0)	*346(100.0)

*Some patients have multiple findings. GB = Gallbladder. $\chi^2 = 4.303$; df = 5. P = 0.507.

Table 9. Distribution of abnormal Ultrasound findings in relation to age group frequency (%).

Ultrasound findings	Age group (years)				
	≤30	31-40	41-50	>50	Total
Enlarged GB	35(38.9)	39(37.9)	30(28.6)	12(25.0)	116(33.5)
Increased liver echogenicity	22(24.4)	28(27.2)	26(24.8)	15(31.3)	91(26.3)
Hepatomegaly	22(24.4)	21(20.4)	24(22.9)	15(31.3)	82(23.7)
Thickened GB wall	7(7.8)	4(3.9)	13(12.4)	3(6.3)	27(7.8)
Sludge	4(4.4)	4(3.9)	12(11.4)	3(6.3)	23(6.6)
Gall stone	0(0.0)	7(6.8)	0(0.0)	0(0.0)	7(2.0)
Total	90(100)	103(100)	105(100)	48(100)	*346(100)

*Some patients have multiple findings. GB = Gallbladder. $\chi^2 = 32.507$; df = 15; P = 0.005.

Table 10. Relationship between ultrasound findings and CD_4^+ classification.

CD_4^+Classification	Ultrasound findings			χ2	df	P
	Normal frequency (%)	Abnormal frequency (%)	Total frequency (%)			
Not significant(≥500)	110(50.9)	85(43.8)	195(47.6)	37.023	3	0.001
Mild (350-499)	60(27.8)	46(23.7)	106(25.9)			
Advanced (200-349)	36(16.7)	35(18.0)	71(17.3)			
Severe (<200)	10(4.6)	28(14.4)	38(9.3)			
Total	216(100)	194(100)	410(100)			

Table 11. Distribution of Ultrasound findings in relation to CD_4^+ classification frequency (%).

Ultrasound findings	CD4+ classification					χ2	P
	Not Sig.	Mild	Advanced	Severe	Total		
Enlarged GB	63(35.8)	29(44.6)	17(30.4)	7(14.3)	116(33.5)	61.517	0.001
Increased liver echogenicity	41(23.3)	13(20.0)	13(23.2)	24(49.0)	91(26.3)	23.066	0.001
Hepatomegaly	41(23.3)	23(35.4)	14(25.0)	4(8.2)	82(23.7)	36.146	0.001
Thickened GB wall	14(8.0)	0(0.0)	6(10.7)	7(14.3)	27(7.8)	4.222	0.121
Sludge	10(5.7)	0(0.0)	6(10.7)	7(14.3)	23(6.6)	1.130	0.568
Gall stone	7(4.0)	0(0.0)	0(0.0)	0(0.0)	7(2.0)	-	-
Total*	176(100)	65(100)	56(100)	49(100)	*346(100)	-	-

*Some patients have multiple findings. GB = Gallbladder.

findings and age (P=0.005) (Table 9).

CD4 count and Ultrasound findings

Of the patients studied, 52.7% had normal ultrasound findings. In those with abnormal ultrasound findings, 43.8% were in the 'not significant' CD_4group and the frequency of abnormal ultrasound findingsreduced with decreasing CD_4^+ count and increasing disease severity, implying a negative correlation with disease severity (P = 0.001) (Table 10).

In those with abnormal ultrasound findings, patients with hepatomegaly and enlarged gallbladder were commoner in the mild CD_4^+ class while those with increased liver echogenicity, thickened gallbladder wall and sludge were most frequent in the severe CD_4^+ category. Gall stone was present only in the not significant CD_4^+class. Enlarged GB, increased liver echogenicity and hepatomegaly correlate significantly with CD_4^+ count (P = 0.001). However, thickened gallbladder wall and gallbladder sludge did not individually correlate significantly with CD_4^+ count (P = 0.12 and 0.57 respectively) (Table 11).

Liver enzymes and ultrasound findings

Majority (46.1%) of the patients who had normal ultrasound finding had normal serum liver enzymes however, some patients with normal serum liver enzymes also had abnormal ultrasound findings and vice versa. Hepatomegaly was seen in various grades of liver enzymes abnormalities except grade 2 ALT. It was most frequent in grades 3 for ALT and AST but in grade 1 for ALP. This finding was statistically significant (P = 0.001) (Table 12). Increased liver echogenicity varied with degree of elevated liver enzymes but was worse in grade 2 for ALT, grade 3 for AST and grade 2 for ALP. This finding was also statistically significant (P = 0.001, Table 12). Enlarged GB was seen in all grades of elevated liver enzymes and was most frequent in grade 2 for ALT, grade 3 for AST and grade 1 for ALP. This finding was statistically significant (P = 0.001, Table 12). Thickened GB wall was seen in grades 0 and 1 for ALT, and grades 0-2 for AST and ALP. The finding was statistically significant (P = 0.013 and 0.017 for ALT and AST respectively) (Table 12).

Among the three (3) liver enzymes studied, ALP has the highest sensitivity (68) to truly detect those patients with sonographic detectable hepatobiliary disease followed by AST (55.7). However, the ALT has the highest specificity (75.9) to detect correctly absence of hepatobiliary diseaseon ultrasound. ALT also has the highest positive predictive value implying those with abnormal ultrasound truly have the hepatobiliary disease while ALP has the highest negative predictive value implying that those with normal ultrasound truly have no hepatobiliary disease (Table 13).

Logistic analysis

Logistic regression shows that hepatomegaly was the most likely abnormal ultrasound finding (OR=4.08) to be seen in HIV/AIDS patients with advanced/severe CD_4 counts followed by enlarged gallbladder (OR=2.83) while increased liver echogenicity was the least probable. However these findings were not statistically significant with P = 0.08 or 0.48 (Table 14). Among the three (3) liver enzymes studied, abnormal ALP enzyme value (OR=1.3) was the most likely to be seen in patients with advanced/severe CD_4 counts followed by AST (OR=0.63). However these findings were also not statistically significant with P = 0.61 or 0.34) (Table 15).

DISCUSSION

The number of people living with HIV/AIDS worldwide was estimated to be 36.9 million at the end of 2014 according to World Health Organization (Worldwide AIDS and HIV statistics, 2011). The use of highly active antiretroviral therapy (HAART) has modified the disease pattern. Clinical and ultrasound findings in HIV/AIDS depend on the immune status of the patient as represented by their CD_4^+ count (Igbinedion et al., 2009). Abnormalities of liver enzymes are common in HIV/AIDS patients and these may be due to direct inflammation induced by HIV on the liver cells and gall bladder or due to complications of antiretroviral drugs (Ejilemele et al., 2007). A total of 410 HIV positive patients on HAART were studied, majority of the patients were females (65.8%) with male to female ratio of 1: 2. This is in agreement with a study done in South Western Nigeria by Obajimi et al. (2008) which documented a female preponderance of 66.5%. This finding is also similar to the report of the Joint United Nations Programme on HIV/AIDS (UNAID), 2010) which documented that the percentage of women living with HIV/AIDS increased from 43% in 1999 to 50% in 2010 and that Sub-Sahara African women comprised 59% of adults infected with HIV (Global Fact sheet, 2012). The sex ratio in this study was however at variance with the study documented in Sudan by Mahmoud et al. (2012) which showed a higher male preponderance of 66%. The finding of female preponderance in this study could be due to the fact that females' genital anatomy places them more at risk for sexually transmitted infections, compared to men (Center for Disease Control and Prevention Fact sheet, 2017). Females also have better health seeking behaviours and are routinely counseled during antenatal care for HIV testing leading to the discovery of their HIV status (Center for Disease Control and Prevention Fact sheet, 2017).

Approximately 83% of the patients were within the age range of 18 to 47years, thereby constituting the majority of the patients in this study population. This age range was described by Adeoye (2005) as the economically productive segment of Nigerian society and also the age group at the greatest risk of HIV/AIDS. However, there were few patients above 50 years old, possibly because most of the older populations are less sexually active (Adeoye, 2005). Finding is in agreement with a study done by Igbinedion et al. (2009) in Benin-Nigeria which documented about 89% of the patients to be between 18 to 47 years. The mean age for females and males were 37.59 years and 42.84 years respectively. This mean age was also consistent with the study done in South Western Nigeria by Obajimi et al. (2008) which documented mean age of female HIV patients to be 38.02 years. This may be due to the fact that females are sexually active at an early age compared to males (Abubakar et al., 2014).

