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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Journal of AIDS and HIV Research

Full Length Research Paper

Metabolic factors associated with the development of lipodystrophy in patients on long-term highly active anti-retroviral therapy (HAART)

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Dyslipidemia, insulin resistance and diabetes are frequent in patients on highly active anti-retroviral therapy (HAART) and especially in patients with lipodystrophy, and may lead to atherosclerosis. This study described the metabolic alterations associated with lipodystrophy in adults on chronic HAART in Kenya. The prevalence of dyslipidaemia amongst the study participants was (211) 79.6%. Elevated total cholesterol was found in 129, high low-density-lipoprotein cholesterol (LDL-C) in 107, low High-density lipoprotein cholesterol (HDL-C) in 110 and high triglycerides in 131 participants. Lipodystrophic patients were more likely to have dyslipidemia than normal lipids (55.4 versus 35.1%, p = 0.007 OR 2.2 CI 1.3 to 4.6) with 57, 45.9, 65.9 and 45.2% having elevated total cholesterol, elevated LDL-C, elevated triglycerides and low HDL-C, respectively. Hypertriglyceridemia and hypercholesterolemia were significantly associated with lipodystrophy (OR 3.8 CI 2.3 to 6.4; p = 0.000) and (OR 1.94 CI 1.2 to 3.2; p = 0.008), respectively. The odds of lipodystrophy was 2.913 times higher for patients with elevated triglycerides than for those with normal triglycerides (p < 0.001). Sixty-four (24.3%) participants had dysglycemia, with 3.5% having diabetes and 20.8% having impaired fasting glucose (IFG). Among patient with lipodystrophy, 69.8% had normal fasting glucose, 25.1% had IFG and 5.1% were diabetic. Lipodystrophic patients were not more likely to have abnormal blood sugars than normal blood sugars (p value 0.125).

Key words: Dyslipidemia, atherosclerosis, fasting glucose, dysglycemia, lipodystrophy, chronic highly active anti-retroviral therapy (HAART), metabolic alterations.

INTRODUCTION

Dyslipidemia and dysglycemia are very frequent in patients with lipodystrophy (Mercier et al., 2009; Jevtovic et al., 2009). The lipid disturbances, insulin resistance and increased risk of diabetes coupled with fat redistribution seen in lipodystrophic patients have been shown to predispose to premature and accelerated atherosclerosis and thus an increased risk of acute myocardial infarction (DAD study group, 2007).

Saves et al. (2002) reported the prevalence of hypertriglyceridemia in patients without lipodystrophy, those with 1 to 3 signs and those with more than 4 prevalence of hypercholesterolemia were 48, 62 and 62%, respectively. Samaras et al. (2007) found hypertriglyceridemia to be twice as prevalent (61%) in patients with lipodystrophy

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as compared to those without. The pathogenesis of hypertriglyceridemia in HIV-associated lipodystrophy appears to arise predominantly from increased hepatic secretion of very low density lypoprotein tryglycerides (VLDL-TG) rather than reduced clearance. De novo lipogenesis, resting lipolytic rate and hepatic triglyceride stores are also increased in HIV-associated hypertriglyceridemia. Triglyceride clearance may also be impaired in these patients because of reduced lipoprotein lipase activity; however, this appears to play a minor role (Grunfeld et al., 1992).

Insulin resistance and impaired glucose tolerance have been observed with regimens containing protease inhibitors especially Indinavir and regimens containing nucleoside reverse transcriptase inhibitors, chiefly stavudine (Carr et al., 1998; Lawrence, 1946). While Indinavir has been demonstrated *in vitro* to have a direct effect on glucose metabolism and may induce insulin resistance by inhibiting glucose movement through the GLUT4 transporter, the emergence of insulin resistance during antiretroviral therapy is a complex process that is not completely understood. Fasting glucose levels from a group of 1,278 men in the MACS cohort showed that 14% of HIV-infected men on antiretroviral therapy had diabetes mellitus compared with 5% in HIV-negative men adjusted for age and body mass index (BMI) (Palella Jr et al., 2004).

Insulin resistance has been demonstrated in patients with fat redistribution, even in patients not receiving protease inhibitors. In an Australian study on the prevalence of metabolic syndrome in patients on HAART, the prevalence of fasting glucose greater than 5.6 mmol/L was 19% in those with lipodystrophy versus 11% in those without lipodystrophy (Samaras et al., 2007).

Lipodystrophy and the associated metabolic dysregulation has similar features as those seen in metabolic syndrome and has been shown to put HIV infected patients at risk of premature and accelerated atherosclerosis (Friis-Møller et al., 2003a, b). Samaras et al. (2007) in a multicenter cross-sectional study of 788 HIV positive patients found a prevalence of metabolic syndrome in patients on HAART to be 14% by International Diabetes Federation (IDF) criteria and 18% by National Cholesterol Education Program Adult Treatment Panel III (ATPIII) criteria (NCEP/ATP III, 2001); lipodystrophy was present in the majority of patients with metabolic syndrome: 73% by IDF criteria and 79% by ATP III criteria (Samaras et al., 2007).

The prevalence of lipodystrophy is high in Kenya. There are few studies on its prevalence and on the metabolic alterations associated with its development. The increased cardiovascular risk associated with these changes may lead to increased morbidity and mortality in affected patients who may benefit from intervention strategies such as dietary modification, physical exercise and lipid lowering therapy to reduce their risk constellation (Behrens et al., 2003). We set out to investigate the prevalence and type of dyslipidemia and dysglycemia in patients on chronic HAART in Kenya.

MATERIALS AND METHODS

Ethical considerations

The study was conducted after approval by the Department of Clinical Medicine and Therapeutics, University of Nairobi, and the Kenyatta National Hospital Scientific and Ethical Review Committee.

Study site

The study was conducted at the HIV out-patient clinic at Kenyatta National Hospital, a tertiary National referral and teaching hospital in Kenya.

Study population

The participants were HIV-infected adults on HAART for longer than six months, attending the HIV clinic between August 2007 and 2008. The participants were HIV-1 positive adult patients on combination HAART as recommended by the National HIV program and defined as either dual NRTI (d4T or AZT or TDF with 3TC) with a NNRTI (NVP or EFV) or the dual NRTIs with a PI (LPV/r) for 6 to 72 months who attended the HIV clinic between August 2007 and 2008.

Study design

This was a cross-sectional descriptive study. Random sampling was done daily during routine visits until the desired sample size was reached. The minimum sample size required to determine the prevalence of lipodystrophy was determined at 265 patients. The criteria for statistical significance was p value < 0.05.

Inclusion criteria

HIV-1 infected male and female patients aged 15 years and older on HAART, regularly reviewed and compliant with treatment for six months or more were deemed eligible for this study.

Exclusion criteria

Patients on HAART for less than 6 months, patients on anabolic steroids or immuno-modulatory therapy, patients known to have Cushing's disease or other endocrine disorders, pregnant patients and moribund patients such as patients with malignancy or HIV wasting syndrome were excluded.

Patient assessment

The Comprehensive Care Centre operates five days in a week. All patients underwent full evaluation at initial and subsequent follow-up visits. Data on patient characteristics such as age, gender, marital status, occupation, level of education, WHO clinical staging, current and prior anti-retroviral therapy, physical examination findings and baseline and subsequent laboratory investigations including full blood count, liver and renal function tests, CD4 and CD8 counts were recorded in the patients' charts. Patients deemed eligible for antiretroviral therapy commenced treatment and thereafter

Table 1. Demographic characteristics of the study population.

Variable		No. of patients	Mean/ (%)
Age	All patients aged ≥ 15 years	265	40.69±23.41
Gender	Female	158	(59.6)
	Male	107	(40.4)
WHO stage	I	24	(8.9)
	II	47	(17.3)
	III	99	(37.3)
	IV	95	(36)
CD4 counts	Nadir	256	119±49
	Most recent	265	335±76.50
Duration of HAART (Months)	6-18	83	(31.2)
	19-36	123	(46.4)
	>3	59	(22.4)
HAART combinations	d4T based	188	(70.9)
	AZT based	41	(15.5)
	TDF based	36	(13.6)

were given individualized appointments depending on their clinical condition. They also returned to the clinic monthly for supply of antiretroviral medication. Recruitment was done among patients who had been on anti-retroviral therapy for more than six months. The patients were informed about the study and their eligibility assessed. Those who met the inclusion criteria and gave signed informed consent were recruited. A study questionnaire was used to collect baseline and clinical data. Lipodystrophy was assessed by patient report and physician examination using a modified version of the lipodystrophy case definition questionnaire (Carr, 2003). After an overnight fast of 9 to 12 h, blood was taken from all the patients for measurement of lipid profiles and fasting blood glucose. Lipid profile was done at the Pediatric Laboratory, University of Nairobi using the HUMALYZER 2000 machine through a direct method to measure LDL-C and HDL-C and enzymatic hydrolysis to measure triglycerides and total cholesterol. Fasting blood glucose was measured by the glucose oxidase method on the medisense glucometer (Gochman et al., 1972).

Outcomes

Lipid disturbances were classified as per NCEP/ATP III guidelines as high total cholesterol, high LDL-cholesterol, high triglycerides or low HDL-cholesterol. Study participants were considered diabetic if they had history of self report of diabetes, or use of hypoglycemic medication, or Fasting plasma glucose (FPG) ≥ 7.0 mmol/L. Impaired fasting glucose (IFG) was defined as FPG of 5.6 to 6.9 mmol/L.

Statistical analysis

All data was entered into data base using Microsoft Excel. Qualitative variables were described in frequencies or percentages and compared between groups using Chi square (χ^2) test. Quantitative variables were described with medians or means and compared between groups using Wilcoxon rank sum test. Cox proportion hazard regression modeling was used to determine variables that predicted the outcomes. Statistical analysis was performed using Statistical Package for Social Sciences, version 15.0. Results were presented in form of tables. The criteria for statistical significance was p value < 0.05

RESULTS

We screened 318 HIV-seropositive patients on chronic HAART therapy and excluded 53 (16.6%) patients; 40 had been on HAART for less than 6 months, 5 had opportunistic infections, 3 were moribund, 2 had HIV wasting syndrome, 2 declined consent and 1 had a malignancy. Two hundred and sixty five patients were thus enrolled at the Comprehensive Care Centre at the Kenyatta National Hospital.

Patients' baseline characteristics

The mean age of the study population was 40.69 years with 59.6% being female. The mean baseline CD4 count and reconstituted CD4 count of the study participants was 119 and 335/mm^3, respectively as depicted in Table 1. Majority of the patients (73.3%) were in WHO stage III and IV at initiation of HAART. The mean duration of treatment of the study participants was 29.7 months, with 66.8% having used HAART for longer than 18 months. Stavudine based regimens were in use by 70.9% of patients whereas 15.5% of patients were on AZT-based regimen. Twenty six of these patients had switched from a d4T based regimen prior to enrolment into the study. It

Table 2. Metabolic variables of the 265 study participants.