Based on the World Health Organization (WHO, 2006) classification of CD_4^+ immunological profile in adult HIV infected patients, 73.4% of the patients had CD_4^+ count of350 cells/µl and above making up 47.6 and 25.8% for the "Not significant" and "Mild" categories respectively while only 9.3% were in the "Severe" category (that is CD4+ count<200cells/µl). This was at variance with the findings of Igbinedion et al (2009) who found almost half (46.3%) of the patients to be in the "Severe" CD4+ class.

Table 12. Distribution of Ultrasound findings in relation to liver enzymes classification.

Ultrasound findings	Liver enzymes grading						χ2	P
	Normal	0	1	2	3	Total		
	N(%)	N(%)	N(%)	N(%)	N(%)	N(%)		
				ALT				
Normal	164(43.6)	11(23.9)	41(33.9)	0(0.0)	0(0.0)	216(38.4)	-	-
Hepatomegaly	45(12.0)	11(23.9)	23(19.0)	0(0.0)	3(25.0)	82(14.6)	48.927	0.001
Increased liver echogenicity	57(15.2)	8(17.4)	16(13.2)	4(57.1)	6(50.0)	91(16.2)	107.956	0.001
Enlarged GB	76(20.2)	8(17.4)	26(21.5)	3(42.9)	3(25.0)	116(20.6)	67.709	0.001
Thickened GB wall	16(4.3)	4(8.7)	7(5.8)	0(0.0)	0(0.0)	27(4.8)	8.667	0.013
Sludge	15(4.0)	4(8.7)	4(3.3)	0(0.0)	0(0.0)	23(4.1)	10.522	0.005
Gall stone	3(0.8)	0(0.0)	4(3.3)	0(0.0)	0(0.0)	7(1.2)	0.143	0.705
Total	376(100)	46(100)	121(100)	7(100)	12(100)	*562(100)	-	-
				AST				
Normal	126(45.7)	41(39.4)	39(28.7)	10(29.4)	0(0.0)	216(38.4)	-	-
Hepatomegaly	30(10.9)	15(14.4)	31(22.8)	3(8.8)	3(25.0)	82(14.6)	46.293	0.001
Increased liver echogenicity	36(13.0)	20(19.2)	18(13.2)	11(32.4)	6(50.0)	91(16.2)	28.615	0.001
Enlarged GB	55(19.9)	19(18.3)	32(23.5)	7(20.6)	3(25.0)	116(20.6)	76.586	0.001
Thickened GB wall	13(4.7)	3(2.9)	8(5.9)	3(8.8)	0(0.0)	27(4.8)	10.185	0.017
Sludge	9(3.3)	6(5.8)	8(5.9)	0(0.0)	0(0.0)	23(4.1)	0.609	0.738
Gall stone	7(2.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	7(1.2)	-	-
Total	276(100)	104(100)	136(100)	34(100)	12(100)	*562(100)	-	-
				ALP				
Normal	100(49.0)	68(33.7)	45(32.8)	3(15.8)	-	216(38.4)	-	-
Hepatomegaly	23(11.3)	33(16.3)	23(16.8)	3(15.8)	-	82(14.6)	23.171	0.001
Increased liver echogenicity	28(13.7)	39(19.3)	17(12.4)	7(36.8)	-	91(16.2)	25.176	0.001
Enlarged GB	26(12.7)	46(22.8)	41(29.9)	3(15.8)	-	116(20.6)	38.552	0.001
Thickened GB wall	10(4.9)	10(5.0)	4(2.9)	3(15.8)	-	27(4.8)	6.333	0.096
Sludge	13(6.4)	3(1.5)	7(5.1)	0(0.0)	-	23(4.1)	6.609	0.037
Gall stone	4(2.0)	3(1.5)	0(0.0)	0(0.0)	-	7(1.2)	0.143	0.705
Total	204(100)	202(100)	137(100)	19(100)	-	*562(100)	-	-

*Some patients have multiple findings. GB = Gallbladder; ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase.

Table 13. Sensitivity, Specificity, Positive predictive value and Negative predictive value using ultrasound as outcome variables.

Parameters	ALT	AST	ALP
Sensitivity	37.1	55.7	68.0
Specificity	75.9	58.3	48.3
Positive predictive value	58.1	54.5	53.2
Negative predictive value	57.3	59.4	61.7

ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase.

The higher CD4+ count and thus milder/not significant disease in this study maybe due to incessant public enlightenment, with resultant decreased stigmatization, earlier diagnosis and follow up as well as improved availability of anti retroviral drugs unlike in the past when most patients were well stigmatized, presented late and were unable to access or procure anti retroviral medications. This might also indicate the improved

Table 14. Logistic regression abnormal ultrasound findings and advanced/severe CD_4 counts.

Findings	Odd Ratio (OR)	95% C.I. for OR	P
Hepatomegaly	4.08	0.86-19.37	0.077
Increased liver echogenicity	0.63	0.17-2.28	0.483
Enlarged GB	2.83	0.70-11.51	0.143
Thickened GB wall	1.00	0.21-4.67	1.000
Sludge	1.00	-	-

GB = Gallbladder. C.I = Confidence Interval.

Table 15. Logistic regression liver enzymes and advanced/severe CD_4 counts

Findings	Odd ratio (OR)	95% C.I. for OR	P
ALT	0.187	0.07-0.48	0.060
AST	0.63	0.24-1.63	0.342
ALP	1.30	0.48-3.53	0.610

ALT = alanine transaminase; AST = aspartate transaminase; ALP = alkaline phosphatase. C.I = Confidence Interval.

effectiveness of government interventions such as public health education, Voluntary Confidential Counseling and Testing (VCCT), and availability of free Antiretroviral Drugs (ARVS).

Based on the grading system developed by the Acquired Immune Deficiency Syndrome (AIDS) Clinical Trials Group (CTG) in assessing the serum level of liver enzymes of infected individuals. In this study, 53.7% of the patients had normal liver function tests while 46.3% of the patients had various abnormalities of their liver enzymes (ALT, AST and ALP). This is not in agreement with the study done by Ejilemele et al. (2007) in Port Harcourt Nigeria who documented a higher (87.6%) abnormal liver enzyme. This study is also at variance with the finding of Savita et al. (2015) in India which also documented a higher (63%) abnormal liver function tests (LFT). In this study, out of the patients with abnormal (46.3%) liver enzymes, 39.4% were classified as hepatocellular liver (elevated AST and ALT) injury while 60.6% were classified as cholestatic liver (elevated ALP) injury. This is not in agreement with the study done by Ejilemele et al. (2007) in Port Harcourt Nigeria who recorded a lower (14.5%) cholestatic liver injury and a higher (85.5%) hepatocellular injury (Ejilemele et al (2007). This study is also not in agreement with the finding of Savita et al (2015) in India which documented 63% of their patients with abnormal liver function tests (LFT). They recorded a lower (6%) cholestatic liver injury, a higher (29%) hepatocellular injury and 28% had a mixed pattern of liver injury. The reason for the higher prevalence of abnormal liver function tests in the Port Harcourt and India studies is most likely due to the fact that the patients studied were both not on antiretroviral therapy at the time of data collection.

Different hepatobiliary sonographic abnormalities such as enlarged gallbladder (GB), increased liver echogenicity, hepatomegaly, thickened GB wall, gallbladder sludge and stone were observed in this study which were comparable with those documented by other authors (Obajimi et al (2008); Igbinedion et al (2009); Pawar et al. (2013); Mahmoud et al (2012). Some of these abnormal ultrasound findings (enlarged GB, increased liver echogenicity and hepatomegaly) correlated significantly with CD_4^+ count (P = 0.001). The "Not significant" CD_4^+ class recorded 43.8% abnormal hepatobiliary ultrasound findings as compared to 14.4% noted in the "severe" CD_4^+ class. In a similar study, Pawar et al. (2013) documented 43.3% abnormal hepatobiliary ultrasound findings in the "Not significant" CD_4^+ (>350 cells/µl) category and 56.7% in the "Significant" CD_4^+ (≤350 cells/µl) class. This was in contrast with the finding of Igbinedion et al. (2009) who documented a higher abnormal abdominal ultrasound finding of 85.7% in the "Not significant" CD_4^+ class and 89.9% in the "severe" CD_4^+ category. Hepatomegaly was the third most common abnormal hepatobiliary sonographic findings in this study as seen in 23.7% patients. Different studies had documented figures such as 13.3, 39.0, 40.0, 35.0 and 41.0% respectively for hepatomegaly; (Obajimi et al., 2008; Igbinedion et al., 2009; Javier et al., 2005; Tshibwabwa et al., 2000; Grumbach et al., 1989). According toIgbinedion et al. (2009), hepatomegaly in these patients could be due to infections, non specific response to infective hepatitis, fatty infiltration or neoplastic infiltration from lymphoma or Kaposi sarcoma. Increased liver echogenicity was the second most common finding as seen in 26.3% patients. Grumbach et al. (1989) documented a higher finding of

45.5%. Both hepatomegaly and increased liver echogenicity negatively correlate with CD_4^+ count (Both P = 0.001). This was also in contrast with the study of Igbinedion et al. (2009), whose finding (hepatomegaly and increased liver echogenicity) did not correlate with CD_4^+ count. Increased liver echogenicity in HIV could be due to fatty infiltration, chronic hepatitis, drug and alcoholic induced liver diseases as in the general population (Valentina and Giuseppe, 2012). Gallbladder enlargement was noted in 33.3% of patients while thickened gallbladder wall, gallbladder sludge and gallbladder stones were noted in 7.8, 6.6 and 2.1% of the patients respectively. In similar studies, Igbinedion et al. (2009) documented thickened gallbladder wall in fewer patients (7.3%) and gall stones in a higher proportion (4.7%) of the patients. Pawar et al. (2013) who recorded thickened gallbladder wall and gall stones in a higher proportion(9.0%) and (4.0%) of the patients respectively and gallbladder sludge in fewer patients(6.0%) which did not correlate with CD_4^+ count. Obajimi et al. (2008) recorded gall stones in only 1.3% of their patients. Grumbach et al. (1989) recorded thickened gallbladder wall in 55%, dilated gallbladder in 18%, biliary sludge in 23% and gall stones in 5% of the patients. This difference (lower values in this study) in gallbladder abnormal findings may be attributed to the fact that there is better awareness on HIV/AIDS. Therefore, most patients present early coupled with the availability of HAART and early treatment. Thickened gallbladder wall in HIV could be due to cholecystitis, hepatitis, sepsis and tuberculosis (HIV infection and Gallstones-Risk factors, 2017).

The major risk factors for developing gallstones in HIV include male sex, increasing age (>40years), those on Norvir therapy and high blood pressure (Romano et al., 1988). Gallbladder sludge in HIV is associated with opportunistic infection, cholecystitis and hepatitis (HIV infection and Gallstones-Risk factors, 2017). Hepatomegaly was the most likely abnormal ultrasound finding in HIV/AIDS patients with advanced/severe CD_4 counts while increased liver echogenicity was the least probable. However these findings were not statistically significant (P=0.77 and 0.48 respectively) using logistic regression test. No specific reason could be adduced for this finding and further studies would be required in this regard. Aspartate and Alanine transaminases are excellent markers of hepatobiliary injury while Alkaline Phosphatase is a marker of biliary diseases; however, none of the enzymes are specific for hepatobiliary diseases as similar elevation of these enzymes may be encountered in myocardial infarction and some muscle injuries (Ocama et al., 2008).

In this study, the prevalence of abnormal liver enzymes (ALT, AST and ALP) and ultrasound abnormalities (hepatomegaly, increased liver echogenicity, enlarged GB, thickened GB wall, GB sludge and stone) in adult HIV positive patients was 47.3%. This was lower than in earlier reported studies done by Igbinedion et al. (2009), Pawar et al. (2013) and Mahmoud et al. (2012) which recorded 66.6, 96.0 and 93.3% respectively. This low prevalence may be due to the fact that all the patients at the time of conducting this study were already on regular Highly Active Antiretroviral Therapy (HAART).Some patients with normal liver enzymes had abnormal ultrasound findings and vice versa; an average of 46.3% of the patients had various grades of liver enzymes abnormalities.This was not in agreement with a study done by Savita et al 2015 in India which documented a higher (63%) abnormal liver function tests (LFT). In this study, 47.3% of the patients had various abnormal hepatobiliary ultrasound findings and this was in agreement with the study of Savita et al. (2015) who recorded 47% of various abnormal ultrasound findings in their patients. In this study, increased liver echogenicity was seen in 27.0% which is inconsistent with the finding of Savita et al. (2015) who recorded a higher percentage of 46.3%. This study recorded hepatomegaly in 27.1% of the patients which is also in contrast with a study done by Savita et al. (2015) who recorded 15.5%. In this study, both increased liver echogenicity and hepatomegaly significantly correlate individually with liver enzyme. There was however no correlation between LFTs (ALT, AST and ALP) and ultrasound findings in the study done by Savita et al. (2015). Infection by HIV increases activities of the three enzymes, which may be due to liver cells apoptosis caused by HIV infection, or immune response to HIV replication which subsequently leads to hepatocellular necrosis and inflammation (Housset et al., 1990; Hufert et al., 1993; Gendrault et al., 1991). Low frequency of abnormal liver function tests and various abnormal hepatobiliary ultrasonographic findings in this study could be due to early diagnosis and treatment with readily available antiretroviral therapy. Elevated serum alkaline phosphatase was the most likely liver enzyme abnormality in patients with advanced/severe CD_4 counts followed by aspartate transaminases as indicated by logistic regression analysis but this was not statistically significant (P = 0.61).

Conclusion

Ultrasonography is a versatile tool for evaluating hepatobiliary organs affected by HIV/AIDS. Most abnormal sonographic hepatobiliary findings in patients with HIV/AIDS correlated significantly with CD_4^+ count and liver enzyme levels. Therefore, ultrasound scan could be introduced as part of management/follow-up of HIV/AIDS patients as it may improve management out come in these patients. Ultrasound is easy to perform, non invasive, inexpensive and readily available in this part of the world where HIV/AIDS is most prevalent. Among the liver enzymes studied, ALP has the highest sensitivity to truly detect those patients with sonographic Detectable hepatobiliary disease and highest negative

predictive values implying those with negative abnormal ultrasound truly have no hepatobiliary disease.

RECOMMENDATIONS

Hepatobiliary ultrasound scan should be made an initial routine examination in all HIV clinics for better management of these patients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Case Report

Clinical and biological outcomes in an HIV-positive child treated with the immuno-modulator 6,6'-dithiodinicotinic acid (CPDS) over four years: A case report

Adolphe Ndarabu Igulu[1*], Leon Tshilolo Muepu[1,2], Didier Mbuyi Mukendi [2,3] and Ronald Harvey Goldfarb[4]

[1]Dept of Medicine, Centre Hospitalier Monkole, Kinshasa, Democratic Republic of the Congo.
[2]Centre de Formation et d'Appui sanitaire (cefa), Kinshasa, Democratic Republic of the Congo.
3Department of Statistics and Mathematics, Faculty of Science, University of Kinshasa, Democratic Republic of the Congo
[4]Translational and Immuno-oncology, Infectious Disease and ImmunoEngineering Consulting, Guilford, CT, USA; Executive Consultant for the Grassetti Family Trust (GFT), Berkeley, CA, USA

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The elimination of HIV-AIDS by 2030 is a challenging target for a country such as the D.R. Congo, since currently fewer than 50% of persons living with HIV (PLWH) are under antiretroviral treatment (ART) and have a viral suppression. There also a high rate of death of PLWH in D.R. Congo. Accessible, affordable and sustainable immunotherapy, coupled with ART, can provide a substantial support to eliminating HIV. The purpose of this paper is to describe the measured clinical and biological profiles over a 4-year period of treatment with the immunomodulator 6,6'-dithiodinicotinic acid (CPDS), of a patient who was only 10-months old at the start of treatment. The patient was part of a larger two-year clinical trial of CPDS on HIV-AIDS. This study found that despite his advanced clinical HIV stage (being born to parents with terminal HIV), the patient maintained his weight and lymphocyte counts, and did not experienced any severe HIV-AIDS-related illness during the study period. This suggests a beneficial or protective effect of CPDS treatment. The patient shifted to ARV at 5-years old and is now 17-years old, and under first-line ART. The study concludes that survival of this child could likely be attributed to CPDS. We therefore recommend exploring further the simultaneous use of ART and CPDS immunotherapy for a greater clinical and biological benefit of PLWH. We also recommend further study into the mechanism of action of the compound.

Key words: HIV-AIDS treatment, immunomodulation, 6,6'-dithiodinicotinic acid.