Variable (mmol/l)	Male (n=107)	Female (n=158)
TC Mean± SD	5.2±1.38	5.35±1.32
Median (IQR)	5.08 (2.15-10.08)	5.16 (2.54-9.87)
HDL-C Mean± SD	1.097±0.39	1.179±0.44
Median (IQR)	1.08 (0.24-2.67)	1.11 (0.20-3.03)
LDL-C Mean± SD	3.28±1.26	3.18±1.25
Median (IQR)	3.03 (1.08-7.92)	3.02 (0.08-7.67)
TG Mean± SD	2.00±1.54	2.21±1.79
Median (IQR)	1.62 (0.49-10.6)	1.70 (0.47-11.26)
FBS Mean± SD	5.5 ± 2.1	5.18±0.9
Median (IQR)	5.4 (2.8-20.1)	5.1 (3-9.3)

was also noted that of 36 (13.6%) patients who were on a TDF based regimen, 30 had switched from a d4T based regimen and 6 from an AZT based regimen prior to the time of enrolment.

Metabolic variables of the study population

The median total cholesterol, HDL-cholesterol, LDL-cholesterol and fasting blood sugar were normal whereas the median triglycerides were marginally elevated in females as shown in Table 2.

Prevalence of dyslipidemia

Overall dyslipidaemia was found in 211 (79.6%) patients, of whom 58.3% were females. Elevated total cholesterol (>5.17 mmol/l) was found in 129 (48.6%) patients, elevated LDL-cholesterol (>3.34 mmol/l) in 107 (40.3%) patients, low HDL-cholesterol levels (<1.03 mmol/l) in 110 (41.5%) patients and high triglyceride levels (>1.69 mmol/l) in 131 (49.4%) patients as depicted in Figure 1.

Dyslipidaemia and lipodystrophy

Dyslipidaemia was found in 117 (55.4%) patients with lipodystrophy and normal lipids in 18 (34.6%) patients with lipodystrophy. Presence of dyslipidaemia was significantly associated with lipodystrophy (p = 0.007 OR 2.2 CI 1.3 to 4.6) as shown in Table 3.

Types of dyslipidemia and lipodystrophy

Hypertriglyceridemia was the most common type of dyslipidaemia associated with lipodystrophy occurring in 89 (65.9%) of the patients. The association was found to be statistically significant (OR 3.8 CI 2.3 to 6.4 p = 0.000). Hypercholesterolemia was found in 77 (57%) of the patients with lipodystrophy and this also achieved statistical significance (OR 1.94 CI 1.2 to 3.2 p = 0.008). Neither elevated LDL-C nor low HDL was significantly associated with lipodystrophy. The associations between the various types of dyslipidaemia with lipodystrophy are summarized in Table 4.

Dysglycemia and lipodystrophy

Abnormality in blood glucose was not found to be significantly associated with the presence of lipodystrophy. Among patients with lipodystrophy, 93 (69.8%) had normal fasting blood sugar, 34 (25.1%) had impaired fasting glucose and 7 (5.1%) were diabetic as shown in Table 5.

Logistic regression analysis

A logistic regression model was constructed to find which of the associated factors independently predicted lipodystrophy while controlling for the other factors and to quantify this association (Table 6). From logistic regression analysis model, we estimated that for patients with abnormal triglycerides (that is, triglycerides levels > 1.69) who had the same levels of total cholesterol, the odds of lipodystrophy was 2.913 times higher compared to those with normal triglycerides. This estimate was statistically significant (p < 0.0001). From the same model we estimated that for patients with abnormal total cholesterol levels (that is, total cholesterol levels > 5.17) who had same levels of triglycerides and HAART duration, the odds of lipodystrophy is 1.288 times higher compared to those with normal total cholesterol, though this estimate was not statistically significant (p = 0.388).

In conclusion, we observe from the logistic model that abnormal levels of triglycerides was significantly associated with a higher likelihood of developing lipodystrophy for patients in this study.

DISCUSSION

Prolonged use of HAART has led to recognition of long term complications of these therapies such as lipodystrophy which manifests with distressing morphologic changes in body habitus and has been associated with metabolic abnormalities such as hypertriglyceridemia, hypercholesterolemia, insulin resistance (raised C-peptide and insulin concentrations), impaired glucose tolerance and type 2 diabetes mellitus (Carr, 2000). This study was conducted between August 2007 and 2008 at Kenyatta National Hospital, a referral and teaching hospital in Kenya. It comprised 59.6% females (female to male ratio

Table 3. Association between dyslipidaemia and lipodystrophy.

Variable	Prevalence of lipodystrophy n(%)	Total	P value
Dyslipidemia	117 (55.4)	211	0.007
No dyslipidemia	18 (34.6)	54	

Table 4. Types of dyslipidaemia associated with lipodystrophy.

Type of lipid	Prevalence of lipodystrophy n (%)	Total	Odds ratio (95% CI)	P value
Hypercholesterolemia	77 (57)			
Normal total cholesterol	59 (43)	136	1.94 (1.2-3.2)	0.008
High LDL-C	62 (45.9)			
Normal	74 (54.1)	136	1.5 (0.95-2.6)	0.076
Hypertriglyceridemia	89 (65.9)	136	3.8 (2.3-6.4)	0.000
Normal triglycerides	47 (34.1)			
Low HDL-C	61 (45.2)	136	1.3 (0.8-2.2)	0.257
Normal HDL-C	75 (54.8)			

Table 5. Association between fasting blood glucose and lipodystrophy.

FBS	Prevalence of lipodystrophy n (%)	Total	P value
Normal	95 (69.8)	136	
IFG	34 (25.1)	136	0.124
DM	7 (5.1)	136	

Table 6. Logistic regression model.

Variable	Odds ratio	P	95% CI	
Abnormal Triglycerides	2.913	<0.0001	1.635	5.191
Abnormal total Cholesterol	1.288	0.388	0.725	2.286

1.5:1). Most of the individuals in the study population were young individuals with a median age of 40 years. Females were younger than their male counterparts where 60.7% were below 40 years compared to 44% of males. These findings reflect the National AIDS and STI Control Programme (NASCOP) estimates (Ministry of Health Kenya, 2010) that at least two-thirds of all HIV infected individuals in Kenya are young women. Therefore, the age and gender distribution of this study population is fairly representative of the sample of AIDS patients in Kenya.

Dyslipidemia was found in 79.6% of our study participants. All four types of lipid abnormalities (high total cholesterol, high LDL cholesterol, high triglycerides and low HDL cholesterol) were encountered in our patients and were more prevalent in patients with lipodystrophy.

The pathogenesis of the dyslipidemia is thought to be due to increased apoliporotein B levels, increased dense LDL 2 levels and a shift towards hepatic secretion of VLDL-triglycerides. Circulating cytokines and acute phase reactants may also play a role (Grunfeld et al., 1992).

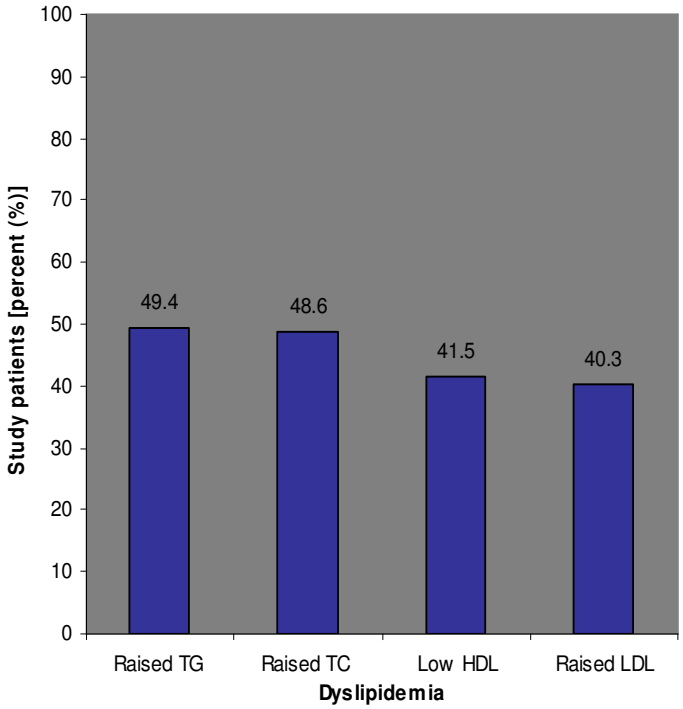

Figure 1. Prevalence of dyslipidemia in the study population.

The commonest type of dyslipidemia in this study was hypertriglyceridemia. This finding is not surprising in this population who had multiple risk factors for development of hypertriglyceridemia. These include HIV infection where the pathophysiology is thought to be due to cytokine mediated (especially IFN-α) suppression of lipases with decreased clearance of triglycerides from blood (Grunfeld et al., 1992). In addition, use of HAART, especially protease inhibitors as well as having lipodystrophy are cited as risk factors for elevated triglycerides.

The prevalence of elevated total cholesterol in our study (>5.17 mmol/l) was 48.6%, high LDL-cholesterol was found in 41.5%. Majority of the patients who had elevated total and LDL-cholesterol had borderline elevation. LDL cholesterol is atherogenic and this is therefore of concern in this young population on long term HAART and who probably have other cardiovascular disease risk factors. It may be important to institute therapeutic lifestyle modification and lipid lowering agents in these patients.

Low HDL-cholesterol was found in 40.3% of patients. This is also expected, as HIV infection has been shown to suppress HDL-cholesterol (Grunfeld et al., 1992). This finding however is in contrast to those reported by Manuthu et al. (2008) who found HDL not to be reduced in patients on HAART.

Patients with lipodystrophy were more likely to have dyslipidemia than normal lipids (55.4 versus 35.1%, p = 0.007 OR 2.2 CI 1.3 to 4.6). Similar findings were reported by the data collection on adverse events from anti-HIV drugs (D: A: D) study group who found dyslipidemia in 57% of patients with lipodystrophy. The D: A: D study also reported on a follow up study that after the initial 7 years of HAART, the risk of myocardial infarction was 27% per year (Friis-Moller et al., 2003).

In our study, triglycerides were 3.8 likely to be elevated and total cholesterol 1.94 likely to be elevated in patients with lipodystrophy. Samaras et al. (2007) found significantly elevated total cholesterol and triglycerides in patients with lipodystrophy who did not have other features of metabolic syndrome. Saves et al. (2002) found the prevalence of hypertriglyceridemia in patients without lipodystrophy and those with lipodystrophy was 20 and 42%, respectively that of hypercholesterolemia was 48 and 62%, respectively. Hypertriglyceridemia was found to be an independent predictor of lipodystrophy in the multivariate analysis with the odds of lipodystrophy being 2.9 times higher in those with hypertriglyceridemia compared to those with normal lipids. These patients may benefit from long-term follow-up with regular cardiovascular risk assessment and institution of intervention strategies to reduce their risk constellation.

Impaired fasting glucose was seen in 24.4% and diabetes mellitus in 3.5% of our study participants. Insulin resistance and diabetes have been seen with regimens containing Indinavir and stavudine (Brown et al., 2005). It is thought to be due to inhibition of glucose movement through GLUT 4 transporter (Murata et al., 2000). Manuthu et al. (2008) and the Rwanda study (Mutimura et al., 2007) also reported the prevalence of dysglycemia as 20.7 and 17.3%, respectively. Abnormalities in fasting blood glucose were not found to be associated with lipodystrophy in this study. IFG was reported in 25.1% and diabetes in 5.1% and normal blood glucose in 68.8% of patients with lipodystrophy. Samaras et al. (2007) found abnormalities in blood glucose to be more common in lipodystrophy than those without (19 versus 11%) while Saves et al. (2002) reported that glucose alteration was 16% in patients without lipodystrophy and 28% in patients with lipodystrophy.

It is noteworthy that although metabolic alterations were more common among patients with lipodystrophy, they were also present in patients without lipodystrophy implicating the role of viral and antiretroviral therapy in the etiology.

Conclusion

Dyslipidemia was common in patients with lipodystrophy and this is likely to increase the risk of cardiovascular disease. However, dysglycemia was not associated with lipodystrophy. Hypertriglyceridemia was found to be independent predictors of lipodystrophy. We recommend that lipid profiles should be performed before HAART initiation and be routinely monitored especially in patients who develop lipodystrophy, and regular cardiovascular risk assessment should be done in patients on HAART and

and institution of intervention strategies done to reduce their risk constellation.

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Lipodystrophy, "social death" and treatment adherence in human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS)

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The lipodystrophy syndrome is the adverse effect to the use of the most important antiretrovirals according to the Ministry of Health of Brazil. Based on this idea, it is sought to emphasize that the lipodystrophy syndrome, as a result of acquired immune deficiency syndrome (AIDS) treatment confirms the "social death" experienced by patients and the possible interference in the process of treatment adherence. The data showed that from 48 patients (n) volunteers, 37.5% decreased their frequency to engage in social activities supported by data from the second instrument (WHOQOL-HIV BREF) regarding the facet for social isolation, in which 39.6% feel partially accepted by their friends and family, suggesting a tendency to be a "social dead". Regarding treatment adherence, 35.4% in this sample needs medicines for their daily life. It can be concluded that, despite the reduction in the social involvement of these individuals, the fact of using the medication as it was prescribed corresponds to a high percentage of people who could not live their daily lives without medication.

Key words: Lipodystrophy syndrome, social death, treatment adherence, human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS).

INTRODUCTION

After 30 years, Brazil is characterized by an epidemic of human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) stable cases, with concentration in some population subgroups in vulnerable situation. According to the latest Epidemiological Bulletin (base year 2010), there were reported [information system for notifiable diseases of AIDS cases (SINAN), YES, laboratory test control system (SISCEL), system for logistic control of drugs (SICLOM)] 608,230 accumulated AIDS cases from 1980 to June, 2011, and 397, 662 (65.4%) in males and 210, 538 (34.6%) in females (Ministry of Health Brazil, 2011a). The number of patients receiving antiretroviral therapy in 2010 was 201, 279, according to system monitoring indicators of the national STD/AIDS (MONITORAIDS) (Souto, 2006). From the total patients in treatment, the National STD/AIDS (National Program) estimates that 49% will develop some type of lipodystrophy syndrome (LDS) (metabolic and anatomical proper usage or not of medication antiretroviral in HIV/AIDS), noting that there is support for comprehensive care provided in Ordinance No. 2.582/GM-MS of December 2, 2004 for this population (Tellini, 2006).

The LDS causes a redistribution of body fat, with peripheral fat loss and fat accumulation in the abdominal

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region, and important metabolic alterations. Facial lipoatrophy, which may affect 19% of people living with HIV/AIDS gives the person an aspect of premature aging that causes a huge impact on quality of life of the person, bringing to them the stigma of “AIDS face”. These changes in body structure (lipoatrophy members, abdominal fat accumulation, hump, association of lipoatrophy with lipohypertrophy) have striking effects on the psychosocial life of HIV/AIDS carrier and they can influence the adherence to treatment (Tellini, 2006).

Study of systematic literature review indicated that the noncompliance occurs universally and their rates are comparable between the developed and developing countries. These rates range from 0.8 to 85.0% according to prescribed antiretroviral takings, which was 75.0 to 100.0% (Rocha et al., 2010). The prevalence of LDS was found between 30 and 80% of patients in treatment with associated factors such as time of use of antiretroviral, low counting of CD4+ cells and high viral load (Fernandes et al, 2005; Santos et al., 2005; Ministry of Health Brazil, 2011b). The Ministry of Health of Brazil (MS) considers SLD as one of the most important adverse effects of highly active antiretroviral therapy (HAART) due to the large value considered by the society to the “body” because it directly affects the individual’s life (Ministry of Health Brazil, 2009). The bodily changes can cause a great impact in the life of a person with HIV/AIDS, since they seem to put in the physical appearance of their ideal health and then maintain secrecy about their status as HIV positive.

Body image and self-perception of the body play an important role in social relations. Thus the thought that the body image is closely related to this concern (which is the judgment they will face), to the extent that they currently do not “see” in their bodies this ideal image, and the ideal of the social environment in which they live, is strengthened. Paraphrasing the thought of Rodrigues, “the body carries in itself the mark of social life (emphasis added by the author), and expresses the concern of the whole society to consider physically, certain changes in it” (Valenca, 2003).

The progress on reducing mortality and morbidity with the use of more potent drugs dramatically changed the natural scenery of the disease and helped enhance the quality of life of people living with HIV and AIDS (PLWHA). When there is a reduction in morbidity, there is also a decrease in the incidence of psychiatric disorders. However, a new challenge has emerged for those people living with HIV: How to re-plan the future that was not expected? It is this new reality that can generate, according to scholars, depressive and anxious states which cannot be overlooked by healthcare professionals, as they may interfere with treatment adherence, given that HIV infection may be accompanied sometimes by poverty, unstable living situations, fragmented families, friends, and family rejection, stigma, social isolation, problems at work and other factors (Rachid and Schechter, 2008).

Currently, it is necessary to maintain informative process on antiretroviral therapy and other complementary therapies, as well as monitoring the multidisciplinary team to prevent or reduce the risk of noncompliance with treatment (Gomes et al., 2011). The treatment regimen, the profile of the bond with the assistance, social integration of the individual, the use of alcohol and drugs, the presence of co-morbidities, use of other medications, the need to support treatment for long-term, disease severity, affective intolerance (impatience, negativism, pessimism) and the patient’s physical concerns regarding HIV and its treatment, as well as prejudice and anguishes, respectively related, were also cited in literature as important factors affecting the adherence to antiretroviral therapy. However, there are conflicts about the impor-tance of age, gender, family income and the expected outcome of treatment in the process of accession (Souto, 2006).

Emphasizing that perception, representations and understanding that a person has of their disease process (the meaning attributed to this process and their cognitive capacity to adapt to different situations of life, accepted as factors related to the educational level) are important components to be considered when addressing the issues of adherence to antiretroviral therapy (Souto, 2006).

Therefore, this article aims to emphasize the lipodystrophy syndrome as an adverse effect of antiretroviral therapy which confirms the “social restrictions” experienced by patients, including interfering in the process of treatment adherence.

METHODOLOGY

This exploratory study was designed as observational, with quantitative and qualitative data taking as an advantage of its feasibility (Fletcher, 2006). The study was approved by the Research Ethics Committee of the University Hospital Onofre Lopes from Federal University of Rio Grande do Norte (CEP-HUOL/UFRN), under number 062/2006, which was developed in the city of Natal, Rio Grande do Norte, Brazil in a state referral center.

The sample selection was non-probabilistic and composed of 48 people selected on the basis of medical and laboratory specialists and the infectious disease physician assistant. The following inclusion criteria were: being a person aged 20 to 60 living with HIV/AIDS, voluntary, presenting LDS, being treated with anti retroviral drug (ARV). The study excluded participants who had some chronic condition that could be an impediment factor for participation in the activities of data collection, as medical evaluation.

Data collection took place between September, 2009 and February, 2010, all participants were volunteers and asked to read and sign the statement of informed consent (SIC), according to the rules of Resolution No. 196/96 National Research Ethics Committee (NREC).

In a study on adherence to antiretroviral treatment covering the

Table 1. Descriptive analysis of socio-demographic data.

Variable	N	%
Gender		
Male	33	68.7
Female	15	31.2
Age range (years)		
32 to 42	25	52.1
43 to 53	18	37.5
54 or more	5	10.4
Level of education		
None	2	4.2
Elementary School	19	39.6
High School	19	39.6
Higher Education	8	16.6
Marital status		
Single	21	43.8
Married	9	18.8
Live as married	8	16.7
Separated	3	6.2
Divorced	3	6.2
Widower	4	8.3
Religion		
Catholic	27	56.2
Spiritist	5	10.4
Evangelical	11	23
Others	4	8.3
No answer	1	2.1
Income (MW)		
1 to 2 MW	25	52.1
3 MW or more	11	23
No answer	10	20.8
Do not work	2	4.1

MW = minimum wage;

period from 2004 to 2009, from the 1,910 related articles (national and international ones), only 176 were within the methodology standard established for the study and from these ones, only 13 studies were conducted in national territory. Regarding the type of adherence measures adopted, most of the studies used as isolated measures, the self-report (71.0%), record-dispensing pharmacy (17.1%), counting pills (6.8%), electronic device [medication event monitoring systems (MEMS)] placed on the packaging of ARV (4.0%), registry in medical records (0.6%) and serum therapy (0.6%).

In these studies it was observed that the main methods used for measuring adherence were subjective measures based on self-reporting by respondents (61.5%); objective measures, based on pharmacy dispensing record (7.7%); devices electronic packaging placed on ARVs (5.9%) and combination of methods (23.1%) (Rocha et al., 2010). For the study, there was a need to structure an instrument that could measure the idea of self-image that these patients have about themselves and morphological changes (LDS). Crossing this information with an instrument previously validated and widely publicized (WHOQOL-HIV BREF) sought to verify the relationship with adherence to treatment or not.

The instruments used were “self-perception in aesthetics of fat redistribution in people infected with HIV/AIDS”, developed by the authors for the referred research. Due to lack of instrumental techniques to be used in this type of evaluation based on socio-demographic characteristics of the target population, this instrument was developed with this special intention. Only after the pilot and the necessary changes that the instrument was available to verify the self-perception of people with HIV/AIDS and LDS. The instrument consists of 17 questions, closed with 6, 8 and 3 open in Likert scale of 5 points: where 1 indicates low perceptions and 5, high perceptions (telling about the difference in appearance and frequency of social activities).

Initially, the pilot was given as 14 participants suffering changes to meet the specifics of the target audience. The variables were divided into socio-demographic data, perception of changes in physical appearance after beginning ARV treatment, social isolation, understanding how it could resolve the noticed bodily changes and the amount of time to achieve the solutions listed in the personal perception.