INTRODUCTION

The clinical management of HIV/AIDS by standard antiretroviral therapy (ART) using the combination of antiretroviral (ARV) drugs alone cannot eradicate HIV reservoirs (Williams et al., 2011; Zhang and Crumpacker, 2013; Harrer et al., 1996). The interest of immunotherapy in HIV treatment is theoretically well established

(Nishimura et al., 2017; Martrus and Altfeld, 2016; Bahr et al., 2003). Some studies showed the practical benefit of use of immunotherapy in treating HIV (Gontran M, et al, 2009; Ndarabu et al., 2017). With IM28, only patients who received it alone or in combination with HAART showed in particular an increase in the levels of CD4 lymphocytes as well as significant reduction of viral load.

In a recent article, we showed that the use of CPDS alone (without ARV) resulted in equivalent effectiveness of ARV treatment in increasing mean body weight and mean CD4 during the first six months of treatment. Weight gain and CD4 increase indicated a recovering immune system. In addition, the study found no difference in mortality rates during the two years of follow-up between the two groups (Ndarabu et al., 2017). CPDS is a biologically active agent (Grassetti, 1986; Grassetti, 1970) that has pleiotropic anti-cancer and anti-metastatic properties, including functionality as a potent immunomodulator that significantly increases the number and activity of NK cells and increases the lymphoproliferation of T lymphocytes. It is devoid of identified side effects and does not have teratogenic or mutagenic effects (Grassetti and Moro, 2013).

In this case report, the focus is on one of the 34 participants, given the particular fact that he was only 10 months old at the beginning of the trial, in 2002 (Ndarabu et al., 2017). He continued to take CPDS alone for 2 years after the completion of the trial before he shifted to ARV treatment at age 5 (a total of 4 years under CPDS-only treatment). He is now 17-years old and regularly followed at Monkole Hospital under ART. The objective of this case report is to highlight the clinical and biological profile of this participant over the 4-year period under CPDS treatment. The clinical parameters considered are: weight, illness episodes or occurrence; the main biological parameter consists of lymphocytic counts (Grassetti and Moro, 2012).

CASE REPORT

The patient was born in December 2001 to HIV positive parents. He was initially admitted at Monkole Hospital in Kinshasa, D.R. Congo at 10-months of age, in October 2002. At that time he was at Stage 2 per the World Health Organization (WHO) Clinical Staging of 1990 criteria, and Stage B2 per the US Centers for Disease Control and Prevention (CDC) pediatrics HIV classification (WHO, 1990; CDC, 1994). There were no medical details about circumstances of birth or the past medical history of the mother and the child. The patient's birth weight was unknown. He was the sixth out of seven siblings. The last-born was premature and died at 6 days after birth. The father died few months later from HIV-AIDS. The mother was previously followed for HIV-AIDS in another medical facility in Kinshasa, without ART. At that time, access to ARV was not universal and required a significant expense that the mother could not afford. The Prevention of Mother to Child Transmission of HIV (PMTCT) policy then in force advocated only a single-dose of Nevirapine (NVP) for the exposed newborn and NVP single-dose/Tritherapy, depending on the mother's eligibility to receive ART (CD4 < 350/µl or WHO clinical stage ≥ 3). Breastfeeding was the rule. When the mother was 36-years old, she started her medical visits at Monkole Hospital (in September, 2002). She was at stage 4 of WHO HIV clinical staging (WHO, 1990), with a severe wasting syndrome, weighing only 38 Kg and displaying low values for counts of lymphocytes: CD4 at 145/µl, CD8 at 392/µl and CD4/CD8 at 0.37. This advanced clinical stage resulted in her death 7 months later, in April 2003. The patient was enrolled in the study following the settled inclusion criteria reported in the previous study with the approval of the Ethical Committee, assigned reference N/Réf: 002/CEFA-MONKOLE/CE/2002 as noted in (Ndarabu et al., 2017), and also with the consent of parents and the research team, considering the expected benefit of the intervention respect to any potential risk which in this case was very unlikely. At the beginning of the treatment, the clinical and biological parameters of the patient were as follows: 8 Kg of body weight at 10-months old, T° 36.8°C. WBC 11,500 mm^3 (N27L69E2M2), HCT 21%. Lymphocytes count (absolute number/µl): CD4 787; CD3 3021; CD8 >2000; CD4/CD8: 0.39. Viral load was not available. The immunomodulator used was CPDS (12 mg/Kg oral capsule twice a day).

Description of clinical and biological parameters

The patient's clinical and biological parameters of weight, ailment episodes, psychomotor development, CD4 count, and clinical evolution were monitored as part of this study. The results are summarized below.

Weight

As per the specified parameters in well-recognized tables for growth (Figure 1) from the World Health Organization (WHO) using the Z-scores of weight-for-age for boys from birth to 5-years old, we recorded the weight of the patient at different medical visits. The weight curve from the

*Corresponding author. E-mail: ado.ndarabu@gmail.com.

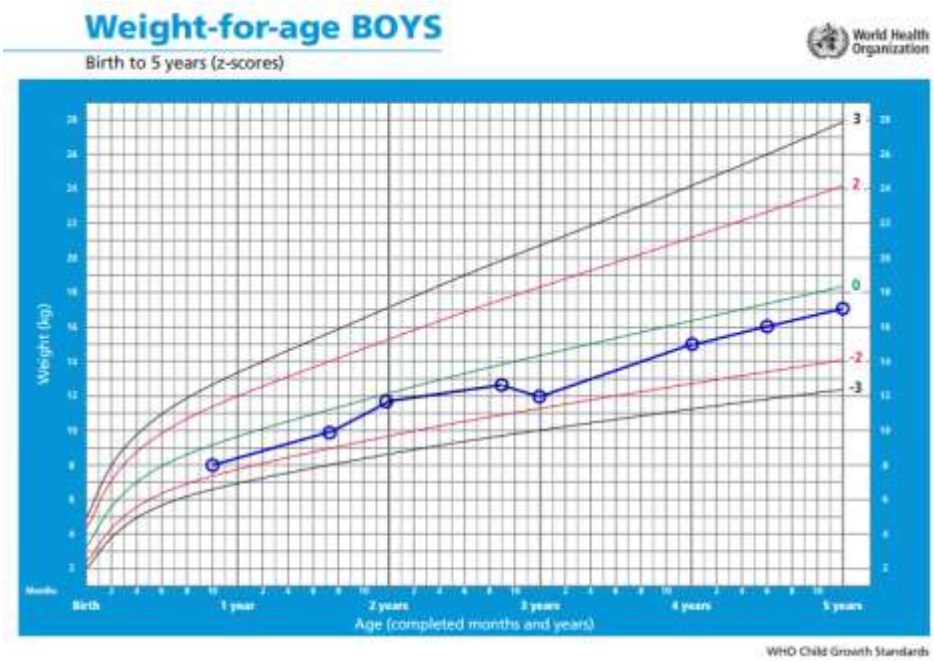

Figure 1. Growth curve of the child (illustrated by the thick blue line), overlain on WHO Child Growth Standards table in use in DR Congo.

beginning to the end of CPDS treatment remained between 0 and -2 Standard Deviation. A slight drop in weight was noticed at 36-months of age.

Ailment episodes

Table 1 shows the different clinical visits of the patient under CPDS treatment, along with the illnesses identified and biological findings. Leukocyte count is in absolute number. The relevant illnesses were made of bronchopneumonia (Figure 2), rhinitis, enteritis, prurigo and shingles. No AIDS-related ailments were noted.

Psychomotor development

The child was awake according to his age throughout CPDS intake. The evidence for spoken language occurred around 12 months of age with a slight stuttering. Walking also occurred at this time.

CD4 count

Numbers of CD4, CD3 and CD8 lymphocytes are expressed by microliter on Table 1. With respect to lymphocytic count, CD4 values remained at a mild (moderate) suppression level at less than 12 months of age, and also from 12 months up to 5-years old, suggesting the maintenance of immune system function under CPDS treatment.

Clinical evolution and comments

The death of the patient's father and mother from HIV-AIDS-related symptoms occurred when he was 13 and 16-months old, respectively, suggesting that they had been already in advanced stages of AIDS at the time he was born. This suggests the possibility that he was potentially infected *in utero*. At 10 months of age, when we started following the patient, the hospital did not have access to a biomolecular tool for HIV testing, and the diagnosis of HIV was made according to the National Policy in force (Minister of Health DRC, 2004), using an algorithm combining the risk factor (HIV exposed infant), the clinical pattern (small for age) and antibody testing (positive Elisa testing). The diagnosis was later confirmed at 18-months old with the concordance of three positive rapid test of HIV: Determine^{TM}; Double-Check^{TM}and Unigold^{TM}. In this context, we could therefore not definitively establish whether the maternal-to-child transmission of HIV occurred between the pre-, peri- or post-natal period.

DISCUSSION

As the patient was at stage B2 of CDC pediatric HIV classification at baseline, this suggests a certain level of

Table 1. Clinical events and biological data during a 4-year follow up period.

Date	Diagnostic Indicator	Lymphocyte count	Blood cell count
15/10/2002	Small size for his age	CD4 787 CD3 3,021 CD8 > 2,000 CD4/CD8 0,39	HCT 29% WBC 11,500 mm^3 FL N27L69E2M2 Sed. Rate 40 $mm/1^{st}$ Hr
04/11/2002	No special finding		
04/12/2002	Bronchopneumonia		HGB 7.5g/dl; HCT 25.1%; WBC 19,200 mm^3; N17M3 E1L69
25/08/2003	Four months of treatment interruption	CD4:716 CD8:2,743 CD3:3,578 CD4/CD8: 0,26	
03/12/2003	Diet related diarrhea		HGB 9.1/dl; HCT 28.8%; WBC 9,300 mm^3; N28 M1 E1 L70
18/03/2004	Flu syndrome	CD4 1,083 CD8 2,740 CD3 3,864 CD4/CD8 0,4	WBC 10,400; Hct 31.5%, HGB 9.9g/dl N24 M1 E4L71
25/06/2004			WBC 7,600 mm^3; RBC 4,58 10^6; HGB 9,3g/dl; HCT 30.2%; PLA 787 10^3; MCV 66 μm^3; TGMH 20.3 pg; MCCH 30.7 g/dl; N45 E3L52%; SR 30mm/H
02/09/2004	Enteritis	CD4:564 CD8:1,281CD3:1,940 CD4/CD8: 0,44	WBC 7,200; Hct 28.1%, HGB 8.9g/dl N67L29 E0 M4
12/12/2004	Prurigo		
15/11/2005	Bronchopneumonia, Prurigo		
13/12/2005	Shingles in process of healing since one week		
17/07/2006	Malaria, teeth decay	CD4 719(19%) CD8 1,784(47%) CD3 2,600(68%) R: 0,4	
24/07/2006	No special findings	CD4 656(16%) CD8 1,805(43%) CD3 2,557(61%) R: 0,36	
24/08/2006	Flu syndrome		

disease advancement (CDC, 1994). Throughout CPDS treatment, the child did not experience any severe ailment episodes that could indicate a Stage 3 WHO pediatrics classification. Because of low weight, the child was considered at Stage 2 (WHO, 1990). The steadiness of the weight curve suggests a certain benefit from CPDS despite the nutritional insecurity generated by the advanced illness of the mother and early death of the father. The child's weight slightly dropped at 36 months-old due to a shingles episode but recovered immediately to its previous upward trend as shown in the weight curve (Figure 1).

Evidence for episodes of fever was very scarce under CPDS treatment. Two fever episodes were recorded, at 2 and 8 months of follow-up, respectively, due to non-life-threatening bronchopneumonia and malaria episodes. The patient was not under Cotrimoxazole prophylaxis. In addition to the bronchopneumonia with fever, the only other observed episode of respiratory tract infection appeared to be from a flu syndrome as reported in Table 1. No lymph node enlargement was observed during the trial. The two diarrhea episodes observed were irrelevant, one of them related to diet. No tuberculosis, meningoencephalitis, or oral thrush episodes occurred (Yotebieng et al., 2010).

Hemoglobin was low particularly at the beginning of the follow up. At two months of CPDS treatment, hemoglobin was 7.5 g/dl. The values available up to 22 months of follow-up treatment were all below 10 g/dl. However, at any time the patient could require blood replacement. This multifactorial anemia was likely influenced by the poor nutritional status of the patient. Being a child in growth, the level of CD4 remained steady throughout CPDS uptake and did not drop (Weiser et al., 2011; Edmonds et al., 2012).

The child did not display an increase of weight during the 3-6 months interval in course of the therapy, as observed in the mean weight of the group in a previous study (Ndarabu et al., 2017); however, the weight did not drop into the red area of growth curve during the four years under CPDS treatment, which is up to 60-months old. This low weight for age in this case can be attributed to a deficient nutritional intake. During breastfeeding, the mother was already in cachexia with concordant, very advanced HIV-AIDS. Moreover, the socio-economic level of the housing where the child grew was one of the low-income. The data therefore suggests a protective effect of CPDS in the maintenance of weight of this child

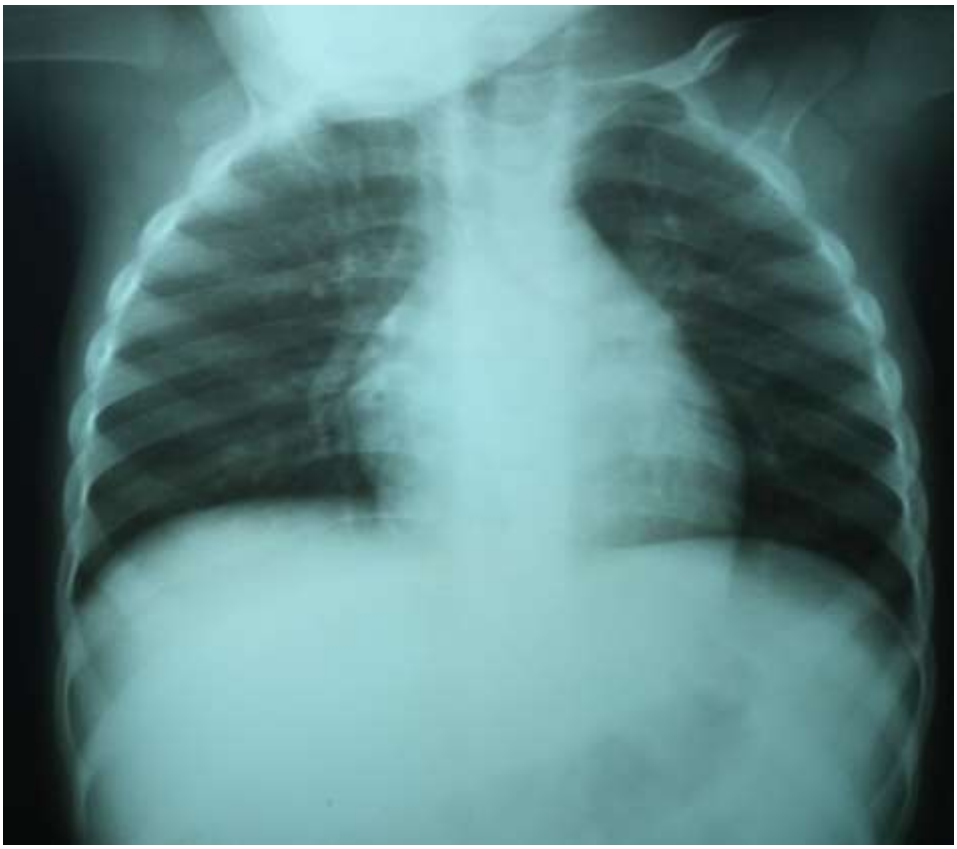

Figure 2. Chest X-ray in December 2002 with upper alveolar infiltrate at the beginning of Immunotherapy.

(Grassetti and Moro, 2012). The illness episodes he displayed were mainly made by rhinitis, enteritis, shingles, prurigo and mild bronchitis. The patient shifted to ART in 2006, when it became universally accessible. At the time of preparation of this paper, at age 17, he is still in first-line therapy made of 1 NNRTI + 2 NRTI, weighing 62 Kg at his last medical visit (September, 2018), with a height of 1.67 m as recorded in his medical history.

Interpretation of CPDS treatment results

Given the fact that, in Africa, 52% of untreated children born with HIV had died by age 2 (Newell et al., 2004), our interpretation of the studies described in this paper is that the survival of this child could likely be attributed to CPDS. This is consistent with the findings in our earlier study reviewing the multi-patient CPDS trial, which found the CPDS-treated group death rate at 23.5% compared to 23.3% of the ARV-treated group, for a 24-month follow-up period (Ndarabu et al., 2017). The rate of death among PLWH in D.R. Congo is still high despite availability of ARV, displaying a value of 24.1% (Catwaba et al., 2017). This has put the D.R. Congo at risk of missing the year 2020 90-90-90 targets (90% of all PLWH will know their HIV status; 90% of all PLWH will receive antiretroviral therapy and 90% of all PLWH receiving antiretroviral therapy will have viral suppression). Patients in the D.R. Congo display, for the two last target parameters, modest values of 42 and 31%, respectively (UNAIDS, 2017). Our clinical use of CPDS suggests that treatment with the compound could contribute substantially towards achieving the 2030 AIDS elimination target (UNAIDS, 2016) by adding immuno-modulation to the viral suppression efforts (Martrus and Altfeld, 2016).