The second questionnaire was the WHOQOL-HIV BREF, the World Health Organization (WHO) (WHOQOL-HIV Group, 2003), using an instrument based conceptually justified, excluding an assessment based solely on criteria associated with clinical components of HIV infection and enhancing also the psychosocial dimensions markedly essential in the lives of infected patients. This instrument consists of 31 questions covering 6 domains of life (physical, psychological, level of independence, social relationships, environment and spirituality) and 5 specific facets of PLHA (symptom, social inclusion, forgiveness and guilt, concern about the future, death and dying). The questions are scored on a 5-point scale (1, negative perceptions and low to 5 high and positive perceptions). Thus, the scores of the domains and facets are arranged in a positive direction, and higher scores denote better quality of life. In some dimensions (pain, discomfort, negative feelings, dependence on medication, death and dying), the scores are not arranged in a positive direction, which means that for these facets, higher scores do not denote better quality of life, these scores need to be reversed so that higher scores reflect better quality of life.

RESULTS

The data in Table 1 show the distribution by sex of 68.7% men and 31.2% women, and from these ones, 52.1% were aged 32 to 42 years, 37.5% between 43 and 53 years and 10.4% between 54 and 66 years, with an average of 43.6 years; studies whose mean age of subjects was 33.9 years old with inclusion criteria, the age should be above 18 years to take part in the research. In summary, these data represent a predominantly male sample formed by young adults with a certain

Table 2. History living with AIDS and antiretroviral treatment time.

Variable	N	%
Time PVHA (years)		
1 to 9	17	35.4
10 to 15	25	52.1
16 to 20 or more	6	12.5
Time TARV (years)		
1 to 9	23	47.9
10 to 15	23	47.9
16 or more	1	2.1
No answer	1	2.1

a certain educational level, alone in most cases; they have involvement with any religious sect or dogma and live with a little more than three minimum wage on average.

Regarding the history of living with HIV/AIDS (Table 2), 52.1% were between 10 to 15 years of diagnosis, with an average of 10.5 years. As for the "use of antiretrovirals", part of the respondents (47.9%) were between 1 to 9 years usage and the rest (47.9%) were between 10 to 15 years, then the average decreased to 8.5 years, which may indicate that the patients started antiretroviral therapy soon after the diagnosis of HIV/AIDS and that this may have been caused by delayed diagnosis. However, it is interesting to note that half of the patients, 50%, had only 1 to 3 hospitalizations and half this period, 50% of hospitalizations has never suffered after initiation of treatment, therefore the average admission which suffered after diagnosis of HIV (0.93) is considered low, and this may be related to treatment efficacy.

In this article, however, we will give emphasis to the data that are related to variables, LDS, which deals with the decrease in the frequency with which people engage themselves in social activities and for interference with the adherence to treatment somehow. For this, it was made a revision of the two instruments mentioned above, so that the data were taken of only variables mentioning social isolation and treatment adherence and also recalling that all participants have some kind of LDS since this data was an inclusion criterion for the study participants.

The instrument that deals with the "self-perception in aesthetics of fat redistribution in people infected with HIV/AIDS" in item 14 refers specifically to "frequency" with which the person engages in social activities, making a comparison between before and after developed AIDS. In this item, from a Likert scale, participants were asked to choose between: Infrequent (I), and Not Just Frequent and Frequent (NJF and F), Frequent (F), and Not Very Frequent and Frequent (F and NVF) and Very Frequent (VF), whose results are shown in Table 3, noting that the frequency with which the volunteers engage in social activities is seemingly minor, represented by the percentage of volunteers (37.5%) who reported they have reduced their social activity when compared with their life before and after the disease AIDS. The WHOQOL-HIV BREF has six domains as described in Table 4. Among its facets were defined five which are considered specific for people living with HIV/AIDS. These are: symptoms of PLWHA, social inclusion, forgiveness and guilt, worries about the future, death and dying.

Table 3. Frequency of Involvement in social activities.

Variable	N	%
I	18	37.5
NJF and F	6	12.5
F	11	23.0
F and NVF	4	8.3
VF	9	18.7

I = Infrequent; NJF = not just frequent; F = frequent; NVF = not very frequent; VF = very frequent.

The domain IV of the WHOQOL-HIV BREF that deals with social relations could corroborate the findings of the body self-perception instrument with regard to the idea of social isolation. Its facets are personal relationships, social support, sexual activity and social inclusion. In this case, we used only social inclusion facet, as this is considered by scholars (WHOQOL-HIV Group, 2003) specific for people living with HIV/AIDS. The values are distributed in a Likert scale ranging from none to total, where values of 1 to 5 are distributed as follows: 1 none, 2 little, 3 average, 4 very much, 5 completely. The results are reported in Table 5.

The data show that 39.6% of participants feel partially (average) accepted by the people who know them, which may mean certain fear of social exposure or who might have lived situations of prejudice that suggest certain reservations about living with people in the social mean. With regard to treatment adherence, the WHOQOL-HIV BREF presents data that were arranged in a Likert scale, which is about how a person needed some medical treatment for their daily life, on a scale that goes from nothing to extremely, whose values are defined as follows: 1 would be nothing, 2 little, 3 more or less, 4 quite and 5 extremely. From Table 6 it is observed that most of the studied group (35.4%) believes that they need medical treatment for their daily life.

DISCUSSION

The respondents "note" themselves different (35.4%)

Table 4. Domains and facets of the WHOQOL-HIV.

Domain	Facets
Domain (I): Physical	Pain and discomfort Energy and fatigue Sleep and rest **Symptoms of PVHAs***
Domain (II): Psychological	Positive feelings Cognition (thinking, learning, memory and concentration) Self-esteem Body (body image and appearance) Negative feelings
Domain (III): Level of independence	Mobility ADL (activities of daily life) Dependency on drugs or treatments Ability to work
Domain (IV): Social Relations	Personal relationships Social support Sexual activity **Social inclusion**
Domain (V): Environment	Physical security Dwelling Finances Care (access and quality of health and social care) Information (acquire new information/learning new skills) Leisure 22 physical environment (pollution/noise/traffic/climate) 23 transport
Domain (VI): Spirituality/religion/personal beliefs	SRPB (spirituality/religion/personal beliefs) **Forgiveness and guilt** **Concerns about the future** **Death and dying**

Overall quality of life and general health perception. *Facets that are highlighted in bold are specific to people living with HIV/AIDS, and as such they were added to the original WHOQOL.
Source: Zimpel and Fleck (2007).

Table 5. Idea of social inclusion.

Variable	N	%
None	0	0
Little	7	14.6
Average	19	39.6
Very much	9	18.7
Completely	12	25
No answer	1	2.1

Table 6. Idea of treatment adherence. "*How much do you need medical treatment for your daily life".*

Variable	N	%
Nothing	8	16.6
Little	8	16.6
More or less	12	25
Quite	17	35.4
Extremely	3	6.2

compared to before and after the disease AIDS, which is confirmed when they "feel" they are viewed differently (35.4%) by individuals, which underscores the tendency to social isolation because the "frequency" of engaging in social activities seems to decrease (37.5%). This study (Silva and Alchieri, 2011) suggests that in evaluating the body self-perception of people living with HIV/AIDS on ARV use (even without specifying usage time and/or class or generation of drugs, and who have SLD), there may be direct interference in the ability to interact, and often the person engages in social activities. This makes them isolate themselves (social death) so that this does not denounce their status as HIV positive (forced disclosure of diagnosis).

The fear of loss of social identity, prejudice and stigma brings to people the need to hide HIV infection, thus maintaining the secret lies in the center of the representations from the carrier. Being "illegal" becomes often necessary to maintain social interactions possible, and not talking about the situation to friends and family is a way of removal of the stigmatizing condition. The secret needs to be kept for fear of social death and the need to be accepted and loved. Therefore, the underground would be the only way to survive in the face of difficulties (Freitas et al., 2010).

When comparing the data obtained in social inclusion tool "self-perception in aesthetics of fat redistribution in people infected with HIV/AIDS", defined as the frequency of these people engaged in social activities, with the data obtained in the WHOQOL-HIV BREF, defined on the basis of how these people felt completely accepted by others (Table 5), it is observed that there is not much difference because the first instrument to decision making in getting involved in social activities became infrequent (37.5%) with the passage of time since the second instrument volunteers felt "average" (39.6%) accepted by others, which could suggest the fear of being exposed for not disclosing their HIV status and fear of been treated with prejudice.

In a study (Gomes et al., 2011) performed in a reference service from Rio de Janeiro with 30 seropositive patients, fear of being noted by society as seropositive was found in the speech of the participants, as follows:

"People choose to conceal HIV-positive serology, considering that HIV has, in its metaphorical history, moral and reprehensible judgment that interferes in the private lives. (...) In general, the negative depiction socially elaborated, referring to those who are living with HIV/AIDS, is reinforced by the language and metaphors used to talk and think about HIV and AIDS. This process increases the fear and, above all, the isolation of those affected ones. Nevertheless, the stigma is extended to family and friends. (...) As a consequence, the study subjects tended to guard or to exclude themselves from society after the discovery of diagnostic. (...)"

Comparing with the data obtained, it is verified that in a (also small) sample of just 48 people, the frequency with which patients decreased social interaction or involvement in social activities was considerable, 37.5% in the first instrument (self-perception) and 39.6% in the second instrument (WHOQOL-HIV BREF), noting that the second instrument refers to "be socially accepted-social inclusion". However, this data could only be confirmed with a larger study which can relate to the SLD aspect of social inclusion.

Regarding adherence to treatment, 35.4% of patients are aware that improvement or maintenance of general health is closely linked to proper use of medication, as well as the fulfillment of the goals proposed for treatment by the multidisciplinary team (WHOQOL-HIV BREF). But fears regarding the metabolic transformations and body (SLD) that may occur as a result of prolonged use of medication is evident when 35.4% of patients feel and notice that others see them differently when compared with before and after the disease AIDS.

Actually, could the data be interpreted as compliance with treatment for this group? Must it take into consideration the time of use of antiretrovirals, 8.5 years on average? In other words, the fact that the research group has already on the average 10.5 years of life carrying the disease HIV/AIDS, and have already gone through some sufferings related to the problems arisen from this experience, could strengthen the thought that even though the idea that antiretroviral drugs can produce certain changes in the body and a person's body, SLD is important to keep the use of drugs and receive the support of the multidisciplinary team as part of treatment for HIV/AIDS.

In the study in Rio de Janeiro service (Gomes et al., 2011), the above exposed becomes evident:

"(...) Respondents demonstrated that to understand the importance of the correct use of HAART, however, there are several factors that influence the inappropriate use of these medications, leading often to treatment dropout (emphasis added by the author). It was identified that one of the reasons that lead to such behavior is related to the side effects of these drugs, especially the change of body image (emphasis added by the author) that can characterize people with HIV as 'AIDS infected' because of lipodystrophy."

The core message about the treatment is that "AIDS equals death", and the drugs included in the daily life ensure survival and a way to resume normalness of their lives, even if it is "difficult". The report below expresses a strikingly affirmative statement.