CONCLUSION

The steady clinical and biological profile of this child during the four years of CPDS treatment suggests a beneficial impact for this immunotherapeutic agent even in a very young patient. Thus, we retain high interest of exploring further the simultaneous use of ART and CPDS immunotherapy for a greater clinical and biological benefit of PLWH. In light of previous findings that CPDS can activate NK cells in standard models of NK cell-mediated cytotoxicity, the significant evidence for the role

of activated NK cells for treating various infectious and malignant diseases, as presented in multiple reviews (Tomin et al., 2016; Goldfarb and Herberman, 1982), and increasingly specific evidence for a role for NK cell-mediated immunotherapy for control of AIDS/HIV (Garrido et al., 2018; Mavillo et al., 2005; Mikulak et al., 2017; Scully and Alter, 2016), the authors believe that additional investigation of the potential beneficial effects of CPDS immunotherapy on treatment of AIDS/HIV is justified.

Further investigation of CPDS also is warranted because this immunomodulator has a very low cost of goods and manufacture that would allow it to be pragmatically available to under-served and economically disenfranchised patient populations including those in the D.R. Congo. This is in stark contrast to currently available drugs that are often financially out of reach to patients, resulting in unmet medical needs. In sum, the data and economics both argue for further investigation of CPDS for its role as an immuno-modulator that could play a role in augmenting treatment of HIV/AIDS. Towards that end, the potential immunologic, mechanism of CPDS in regulating NK cell activities are undergoing more thorough investigation. This will be the subject of an additional communication.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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A mixed methods study of the factors associated with HIV testing among young people in Saudi Arabia

Abdullah Almilaibary[1*] and Mark Hayter[2]

[1]Family Medicine, Faculty of Medicine, Al-Baha University, Saudi Arabia.
[2]Department of Nursing, Faculty of Health Science, University of Hull, United Kingdom.

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Despite recent progress in enhancing the accessibility of HIV-related health services worldwide, opportunities to diagnose patients are often missed due to genuine barriers at different levels. The aim of the study is to explore the factors that affect the HIV testing uptake by young people in Saudi Arabia. For the quantitative strand of the study a newly developed self-completed online questionnaire was used and the study sample was drawn using a convenience sampling technique. Then, a semi-structured interviews were used to gather the perspective of healthcare professionals working in the field of HIV/AIDS in the country. 394 participants completed the questionnaires: 30% male and 70% female. Only 20 participants had previously been tested for HIV. On HIV/AIDS-related knowledge scale, the male participants scored higher than the females (6.4 V 5.7). For the risk perception scale, female participants appeared to have lower levels of risk perception than male participants (10.5 V 11.7). The female participants showed slightly more positive attitudes towards HIV testing than male participants (111.32 V 108.14). On the other hand, healthcare professionals indicated; stigma, HIV/AIDS knowledge gap and fear of positive result consequences as the main factors hindering the HIV test uptake. Knowledge, attitudes and HIV risk perception are critical factors that inform the decision to undertake HIV testing however, socio-cultural constraints are significant additional burden that hinder the efforts to scale up the HIV testing uptake in Saudi Arabia.

Key words: HIV testing, young people, attitude, knowledge, perception.

INTRODUCTION

Acquired Immune Deficiency Syndrome (AIDS) is a major health problem worldwide, with approximately 42 million people living with the virus. Each day, the number of young people and adolescents living with HIV increases by 2,100 (UNAIDS, 2014a, b, c). Of the four million people infected with HIV in the 15-24 years age bracket in 2013, almost 30% were under the age of 19 (UNAIDS, The GAP Report, 2014). Low and middle income countries have the greatest number of cases, with a large proportion (85%) occurring in the Sub-Saharan region. The population in this region has a high proportion of young people, and this is likely to continue to rise until 2050 (Idele et al., 2014). In South Sudan, for instance, over half the population is under the age of 18 (UNAIDS, 2013). This means that the HIV infection rate among young people, which is already high, is likely to increase.

*Corresponding author. E-mail: aalmilaibary@bu.edu.sa.

Although there has been an overall 30% drop in the number of people living with HIV, the number of adolescents who died from the disease increased by 50% during the 7-year period from 2005 to 2012 (UNICEF, 2013).

Of the regions with the most rapidly growing HIV epidemics, the Middle East and North Africa (MENA) region is one of the two highest; this region is considered high-risk as it is highly susceptible to the spread of the disease (UNAIDS, 2014a, b, c). Although there are significant variations across the region with regard to epidemiological patterns, trends and typologies, the severity of the epidemic is increasing due to the infection becoming more widespread, with the number of new cases increasing and more deaths occurring due to AIDS-related causes. According to UNAIDS (2014a, b, c), the number of people infected with HIV during 2013 was in excess of 25,000. This was a 7% increase from levels in 2005, bringing the total number of people in the region living with HIV to nearly a quarter of a million [CI: 160,000-330,000]. At the same time, the number of AIDS-related deaths in the region rose by an astonishing 66 per cent, bringing the total to 21,000 infected individuals (UNAIDS, 2014a, b, c).

Central to this problem is the level of testing for HIV. This has increased in its coverage, particularly in countries with high levels of infection, but there still appears to be significant barriers to participation (Bajunirwe and Muzoora, 2005; Carr and Gramling, 2004). This is a significant issue as testing can prove important. Not only can it help prevent further transmission of the virus, it can also help in effective treatment being provided to the sufferer (Deblonde et al., 2010). Thus, establishing what these barriers are and exploring effective ways in which they can be overcome is of paramount importance in attempts both to treat HIV-infected patients and prevent HIV transmission.

The prevalence of HIV in Saudi Arabia is currently low; however, there are still cases of HIV transmission within selected groups in Saudi communities that are considered to be at high risk of acquiring the infection. The most recent report on the HIV/AIDS situation in Saudi Arabia revealed that since the identification of the first case of HIV in 1984 and until December 2014, the total number of HIV cases reported was 21,761 (UNAIDS/KSA, 2015). The report also showed that the reported number of HIV-infected Saudi nationals has increased considerably from only 125 individuals in 2000 to 444 in 2014. This official report also pointed out the limitation of its figures in describing the true picture of HIV/AIDS in Saudi Arabia, as it only includes information from limited sources. The government of Saudi Arabia is actively seeking to develop programmes to promote education and awareness regarding HIV and its transmission within Saudi Arabia in order minimise the incidence of HIV. However, the lack of research in the area of HIV/AIDS in Saudi Arabia has resulted in the application of imported policies and guidelines which may be unsuitable for Saudi Arabia in tackling HIV/AIDS. Thus, conducting research in the field of HIV/AIDS within Saudi Arabia is the first effective step in controlling the disease through contextual, evidence-based strategies.

MATERIALS AND METHODS

Setting and participants

The field work of the current research was held in the western province of Saudi Arabia in Makkah and Jeddah. The quantitative strand of the study took place in Umm AL-Qura University in Makkah. However, the qualitative interviews was conducted within National AIDS Programme centres in both Makkah and Jeddah.

Umm Al-Qura University

One of the oldest university in the country established at 1981. In 2014 the number of undergraduate students enrolled in the university were 87 thousands students. Female students were slightly higher than male students which represent 54.5 per cent of the total undergraduate students at the university (University, U.A. www.uqu.edu.sa, 2017).

Health care facilities (NAP)

The National AIDS Program (NAP) established by Ministry of Health in Saudi Arabia on 1994. The NAP centres are distributed all over the 20 health administration region whereas the main centre is located in Riyadh. In each health administration region there are at least one voluntary counselling and testing (VCT) centre, treatment clinic and mobile VCT facility in some of the health administration centres such as Makkah (MOH. www.napksa.com, 2017).