"It was hard to get used to these medicines, because I struggled and had sickness. But it goes along, goes along, until you get used to them and after you do it, you have to take them properly, at the right time, so reactions can't come up, right? The result is great, because the medicines are pretty good and if you take them properly, the result is awesome. The medication must be taken otherwise you don't have a satisfactory return that doctors want (Freitas et al., 2010)".

The discovery of HIV infection, in most cases translates into solitude, closure, mistrust, secrets, lies and disappointment, even within the family. These aspects of wear, suffering and social exclusion point to difficulties in treatment adherence and emphasize the need for greater investment in health services understanding of AIDS as an event beyond the clinic, requiring the formulation of strategies for integration and intersectoral cooperation in monitoring people living with HIV/AIDS, to promote health and improve the quality of their lives. It is worth noting that social support from family, friends, and even multidisciplinary team are crucial for coping and overcoming the disease, especially regarding treatment adherence (Freitas et al., 2010; Gomes et al., 2011).

Conclusions

The initial proposal of this article was to emphasize the lipodystrophy syndrome as an adverse effect of antiretroviral therapy which confirms the "social death" experienced by patients, which can affect the process of adherence to treatment, being considered one of the most important adverse events due to prolonged use of HAART, because at first sight when they see themselves suffering with the bodily changes, it causes a conflict between preserving life (taking medication) and preserving body image (just when you stop taking that ARV associated to changes). With the data, we can see that there is a relationship between the presence of SLD and social isolation due to the difficulty to deal with inquiries from relatives and friends as the changes in their body image, which is the possibility of denouncing the diagnosis until later. While it can occur also in difficulty with treatment adherence before these changes, however the study corroborates other theorists who note that over time (over two years of treatment) and according to clinical complications which occur, patients tend to use medication properly, even if it costs them their social interaction.

It is observed that there is a relationship between the presence of SLD and occurrence of social isolation, however the phenomenon of adherence (or not) to treatment seems to be extremely related, considering that, even with severe state, SLD patients use drugs and bodily changes seem to be taken as the "price to pay for longevity".

ABBREVIATIONS

AIDS, Acquired immune deficiency syndrome; **HIV,** human immunodeficiency virus; **SINAN,** national notifiable diseases system; **SIM,** mortality information system; **SISCEL,** laboratory testing control system for the national lymphocyte count TCD_4^+/CD_8^+ and viral load network; **SICLOM,** logistical medication control system; **MonitorAIDS,** indicator monitoring system for the national STD/AIDS program; **PN-DST/AIDS,** national STD and AIDS program; **MO,** minister's office; **MH,** ministry of health; **LDS,** lipodystrophy syndrome; **HAART,** highly active antiretroviral therapy; **PLWHA,** people living with HIV and AIDS; **REC,** research ethics committee; **HUOL,** Onofre Lopes University Hospital; **UFRN,** Federal University of Rio Grande do Norte; **ARV,** antiretroviral; **WHO,** World Health Organization; **MW,** minimum wage; **WHOQOL-HIV BREF,** World Health Organization Quality of Life-HIV bref, **SIC,** statement of informed consent; **NREC,** National Research Ethics Committee.

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Full Length Research Paper

Human immunodeficiency virus/Acquired immune deficiency syndrome (HIV/AIDS): Knowledge, attitudes of university students of the State of Azad Kashmir (Pakistan)

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Epidemic of human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) is a significant threat to humankind. This threat is more alarming to developing countries, where poverty and illiteracy may contribute towards higher risk for HIV transmission. To preclude the further feast of HIV, it is very important that people should have a good knowledge of HIV disease. The aim of this study was to examine the level of awareness and attitudes to HIV/AIDS among students at the University of Azad Jammu and Kashmir. A descriptive cross sectional survey was conducted over population samples of 467 students. Significance of data trend was measured by Chi-square test. Our findings showed that 97.9% of the students had heard about HIV/AIDS, 94% respondents considered HIV/AIDS as a fatal disease. About 81.6% of the respondents considered HIV/AIDS as a communicable disease, 84.6% of the respondents agreed that HIV/AIDS can be prevented. 66% of students said that there is no vaccine for HIV. Results of this study show that there is a mediocre level of knowledgeable awareness among the university students in Azad Kashmir. In order to increase the level of awareness to satisfactory level, it is recommended that HIV education should be part of curriculum among all levels of education. Mass media or public media campaigns can not only raise the knowledge in students but also in general public.

Key words: Human immunodeficiency virus/Acquired immune deficiency syndrome (HIV/AIDS), Azad Kashmir, communicable disease, cross sectional study.

INTRODUCTION

Human immunodeficiency virus/Acquired immune deficiency syndrome (HIV/AIDS) is the second widely spread communicable disease worldwide and the sixth common cause of death globally (WHO, 2004). About 65 million people have been affected and more than 25 million people have died of AIDS related causes. The situation situation will even be gloomier, with 29 million new infections estimated by the year 2020, if prevention and treatment efforts are not accelerated. It is estimated that the number of persons living with HIV worldwide is 33.2 million. Deaths related to HIV/AIDS declined from 2.2 million in 2005 to 2.0 million in 2007. However, the number

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of new infections rose to 2.5 times higher than the number of infected persons receiving treatment (UNAIDS, 2008). Global statistics and studies have shown that young people are more vulnerable to HIV and AIDS. At present, the proportion of young people between the ages of 10 and 24 is over 40% in Pakistan. At present, about 36 million children and young people are enrolled in schools, colleges, and universities in Pakistan (National AIDS Control Programme, 2009). Rather than being complacent, this underscores the need for countries to increase their commitment to prevention efforts. This is necessary if this pandemic must start reversal in order to meet the 2015 target by the millennium development goals (MDGs) and to save humanity from an impending scourge.

Situation in Pakistan

Pakistan is perceived as a 'high risk low prevalence country' concerning HIV/AIDS virus. There are 2,622 HIV and 321 AIDS cases in the country. However, HIV/AIDS cases are under-reported in the country and perhaps prevalent among 70,000 to 80,000 people in the country or 0.1% of the adult population (UNAIDS, 2008)

The Pakistan National AIDS Control Programme (NACP) is the one organization that coordinates national AIDS strategies. In the present scenario, HIV/AIDS prevention and control in Pakistan has gained attention due to donor driven pressure and allocations of large amounts of funding ($40 million USD) through a comprehensive, five-year enhanced HIV/AIDS program (2003 to 2008) executed by NACP under the leadership of the Ministry of Health (Government of Pakistan) with financial assistance from the World Bank and other bilateral donors such as the Department for International Development (DFID) and Canadian International Development Agency (Canadian CIDA). The contract for the Enhanced Program was signed in 2002; however, the funds were released to the provinces only in 2004 (Ministry of Health Pakistan, 2009).

Population makes Pakistan vulnerable to the threat of a generalized epidemic. Moreover, the existence of a number of high risk sexual behaviors among the general population, internal and external migration, high level of injecting drug use, unsafe and invasive medical practices and inadequate health and social services are some of the factors increasing the risk of a generalized HIV epidemic in the country. Particular risk behaviors make some groups of people more-at-risk of infection, and these groups include Female Sex Workers (FSWs), Male Sex Workers (MSWs), Injecting Drug Users (IDUs), prison inmates, coal miners, etc. Moreover, denial about risks and vulnerability, and social stigma attached to HIV/AIDS further aggravates the problem (NACP, 2008). Also people visit barber shops for hair cutting and shaving purposes. Barbers are quite ignorant of the fact that they may be the source of HIV transmission from one customer to the other. There is a potential risk of HIV and other blood borne disease transmission among the barbers who use unfavorable sterilization and disinfection procedures as well as poor knowledge, attitude and practices regarding disinfection and sterilization HIV transmission. A comprehensive and intensified public health approach has to be adopted with the involvement of all relevant sectors and barbers should not be neglected (Fantahun and Yeshambel, 2012). Knowledge about HIV/AIDS in barbers, have no result in any risk reduction practice, because barbers perceived HIV/AIDS to be an uncommon disease in our country (Janjua and Nizami, 2006). Even in some case, same syringe is used by multiple patients. These facts make the people of Pakistan more susceptible to HIV disease. Very few surveys have been conducted by individuals in Pakistan to raise the awareness of people regarding HIV. Janjua and Nizami (2006) reported that there should be a campaign to focus on two groups, a high risk group and general population. A study carried out by Ali (2008) through Population Council Pakistan (2008), new and cost effective measures should be introduce against the spread of sexually transmitted infections (STIs) to the general population. The recommendations that emerged from the study suggest a three pronged holistic approach that addresses behavior change through information, education and communication (IEC) strategies, health care delivery and monitoring and evaluation. Activities such as community level messages, workshops and trainings for media procedures, news paper staff, and community activists should be started (NACP). Nafisa (2011) reported that heterosexuality and sex with a sex worker is the most common risk factor while mother to child and injectable drug abuse are the next common risk factors of HIV/AIDS. Zahid (1997) pointed out that public and governmental recognition of the threat of HIV epidemic in Pakistan is recent and implementation of prevention and education programme pertaining to HIV/AIDS is lagging. The status of being a low prevalence but high risk country for HIV make it critical that practical research and HIV prevention efforts targeted at high risk groups be implemented immediately. Khan (2000) pointed out that AIDS is still relatively a new subject in this part of world due to social restrictions on open discussion of sexuality, particularly amongst unmarried youths. The study carried out by Afsar (2002), about awareness of Pakistan adolescents showed disappointing results. This current survey was design to assess the level of awareness about HIV/AIDS in students of University of Azad Jammu and Kashmir.

Objectives of the study

This study was conducted by giving a questionnaire to the students of the University of Azad Jammu and Kashmir

Table 1. Frequency of different age groups of the respondents.

Age group	Frequency	Percentage
18 to 20	184	39.4
21 to 23	158	33.8
24 to 26	66	14.1
27 to 29	43	9.2
30 to 32	16	3.4

Kashmir to meet the following requirements:

1) To measure the level of awareness about HIV/AIDS.
2) To measure the level of knowledge about modes of transmission of HIV/ADIS.
3) To assess the knowledge of precautionary measures about HIV/AIDS.

STUDY METHODOLOGY

A descriptive cross-sectional study was conducted in University of Azad Jammu and Kashmir Muzaffarabad based on simple random sampling. Total sample of 467 (395 (84.6%) boys and 72 (15.4%) girl students) respondents was taken. The level of awareness among university students about HIV/AIDS was assessed through face-to-face interview and the answers given to the questions in the designed questionnaire for the purpose. Design of the close-ended questionnaire (contained 26 questions about general awareness about HIV/AIDS, modes of transmission, precautionary measures and some suggestive measures) was simple and the level was that of a layman's understanding. Data was analyzed by using Statistical Package for Social Sciences (SPSS) software.

RESULTS AND DISCUSSION

As earlier discussed, sample size of 467 students were selected for this survey. These students were classified on the basis of their age. Five age groups were made (18 to 20, 21 to 23, 24 to 26, 27 to 29 and 30 to 32). Majority of the respondents (39.4%) belonged to age group 18 to 20 years, while 33.8% of the respondents belonged to age group 21 to 23 years. Likewise, 14.1% of the respondents belonged to age group 24 to 26 years, 9.2% were in age group 27 to 29 years and 3.4% of the respondents were above the age category of 29 years (Table 1). It was noted that there is almost linear relationship between growing ages with respondent awareness about HIV infection (Figure 1). Younger students have less awareness as compared to older students.

In order to take the feedback of the university student's awareness about HIV disease, questionnaires were distributed. Table 2 shows student's feedback. As shown in Table 2 that majority of the students (about 97.9% of the students had heard about HIV/AIDS, 94% respondents consider HIV/AIDS as a fatal disease, 81.6% of the respondents considered HIV/AIDS as a communicable disease. 95.3% of the respondents agreed that HIV/AIDS infection decreases the immunity, 66% of the respondents were of the view that there is no vaccine available for HIV/AIDS treatment, 87.8% of the respondents agreed that HIV/AIDS can be transmitted by having sexual intercourse with HIV/AIDS infected person, 82.7% of the respondents were aware that HIV/AIDS can be transmitted by transfusion of HIV/AIDS infected blood and blood components, 95.7% of the respondents were aware about HIV/AIDS being transmitted by sharing syringes/needles with infected person, 84.6% of the respondents were agreed that HIV/AIDS is being transmitted from HIV/AIDS infected mother to child, 84.8% of the respondents were aware that HIV/AIDS cannot be transmitted by blades used by HIV/AIDS infected person, 92.9% respondent admitted that individuals having bisexual and heterosexual behavior are at higher risk for contracting HIV/AIDS, 91% respondents were aware that there is test available for diagnosing HIV/AIDS infections whereas 9% respondents were not aware about it) have considerable awareness about HIV disease.

Majority of the respondents (77.3%) agreed that imparting sex education to students play an important role in prevention of HIV/AIDS and 74.5% of the respondents agreed there should be adult education at school and college levels. 70.4% of the respondents agreed that religious institutes can play important role in the prevention of HIV/AIDs while 29.6% did not agree. 79.2% of the respondents agreed that media can play an important role in creating awareness about HIV/AIDS while 20.8% did not agree.

Phase 2 of the study

Phase 2 of the study was designed to check the knowledge of the students about persons which are at high risk for HIV infections and number of possible precautionary steps to avoid HIV infection. At last, possible outcomes of government campaign for producing HIV severity awareness through print and electronic media in general public have been assessed.

Students were asked whether life style of peoples affects the chances of HIV infection or not. Their response was gathered in "yes" and "no" replies. Information obtained is given in Table 3. Table 3 shows that 22.9% of the respondents understand unsafe sex, drug use; heroine and other such addictions, addiction to pornographic material are the high risk life styles.

In order to access the knowledge of students regarding precautionary measures that can prevent the peoples from HIV infections following questions were asked from students. Information obtained is given in Table 4. The respondents were asked what measures should be taken to prevent HIV/AIDS infection, use of new blades, transfusion

Table 2. Response of respondents about HIV awareness survey.

S/N	Question	Response	
		Yes (%)	No (%)
1	Have you heard about HIV/AIDS?	457 (97.9)	10 (2.1)
2	Is HIV/AIDS a fatal disease?	439 (94)	28 (6)
3	Is HIV/AIDS a communicable disease	381 (81.6)	86 (18.4)
4	Do HIV/AIDS infections decrease immunity?	445 (95.3)	22 (4.7)
5	Is HIV/AIDS diagnosed by blood?	411 (88)	56 (12)
6	Is HIV/AIDS diagnosed by urine?	139 (29.8)	328 (70.2)
7	Can HIV/AIDS be transmitted by having sexual intercourse with HIV/AIDS infected person?	410 (87.8)	57 (12.2)
8	Can HIV/AIDS be transmitted by transfusion of HIV/AIDS infected blood and blood components?	386 (82.7)	81 (17.3)
9	Can HIV/AIDS be transmitted by sharing syringes/needles with infected person?	447(95.7)	20 (4.3)
10	Can HIV/AIDS be transmitted by tattoo making?	390 (83.5)	77 (16.5)
11	Can HIV/AIDS be transmitted by sharing toilet with infected people?	95 (20.3)	372 (79.7)
12	Can HIV/AIDS be transmitted by kissing with infected person?	190 (41.7)	277 (59.3)
13	Can HIV/AIDS be transmitted from HIV/AIDS infected mother to child?	395 (84.6)	72 (15.4)
14	Can HIV/AIDS be transmitted by sharing food with HIV/AIDS infected person?	103 (22.1)	364 (77.9)
15	Can HIV/AIDS be transmitted by sneezing or coughing of HIV/AIDS patient?	110 (23.6)	367 (76.4)
16	Can HIV/AIDS be transmitted by shaking hand with HIV/infected person?	85 (18.2)	382 (81.8)
17	Can HIV/AIDS be transmitted by blades used by HIV/AIDS infected person?	396 (84.8)	71 (15.2)
18	Is person having multi sex partner at higher risk for contracting HIV/AIDS infections?	434 (92.9)	33 (7.1)
19	Can precautionary measures save human beings from HIV/AIDS? Answer of 68 (14.6%) was that they do not know.	329 (70.4)	70 (15)
20	Can imparting sex education to students play an important role in prevention of HIV/AIDS?	361 (77.3)	106 (22.7)
21	Can religious institutes play important role in the prevention of HIV/AIDS?	329 (70.4)	138 (29.6)
22	Can media play a vital role in creating awareness about HIV/AIDS among general public?	370 (79.2)	97 (20.8)

Table 3. Understanding of public high risk life styles.

Response	Frequency	Percent
Unsafe sex	236	50.5
Drug use	59	12.6
Heroine and other such addictions	39	8.4
Addiction to pornographic material	14	3.0
None of the above	12	2.6
All of the above	107	22.9
Total	467	100.0

Table 4. Which one is the precautionary measure against HIV/AIDS?

Response	Frequency	Percent
Use of new blades	65	13.9
Transfusion of screened blood	38	8.1
New and disposable syringe	27	5.8
None of the above	73	15.6
All of the above	264	56.5
Total	467	100.0

Table 5. Influence of HIV/AIDS TV messages on students.

Response	Frequency	Percent
Awareness of HIV/AIDS	175	37.5
Awareness of dangerous practices which may lead to HIV/AIDS	94	20.1
Messages are frightening	30	6.4
Have no effect	52	11.1
All of the above	116	24.8
Total	467	100.0

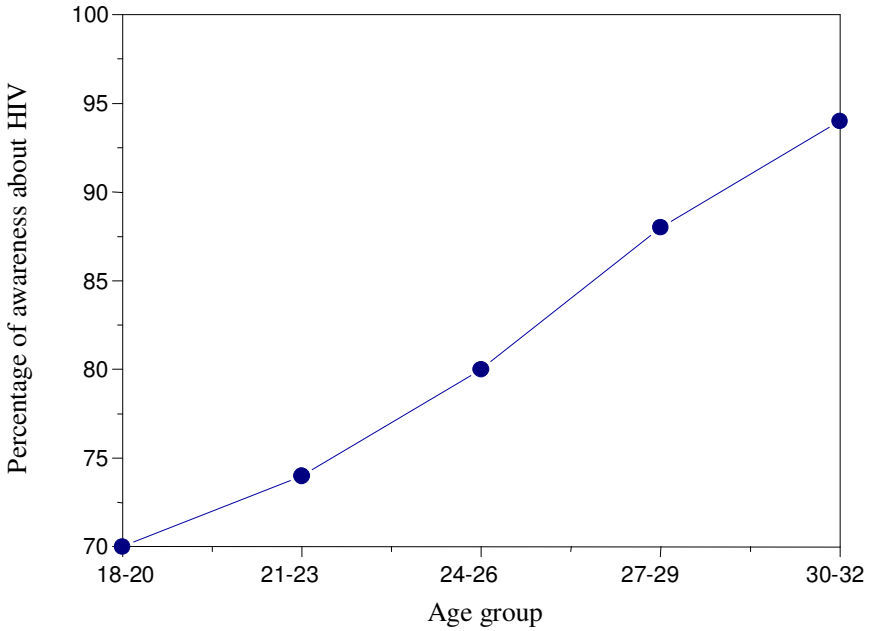

Figure 1. Awareness response of different age groups of respondents.

of screened blood, new and disposable syringe (56.5%), use of new blades (13.9%), transfusion of screened blood (8.1%), new and disposable syringe (5.8 and 15.6%) have no knowledge about precautionary measures.

In order to assess the government campaign outcomes regarding educating the peoples about possible causes of HIV, the followings questions were asked from student. Results obtained through the interviews are given in Table 5. Table 5 shows that 24.8% of respondents got awareness from TV messages about HIV/AIDS and awareness of dangerous practices which may lead to HIV/AIDS; messages are frightening and have no effect. 37.5% awareness of HIV/AIDS, 20.1% awareness of dangerous practices which may lead to HIV/AIDS, 6.4% said the massages are frightening and 11.1% said the messages have no effect.

In order to check the significance of the data, Chi-square (χ^2-test) test of association was used to check the level of significance (P-value). Basic knowledge of student's about HIV/AIDs versus P-values is: have you heard about HIV/AIDS (0.061), do you think AIDS is a fatal (0.004), do you think HIV/AIDS is a communicable disease (0.271), in your opinion can HIV/AIDS infection decrease immunity (0.124), HIV/AIDS is caused by virus named HIV (0.056), HIV virus transmits in the human body and then HIV/AIDS develops (0.588), HIV/AIDS can be prevented (0.778), do you think HIV/AIDS can be cured (0.83), is there any vaccine available for HIV/AIDS (0.032), and can a healthy looking man be infected by HIV/AIDS (0.699); whereas different factors (actions) associated with HIV/AIDS versus P-values HIV/AIDS can be transmitted by sharing syringes/needles with infected person (0.128), HIV/AIDS can be transmitted by tattoo making (0.613), HIV/AIDS can be transmitted by sharing toilet with infected people (0.073), HIV/AIDS can be transmit-ted by kissing with infected person (0.042), HIV/AIDS can be transmitted by mosquito biting (0.768), HIV/ AIDS can be transmitted by sharing food with HIV/AIDS infected person (0.038), HIV/AIDS can be transmitted by sneezing or coughing of HIV/AIDS patient

(0.504), and HIV/AIDS can be transmitted by shaking hand with HIV/AIDS patient (0.052) showing the probabilities of different risk factors (syringes, toilet, food, and shaking hand) are strongly associated with HIV/AIDS

P-values of different factors (actions) associated with HIV/AIDS are: due to own life style risk (0.001), bi-sexual and heterosexual behavior are at higher risk for contracting HIV/AIDS (0.108), having multi sex partner (0.005), individual at higher risk for contracting HIV/ AIDS infection include people who buy sexual services (0.002), knowledge about sexually transmitted diseases are significantly associated with HIV/AIDS (0.001).