Data collection

Quantitative strand

A non-experimental, descriptive, cross-sectional design. The quantitative strand mainly aimed to assess the three globally recognised factors that influence the individual in seeking HIV testing services. These factors include; HIV/AIDS related knowledge, perception of risk and the attitude toward HIV testing. The study sample has been drawn from undergraduate students at Umm Al-Qura University (n = 394). A non-probability, convenient sampling technique has been utilised to recruit the students to complete the questionnaire. The sample size for the quantitative strand was calculated based on the total population of undergraduate students at Umm Al-Qura University which was approximately 87,000 students. The margin of error was set at five percent with a confidence level of 95 percent. Therefore, the sample size calculation revealed that the sample required should be at least 381 participants. Online consent was obtained from all participants in this phase of the study before they could complete the questionnaire.

Measures

The questionnaire included five main sections: demographic information, HIV/AIDS related knowledge, individual's perception of

HIV risk, attitude toward HIV testing, and HIV testing history. A test/re-test strategy was adopted to assess the reliability of the questionnaire which revealed an excellent to good degree of reproducibility for the three scale included in the questionnaire; The HIV related knowledge, HIV risk perception, Attitude toward HIV testing as ICC were (0.68, 0.79, 0.65) respectively. The content validity of the three scales were also assessed and appeared to be acceptable.

The HIV related knowledge: This section of the questionnaire has mainly originated from and influenced by the 18 items HIV knowledge questionnaire developed by Carey and Schroder (2002). 12 items questionnaire assess the participant's knowledge about various aspect of HIV/AIDS which include transmission, preventions, epidemiology, severity and progression.

HIV testing History: In this section of the questionnaire the number of those previously tested as well as those who never been tested for HIV is being counted and the reason behind the testing.

HIV risk perception: Three items were included in this section each of them aim to evaluate the individual's perception of risk contracting HIV infection. The three statements express the thoughts that might deceive a young individual to think he/she is at very low or no risk of HIV infection. Five points Likert type scale were used to measure the individual level of agreement with each statements.

Attitude toward HIV testing: This section is the leading part of the questionnaire which evaluate whether the individual embrace a favourable attitude toward HIV testing or not. This section of the questionnaire is greatly influenced by Boshamer and Bruce (1999) scale developed to measure attitude about HIV Antibody testing. The evaluation is accomplished by rating 37 statements on the level of agreement each individual indicate for each statement.

Qualitative strand

A basic descriptive qualitative research design has been utilized to explore the of HIV health professionals' perspectives about the factors that influence young people HIV testing seeking behaviour which affect the testing uptake in Saudi Arabia. A purposive sampling technique has been applied to recruit HIV/AIDS healthcare professionals (n = 3). Data for the qualitative strands of this study were collected using face to face semi-structured, open-ended, in-depth interview. The interviews have been guided by a topic list which was developed following the preliminary analysis of the quantitative data (questionnaire data). The approximate duration of each interview ranged between 25 to 40 min. Informed consent was obtained from all the participants in the phase of the study.

Data analysis

For the quantitative strand, the descriptive data were first examined and explained. Then, inferential statistical analysis using SPSS software was performed to assess the mean difference between male and female as well as across age groups for; HIV/AIDS related knowledge, risk perception and attitude toward HIV testing scores were measured using parametric test such as (t-test and ANOVA test). The significant level has been set at $p = 0.05$. The qualitative data analysed using the strategy of thematic analysis. The thematic analysis focuses on findings patterns in the data which could be gathered and interpreted through major themes. The interview transcripts were translated to English. Data reduction has been achieved through data grouping, categorising and theme identification which help the researcher in interpreting the data.

RESULTS

Demographic characteristics

The study sample comprised 116 (29.4%) males and 278 (70.6%) females. The ages of more than the half of the study sample fell between 20 to 22 years whereas those who were aged between 17-19 years and 23-25 years represented 34.8 and 14.7% respectively. In terms of marital status, about 93% of the participants were single. Only 11 participants were not hold a Saudi nationality while both parents of about 94 percent of the participants were Saudi nationals (Table 1).

HIV testing information

95% of the participants had never been tested for HIV while only 20 participants had been tested. About 48 % of the participants stated that the main reason for not being tested for HIV was because it was unlikely that they had been exposed to HIV. Nearly 36 % of the participants claimed that no one had offered them the HIV test as the main reason for not being tested while, for about 16 % of the participants, their main reason was that they did not know where to get tested. On the other hand, about 25 percent of those who had been tested previously for HIV selected the mandatory pre-marital test as the main reason for undertaking an HIV test (Table 2). The participants' willingness to be tested for HIV during the following year was recorded in five categories which can be seen in Table 1.

HIV/AIDS related knowledge

The responses showed that misconceptions about HIV/AIDS were apparent in the responses of the respondents. Males and females' participants had similar level of knowledge and misconceptions as T-test result showed non-statistically significant difference ($p = 0.107$). While the ANOVA test revealed statistically significant differences in the mean HIV knowledge scores across the age groups (Table 3).

HIV risk perception

The risk perception score ranged from 3 to 15 and the mean risk perception score was 10.9 with a standard deviation of 2.5. 49.2% of the participant's score ranged between 8 and 11 whereas those who scored 12 or above represented 41.8%. In addition, only 9% of the participants' scores were under 8. The difference between the mean scores of the male and female

Table 1. Demographic information presented across men and women.

Variable	Demographic information		
	Men (%)	Women (%)	Total
Number of participants	116 (29.4)	278 (70.6)	394
Age group			
17-19	26 (22.4)	111 (39.9)	137
20-22	73 (62.9)	126 (45.3)	199
23-25	17 (14.7)	41 (14.7)	58
Marital status			
Single	112 (96.6)	255 (91.7)	367
Married	3 (2.6)	19 (6.8)	22
Divorced	1 (0.9)	4 (1.5)	5
Nationality			
Saudi	113 (97.4)	270 (97.1)	383
Non-Saudi	3 (2.6)	8 (2.9)	11
HIV test history			
Tested for HIV	10 (9.4)	10 (3.8)	20
Not Tested	96 (90.6)	250 (96.2)	346
Willingness to be tested			
Strongly willing	28 (26)	47 (18.4)	75
willing	32 (30.8)	78 (30.5)	110
Uncertain	29 (27.9)	85 (33.2)	114
Unwilling	10 (9.6)	20 (7.8)	30
Strongly unwilling	5 (4.8)	26 (10.2)	31

participants appeared to be statistically significant $p < 0.05$. In addition, ANOVA test indicate a statistically significant difference in the mean scores across age groups (Table 3).

Attitude toward HIV testing

The mean score of the attitude towards HIV testing was 110.4 with a standard deviation of 17.5. T-test and ANOVA test revealed no statistically significant difference either across genders or age groups for the attitude toward HIV testing mean scores (Table 3).

Interviews findings

Four main themes were identified and a number of sub-themes were also recognised under each theme. The themes were as follows: HIV testing facilitators, HIV testing barriers, HIV/AIDS related knowledge, and an action plan to increase the uptake of HIV testing by young people in Saudi Arabia (Figure 1).

DISCUSSION

One of the central aspects of this research is the exploration of the factors that affect the utilisation of HIV testing in Saudi Arabia, using a mixed methods approach and with two distinct groups of individuals: service users (young people) and service providers (HIV/AIDS health care professionals). Although the two groups of individuals are different, their interaction is inevitable because undertaking HIV testing mandates contact with the Saudi Arabian health care system. Thus, both young people and HIV/AIDS health care professionals were included in the study. In this study, it appeared that the individual-level factors that shaped how an individual respond to HIV/AIDS-preventive measures, particularly HIV testing, were related to a complicated multi-dimensional set of problems. A person's attitude is not merely driven individually; environmental components, such as culture and the health care system and

Table 2. Reasons for being tested or not tested for HIV previously.

Reasons for undertaking the HIV test or not	
Reasons for Not tested for HIV	**N (%)**
It's unlikely you've been exposed to HIV.	162 (47.6)
Fear of a positive result consequences.	7 (2.1)
You were worried your name would be reported to the government if you tested positive	3 (0.9)
You don't trust the results to be confidential.	1 (0.3)
No one had offered you an HIV test.	121 (35.6)
You were afraid of losing friends and family, if people knew you were HIV positive.	6 (1.8)
You were afraid of being discriminated and stigmatized.	3 (0.9)
You didn't know where to get tested.	16 (4.7)
You need to travel for a long distance to get tested	1 (0.3)
Fear of needle	4 (1.2)
Other reason	16 (4.7)
Reasons for getting tested for HIV	**N (%)**
Mandatory Pre-marital test.	5 (25)
Worried that you may have been infected.	2 (10)
Because you practiced unprotected sex.	2 (10)
Because a doctor, nurse or other health care professional asked you to	2 (10)
For hospitalization or surgical procedure	2 (10)
For employment purposes	2 (10)
Other reason	5 (25)

Table 3. Scales mean scores and difference across genders and age groups.

Scales mean scores and difference across genders and age groups				
Scale	**Men**		**Women**	**P-value**
	Mean (Yasin et al., 2013)		**Mean (Yasin et al., 2013)**	
HIV/AIDS Related Knowledge	6.17 (2.7)		5.7 (2.5)	0.107
HIV Risk perception	11.67 (2.5)		10.52 (2.4)	0.000
Attitude toward HIV testing	108.15 (17.9)		111.32 (17.3)	0.082
Scale	**Age group**		**Sig.**	**95 % CI**
HIV/AIDS related knowledge	17-19	20-22	0.000	(-1.93 - 0.56)
		23-25	0.000	(-2.90 - 0.97)
HIV risk perception	17-19	20-22	0.011	(-1.54 - 0.15)
		23-25	0.024	(-2.06 - 0.11)

healthcare professional's attitude, influence these attitudes. It was also evident that health care professionals were facing logistical, bureaucratic and societal pressures which played an important role in the low uptake of HIV testing in general.

The three most common reasons indicated by the participants in the current study were in agreement with the literature. However, one of these reasons was widely mentioned globally as the main reason for not being tested for HIV across various populations; this is the low perception of HIV risk. The most common reason indicated for not being tested for HIV (low HIV risk perception) was congruent with other research conducted worldwide in both developed and developing countries; 48% of the participants in the current study stated that exposure to HIV was considered unlikely. The HIV testing was not offered is the second most common reason given for not being tested for HIV, indicated by 36% of the participants. This was also demonstrated as a barrier that affected the utilisation of HIV testing by young people aged 12-24 in the USA (Peralta et al., 2007). In addition, another study conducted in the USA by Johnson et al. (2011) claimed that health care providers were not offering HIV tests as a result of either time constraints or

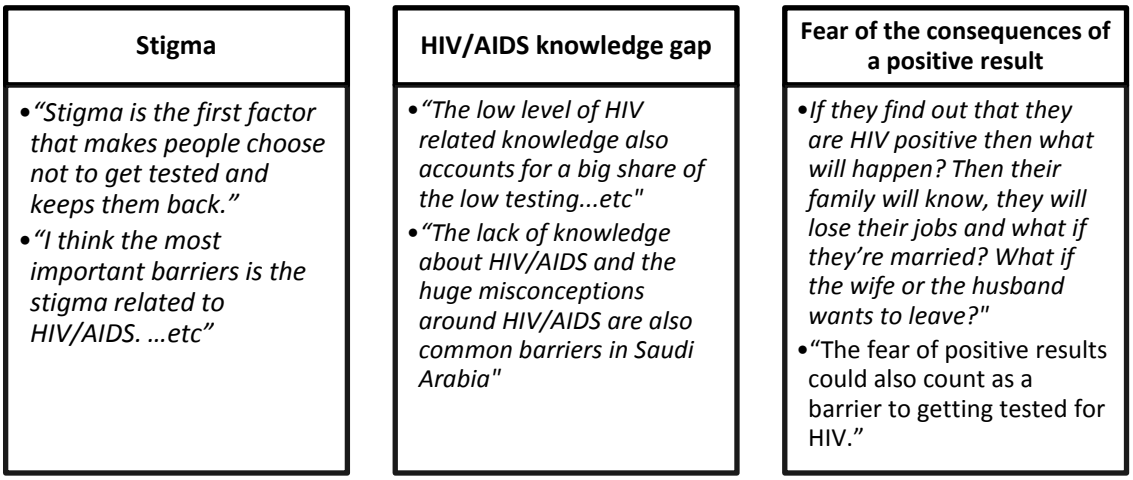

Figure 1. The main factors emerged from the interviews with Healthcare professionals.

an inadequate level of awareness that health care personnel had regarding HIV testing guidelines. Another less common reason for not being tested shown in the current study, which was indicated by 4.6% of the participants, was not knowing the location of HIV testing centres. This was also indicated by young people in the Balkans as a barrier they faced in undertaking HIV testing (Delva et al., 2008). In addition, Payne et al. (2006) stated that African-American college students aged 18-24 lacked information about testing sites. So, it added to the obstacles that hindered their uptake of HIV testing.

Lack of proper knowledge about HIV/AIDS was demonstrated in the current study, which may lead to the reluctance of young people to take up HIV testing and other HIV/AIDS health-related services. Misconceptions were apparent in almost all aspects of HIV/AIDS-related knowledge, including preventive measures, severity, epidemiology and routes of transmission. Although almost more than three decades have passed since the first case of HIV was detected, HIV being transmittable by a mosquito bite is still one of the most common misconceptions identified in the current study. The participants also considered HIV to be a curable disease if detected early and believed that a vaccine is available. Meena et al. (2013) also found that HIV is curable disease is apparent misconception within their study.

The majority of the participants in the current study demonstrated moderate HIV risk perceptions. Sexuality and its related behaviours vary across populations and cultures and, as most of the high-risk behaviours for contracting HIV are strongly disapproved of and regarded as immoral in the Saudi Arabian context, most of the participants perhaps responded to that item in accordance with socio-cultural norms. Most of the literature about young people's HIV risk perceptions shows a low risk perception across young populations worldwide, regardless of the state of HIV epidemics.

The current study suggested a reasonably supportive attitude among the participants. Similarly, Peltzer et al. (2004) indicated that university students in India, South Africa and the USA had a moderately favourable attitude toward HIV testing, although students from the USA held a more positive attitude towards HIV testing in comparison to those from India and South Africa. Although the attitude toward HIV testing was reasonably positive, HIV testing was very low among the participants in the study. The core elements of cognitive theory, which asserts the need to recognise that human beings are not always rational when they make decisions could explain this finding. The decision-making process is usually influenced by an individual's beliefs, experiences and cultural norms. Although the findings of this study demonstrated that attitudes towards HIV testing were reasonably positive, the majority of the participants had not undertaken the test. Accordingly, scaling up HIV testing requires multidisciplinary action, as attitude is just one piece of the picture.

Stigma related to HIV/AIDS was the most common subtheme agreed upon by the HIV/AIDS health care professionals who participated in the study as a barrier to young people undertaking HIV testing in Saudi Arabia. The pervasiveness of stigma as a major barrier to increasing HIV testing among various subgroups has been well documented in the literature worldwide. In the Middle East the impact of stigma on HIV/AIDS prevention programmes has also been highlighted, hindering their progression (Abu-Raddad et al., 2010; Akala, 2005; DeJong and Mortagy, 2013). Although stigma related to HIV/AIDS is observed and acknowledged worldwide, it is understood that stigma is more intense in countries dominated by Islam due to Islamic constraints on sexuality (Kaadan, 2004). The main reason for using a combination of quantitative and qualitative methods was to explain the quantitative data set and enhance

understanding regarding the situation in Saudi Arabia about the utilisation of HIV testing services. The interviews offered another perspective to the main question of the study, as well as providing an action plan to increase the uptake of HIV testing by young people in Saudi Arabia.

Conclusion

The complexity of the research inquiry was clearly demonstrated when attempting to integrate the two sets of data. Tackling HIV/AIDS in general in Saudi Arabia requires a multidisciplinary approach and the engagement of legislators, community leaders, healthcare policy makers, HCPs and representatives of the at-risk groups, such as young people.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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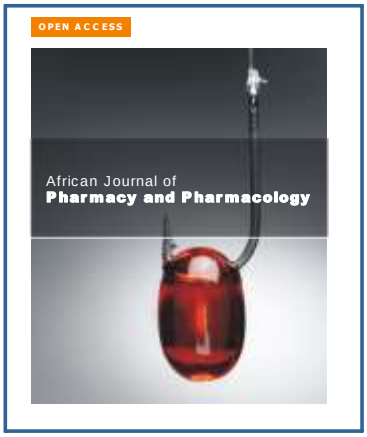

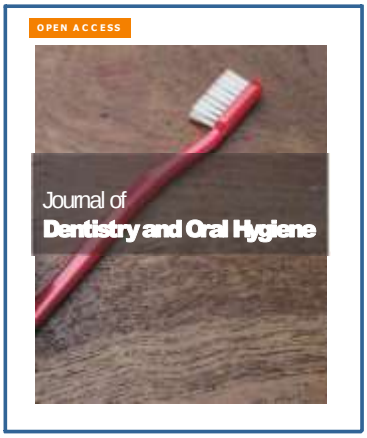

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