Significance of probabilities of different precautionary measures and some suggestive measures against HIV/AIDS can be seen for different factors. Precautionary measures against HIV versus P-values are: PM (Do you agree that precautionary measures can save human beings from HIV/AIDS?) (0.279), media campaign is associated positively with HIV awareness (0.067).

Conclusion

The results of this study indicate that carefully planned information, education and communication can be used to correct misunderstandings about HIV/AIDS and HIV/AIDS prevention practices. Mass media or public media campaigns can raise the bar of knowledge in students. AIDS awareness in curriculum should be included in all levels of education as researchers supported the idea of school-based education of HIV/AIDS being provided, it appears that these curricula were instrumental in increasing students' knowledge about AIDS, dispelling misconceptions about casual contact as a route of disease transmission; and decreasing student's fear and vulnerability about having classmates with AIDS or HIV infection especially at college level. Similarly, education has been recommended as the best line of defense against the spread of the AIDS. There is need to expand and strengthen existing preventive interventions introduced by the government of Pakistan, as well as to introduce new and cost-effective measures to reduce the spread of STIs to the general general population (Ali, 2008). One way of promoting health practices in children is helping them have compassion and understanding of those who are infected, and coping with illness which is appropriate to their level of cognitive development. A study among secondary school students in Islamabad reported that 95% of boys and 100% of girls knew about HIV/AIDS through television. The awareness through media has a strong impact on the knowledge regarding HIV/AIDS in Pakistani population (Shaikh, 2001). In conclusion, there is a satisfactory awareness among the university students entering into the profession. HIV education should be part of curriculum among all levels of education.

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Full Length Research Paper

Medication adherence and cluster of differentiation 4 (CD4) cells response in patients receiving antiretroviral therapy

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This study evaluated medication adherence following interventions and its association with cluster of differentiation 4 (CD4) cells response among patients receiving antiretroviral therapy (ART) in Specialist Hospital Sokoto, North Western Nigeria. Longitudinal study design was used. Interventions included training of health workers on adherence counseling; pre- and post-ART adherence counseling were provided to patients. Out of 1300 patients on ART who were provided interventions, 365 patients were selected using simple random technique. The CD4-cell measurements at months 0, 6, 12, 18 and 24 were extracted from the patients' hospital records; and announced pill counts were conducted. Chi square was used to test the association between groups of variables; and P < 0.05 indicated statistical significance. Out of 365 participants sampled, data from 297 (81.4%) participants were valid for analysis. The mean age of participants was 34.7 (95%CI, 33.6 to 35.8) years; 60.9% were females and 76.4% received Zidovudine/Lamivudine/Nevirapine (AZT/3TC/NVP) regimen. The mean percent adherence was 83.4% (95%CI, 80.8% to 86.0%). The mean CD4-cells count (cells/mm$_3$) at ART initiation increased from 198.9 (95%CI, 180.7 to 217.1) to 396.5 (95%CI, 368.3 to 424.7) at 6 months, 428.0 (95%CI, 400.4 to 455.6) at 12 months, 427.2 (95%CI, 405.6 to 448.8) at 18 months, and 501.4 (95%CI, 469.5 to 533.3) at 24 months. This increase was statistically significant (P< 0.05). Participants' employment and educational status, age, sex and type of ART regimens received had no significant association with medication adherence (P > 0.05). This study reported a mean adherence level that is below the required >95% necessary to achieve the goals of ART. The increase in CD4 cells count over the observation period was statistically significant at the estimated adherence level. Better immunologic outcomes may be achieved with higher adherence level.

Key words: Medication adherence, pill counts, human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), antiretroviral therapy (ART), Nigeria.

INTRODUCTION

Life-long near-perfect adherence, >95%, is necessary to achieve the goals of antiretroviral therapy (ART) which

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include the suppression of human immunodeficiency virus (HIV) replication (HIV-RNA < 400 copies/ml) (Chalker et al., 2010; Arnsten et al., 2001; Paterson et al., 2000). It has also been suggested that 100% adherence rate is necessary to achieve even greater benefits (Lucas et al., 1999). Studies have shown that achieving medication adherence rates greater than 80% in chronic therapy has been problematic, even in resource-rich countries (DiMatteo, 2004; Osterberg and Blaschke, 2005). Non-adherence is the term use to describe a patient's inability to take his or her drugs in the collaboratively agreed manner. Non-adherence to ART poses a risk of HIV progression, a worsening of immunological and clinical states, development of drug resistance and consequently narrowing future treatment options.

A number of methods are employed, alone or in combination, to measure medication adherence. The common methods for measuring adherence to ART include patient self-reports, pill counts, pharmacy dispensing records and electronic medication monitors amongst others (Chalker et al., 2010); and each method has its strengths and limitations. Studies have also shown that the objective measures used in research, although impractical for most clinical settings, are more sensitive than patient self–report for detecting medication non-adherence (Chalker et al., 2010; Doung et al., 2001). Pill counts adherence rates have been shown to have reasonable correlations with electronic drug monitoring (EDM) and HIV viral load (Berg et al., 2006; Liu et al., 2001; Bangsberg et al., 2000). There are additional reports that data from pill count method agreed with data from viral load measurement; and pill counting was recommended as a reliable and economical tool for adherence measurement in a resource-limited setting (San Lio et al., 2008). Studies in Nigeria reported varying levels of adherence to ART ranging from 49.2% (Nwauche et al., 2006), 58.1% (Erah and Arute, 2008), 73.3% (Bello, 2011), 73.8% (Agu et al., 2010), and 44% (Afolabi et al., 2009) in Southern Nigeria, to 79.1% (Agu et al., 2011) and 80% (Mukhtar et al., 2006) in Northern Nigeria.

With effective ART, the cluster of differentiation 4 (CD4) cells count increases by >50 cells/µl within weeks after viral suppression, and increases by 50 to 100 cells/µl per year thereafter. The CD4 cells counts of some patients may not increase that steadily or quickly, even with durable viral load suppression. Additionally, patients who are older (age >50) and those with lower baseline CD4 cell counts are more likely to have reduced CD4 count responses (AETC, 2012). The mean CD4 counts of patients starting ART with baseline CD4 cell counts >350 cells/µl increased by over 500 cells/µl after one year of therapy (Wright et al., 2011). The median increases in CD4 cell counts after 6, 12, and 24 months of ART were 114, 181, and 248 cells/mm^3, respectively (Smith et al., 2004). In Nigeria, patients on ART reported a significant increase of baseline CD4-cell count (cells/µl) by over 100 cells/µl at 3, 6, 12 and 18 months of therapy (Agu et al., 2010). However, studies that correlated the CD4 cells response of patients on ART with medication adherence are scanty in Nigeria. This study evaluated medication adherence following the interventions and its association with CD4 cells response among HIV-positive patients on ART in Specialist Hospital Sokoto, North Western Nigeria.

METHODOLOGY

Setting

This study was conducted in Specialist Hospital Sokoto, a secondary public health care facility in the North Western Nigeria. The hospital offers comprehensive HIV care services including ART for HIV-infected patients. It has a 540-bed capacity and serves the people living in the Sokoto metropolis and its environment including the bordering country, Niger Republic. The HIV-infected patients identified at the HIV counseling and testing service points were enrolled into a pre-ART register for follow-up and only those who meet the ART eligibility criteria are commenced on treatment. At the time of this study, there were 1300 HIV-infected patients currently receiving ART in this facility. The patients were required to come with their medication pill containers at every medication refill visit as a pre-condition for getting a refill. The pharmacist retrieves the patients' pill container during this visit, and counts the remaining medications before dispensing additional pills as an adherence quality improvement strategy instituted in this hospital. Medication adherence is reinforced through adherence counseling and education especially for those who were found to be non-adherent by pill counts; although these were poorly documented as a routine. HIV care and treatment services were supported by Global HIV/AIDS Initiative Nigeria (GHAIN) project and provided at no cost to the patients with funding from the United States (US) President's Emergency Plan for AIDS Relief (PEPFAR) through US Agency for International Development (USAID).

Research design

This was a longitudinal study. The CD4 cells count (cells/mm^3) of patients receiving ART was assessed at months 0, 6, 12, 18 and 24. An announced pill count was conducted to assess medication adherence of study participants following adherence intervention.

Intervention

The intervention included training of health workers on patient adherence counseling and education. These trained adherence counselors provided group education and individual counseling to the patients on every clinic day. All HIV-infected patients who were eligible to commence ART in the facility went through at least three different sessions of adherence counseling and education before being placed on antiretroviral drugs. The patients' commitment to adhere to the medications was obtained in the last counseling session before ART commencement. Ongoing medication adherence counseling and pill counts were also provided at every medication refill visits. The copies of standard operating procedures (SOP) for patient adherence counseling and education were also provided to the adherence counsellors in the facility. There is a strong support group of People Living with HIV and AIDS (PLWHA) that serves as a self help group, and members also function as adherence counselors and treatment supporters.

Outcome measures

The main variables of interest were measured as the percent adherence by pill counts and the CD4-cell counts (cells/mm^3) from each patient's laboratory results obtained across multiple time points.

Selection criteria

All adult HIV-infected patients who had received antiretroviral therapy for at least 6 months, benefited from the medication adherence interventions and refilled their medications between April 1, 2011 and July 18, 2011 were eligible to be included. Only patients that consented to participate were included in the study. Any patients who did not meet each of these criteria were excluded from the study.

Study population

The study population included 1300 HIV-infected patients who were receiving ART and met the selection/inclusion criteria in the hospital.

Sample size calculation and sampling

The sample size (n) was calculated as follows (Yamane, 1967):

$$n = \frac{N}{1 + N\,(e)^2}$$

where n = the sample size; N = the study population= 1300; *e* = the level of precision (± 5%). Therefore,

$$n = \frac{1300}{1 + 1300\,(0.05)^2} = 305.9 \approx 306 \text{ patients}$$

The calculated sample size (n) was a total of 306 ART patients; however, the sample included 365 HIV-infected patients to accommodate for losses due to incomplete or missing data on important variables of interest. Out of the study population, 365 HIV-infected patients were selected using simple random technique.

Ethical consideration

Ethical approval for this study was obtained from National Health Research Ethics Committee (NHREC), Federal Ministry of Health Abuja Nigeria. Informed consent was obtained from the participants and confidentiality was assured by excluding identifiers during analysis.

Data collection

Eight-item study-specific instrument was administered to the participants at the point of medications refill by trained research assistants. The items included patients' identity number (ID), sex, age, educational level, occupation, antiretroviral drug regimens, the expected number of pills remaining based on the last medications refill information and the actual number of pills remaining at this refill visit. The last medications refill information included the number of pills dispensed at the last medication refill visits, taking into account the extra pills dispensed and missed coverage due to stock-outs. The research assistants retrieved the pill containers from the patients, counted and documented the number of pills in the container in the instrument. Using the patients' ID, the research assistants retrieved laboratory results from the patients' case folder and extracted their CD4 cells counts at months 0, 6, 12, 18 and 24.

Data analysis

The data were analyzed using Predictive Analytics SoftWare (PASW) Statistics 18. Descriptive statistics such as frequency distribution were used to present sample characteristics. The percent adherence for each participant was calculated using the following formula: (no. of days of pills dispensed – no. of days of pills returned) / (no. of elapsed days between dispensed date and return date) × 100. The mean percent adherence of the participants was calculated by taking the average of percent adherence for all participants. Paired samples t-test was used to compare the CD4-cell counts at months 0, 6, 12, 18 and 24. Chi-square was used to test the association of the percent adherence and groups of variables. All reported P-values were 2-tailed and $P < 0.05$ indicated statistical significance.

RESULTS

Characteristics of patients

Of the 365 ART patients sampled in this study, data from 297 (81.4%) participants were valid for analysis; while data from 68 (18.6%) participants were not used due to missing data on some core variables. The mean age of participants was 34.7 (95%CI, 33.6 to 35.8) years; 56.6% were 30 to 44 years old; and 60.9% were females. Of the participants, 42.4% had Islamic education, 38.4% were self-employed and 76.4% were receiving AZT/3TC/NVP regimen (Table 1).

Medication adherence

The mean percent adherence level reported among the participants was 83.4% (95%CI, 80.8% to 86.0%). The mean percent adherence for male participants was higher as compared to female participants and the difference was not statistically significant ($P > 0.05$). The participants' ART regimens, age, employment and educational status were not associated with adherence ($P > 0.05$). Of the participants, 52.2% and 16.8% of them reported mean percent adherence of 100% and <60%, respectively (Table 2). Of the 142 (47.8%) participants that reported <100% adherence, the mean percent adherence rate was 65.3% (95%CI, 61.9% to 68.7%).

Medication adherence and CD4 response

At the mean percent adherence level (83.4%) reported, the mean CD4-cell count (cells/mm^3) at ART initiation increased

Table 1. Characteristics of the study participants segregated by reported adherence rates (n = 297).

Characteristic	Number of participants (%)	Mean percent adherence (95%CI)	P-value
Sex			
Male	116.0 (39.1)	84.4 (80.4 – 88.4)	0.279
Female	181.0 (60.9)	82.5 (79.1 – 85.9).	-
Age (years)			
15 – 29	81.0 (27.3)	85.1 (80.6 – 89.6)	0.889
30 – 44	168.0 (56.6)	81.6 (77.8 – 85.4)	0.911
45 – 59	42.0 (14.1)	86.2 (80.8 – 91.6)	0.668
> 59	6.0 (2.0)	82.1 (64.6 – 99.6)	-
Educational status*			
None	24.0 (8.1)	83.8 (75.5 – 92.1)	0.834
Primary	34.0 (11.4)	85.2 (79.0 – 91.4)	0.516
Secondary	60.0 (20.2)	85.8 (80.5 – 91.1)	0.178
Tertiary	43.0 (14.5)	84.5 (78.1 – 90.9)	0.896
Islamic	126.0 (42.4)	81.0 (76.5 – 85.5)	-
Employment status**			
Employed	41.0 (13.8)	78.9 (72.2 – 85.6)	0.719
Self-employed	114.0 (38.4)	85.2 (81.0 – 89.4)	0.934
Unemployed	98.0 (33.0)	80.4 (75.5 – 85.3)	0.785
Student	15.0 (5.1)	91.2 (82.2 – 100.0)	-
ART regimen (irrespective of the type of formulation)§			
Zidovudine/Lamivudine/Nevirapine (AZT/3TC/NVP)	227 (76.4)	84.5 (81.7 – 87.3)	0.100
Tenofovir/Lamivudine/Efavirenz (TDF/3TC/EFV)	8 (2.7)	90.0 (75.2 – 100.0)	0.098
Tenofovir/Lamivudine/Nevirapine (TDF/3TC/NVP)	15 (5.1)	83.4 (71.5 – 95.3)	0.667
Tenofovir/Emtricitabine/Efavirenz (TDF/FTC/EFV)	29 (9.8)	75.8 (65.9 – 85.7)	0.111
Tenofovir/Emtricitabine/Nevirapine (TDF/FTC/NVP)	10 (3.4)	72.4 (55.1 – 89.7)	0.306
Tenofovir/Emtricitabine/Lopinavir-boosted with Ritonavir (TDF/FTC/LPVr)	5 (1.7)	86.8 (69.8 – 100.0)	

CI, Confidence interval; *Ten participants did not indicate their educational status; **Twenty-nine did not indicate their employment status; §Three participants did not indicate the ART regimen received; and were excluded in the analysis. ART regimen presented.

Table 2. Mean percent adherence reported by the participants; n = 297.

Mean percent adherence	Frequency	Percentage
100	155.0	52.2
90 – 99	13.0	4.4
80 – 89	35.0	11.8
70 – 79	30.0	10.1
60 – 69	14.0	4.7
< 60	50.0	16.8
Total	297.0	100.0

from 198.9 (95%CI, 180.7 to 217.1) to 396.5 (95%CI, 368.3 to 424.7) among the 165 of 297 (55.6%) patients who had documented CD4 cells measurement at 6 months, 428.0 (95%CI, 400.4 to 455.6) among the 91 of 297 (30.6%) patients who had documented CD4 cells measurement at 12 months, 427.2 (95%CI, 405.6 to 448.8) among the 30 of 297 (10.1 %) patients who had documented CD4 cells measurement at 18 months, and 501.4 (95%CI, 469.5 to 533.3) among the 13 of 297 (4.4%) patients who had documented CD4 cells measurement at 24 months. The increase in CD4 cells count was statistically significant at 6, 12, 18 and 24 months (P<

Table 3. Adherence rates of the participants segregated by mean CD4-cell counts (cells/mm^3) at different intervals; n = 297.

Mean percent adherence	Mean CD4-cell counts (cells/mm^3) at 95% Confidence Interval					P-value
	Month 0,	Month 6	Month 12	Month 18	Month 24	
100 Adherence	192.8 (166.3 – 219.3)	399.2 (353.9 – 444.5)	435.1 (391.1 – 479.1)	499.2 (470.9 – 527.5)	379.4 (347.9 – 410.9)	0.000
90 – 99 Adherence	243.0 (133.9 – 352.1)	604.0 (442.5 – 765.5)	622.5 (621.5 – 623.5)	360.5 (359.5 – 361.5)	953.5 (952.5 – 954.5)	0.000
80 – 89 Adherence	229.2 (178.1 – 280.3)	430.1 (344.5 – 515.7)	460.8 (350.0 – 571.6)	477.4 (324.8 – 630.0)	317.5 (251.8 – 383.2)	0.000
70 – 79 Adherence	213.6 (162.5 – 264.7)	374.1 (288.5 – 459.7)	335.8 (225.0 – 446.6)	330.4 (177.8 – 483.0)	615.5 (549.8 – 681.2)	0.000
60 – 69 Adherence	173.9 (105.1 – 242.7)	373.7 (234.9 – 512.5)	329.3 (227.7 – 430.9)	224.3 (117.3 – 331.3)	608.0	0.000
< 60 Adherence	182.2 (139.8 – 224.6)	367.7 (303.1 – 432.3)	447.6 (353.0 – 542.2)	469.5 (298.1 – 640.9)	370.5 (132.4 – 608.6)	0.000

0.001). Table 3 shows the participants' CD4 responses over time segregated by adherence level. The CD4 response over time increased significantly from the baseline value for both participants that reported 100% and <100% adherence levels in the first 12 months (Table 3).

DISCUSSION

This study evaluated medication adherence and its association with CD4 cells response among HIV-positive patients receiving ART. The study reported mean adherence level (83.4%) that is below the required >95% necessary to achieve the goals of ART (Chalker et al., 2010; Arnsten et al., 2001; Paterson et al., 2000). This is similar to previous reports in Nigeria (Mukhtar et al., 2006; Olowookere et al., 2008; Agu et al., 2011). Over one-half of the participants reported 100% adherence level. This is highly desirable as 100% adherence rate is necessary to achieve even greater benefits of ART (Lucas et al., 1999). Participants' educational and employment status, age and sex were not found to have any association with medication adherence. This is contrary to previous reports that employment status (Kyser et al., 2011; Agu et al., 2010; Agu et al., 2011) and age (Reda and Biadgilign, 2012; King et al., 2012; Sullivan et al., 2007) were associated with poor adherence but consistent with reports by Silva et al. (2009). These may be explained by previous reports that forgetfulness, being unemployed and looking for work were among the major causes of non-adherence among patients receiving ART (Kyser et al., 2011; Agu et al., 2011; Olowookere et al., 2008; Sullivan et al., 2007). However, further studies may be needed to investigate the differences in the various study findings.

The type of antiretroviral drugs regimens received by the participants had no association with medication adherence in this study. Toxicities/side effects and pill burden are known factors related to the type of antiretroviral regimens that adversely affect medication adherence (Olowookere et al., 2008; Agu et al., 2010). Drug toxicities/side effects were associated with poor medication adherence (Olowookere et al., 2008); whereas pill burden was not (Agu et al., 2010). This may be associated with the advent of fixed dose combinations and subsequently reduced number of pills in ART. The relationship between these specific factors and medication adherence was not assessed in this study; hence, we may not be able to make reasonable inference.

A comparison of adherence levels to CD4 cells count at 6 months intervals shows a steady increase in mean CD4 cells count from baseline in most adherence sub-levels. There are reports that the CD4 cells counts of some patients may not increase steadily or quickly, even with durable viral load suppression (AETC, 2012). Patients who are older (age >50) and those with lower baseline CD4 cell counts are more likely to have reduced CD4 count responses (AETC, 2012). However, the increase in CD4 cells count over the observation period was statistically significant. This is consistent with previous research findings (Smith et al, 2004; Wright et al, 2011; Agu et al, 2010); however, >95% adherence level is highly desirable for better immunologic and clinical outcomes.

There are some limitations which need to be acknowledged. As noted in previous studies, announced pill counts can be inaccurate if any of the following occurs: (a) patients empty pill containers without ingesting any pills ("pill dumping"), (b) the accurate start date for the pill supply cannot be determined, or (c) patients use multiple pill containers (Berg and Arnsten, 2006; Rudd et al., 1989; Pullar et al., 1989). These may cause overestimation of adherence rates by pill counts. The baseline adherence rates were not assessed before the study interventions. This limited the

comparison of adherence rates before and after the interventions. The small sample size in some subgroups may results in estimates with wide confidence intervals. This may have led to unreliable estimates in these subgroups.

Conclusion

This study reported mean adherence level that is below the required >95% necessary to achieve the goals of ART. Participants' educational and employment status, age, sex and the type of antiretroviral drugs regimens had no association with medication adherence. The increase in CD4 cells count over the observation period was statistically significant at the reported adherence level. Better immunologic outcomes may be achieved with higher adherence level.

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Short Communication

A novel approach to inhibiting human immune deficiency virus (HIV-1) infection by actively neutralizing the antibodies of reverse transcriptase system

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Human immune deficiency virus (HIV) infection is frequently reported in Egypt. This study introduces a new approach for HIV eradication based on a new enzyme combination reverse transcriptase and DNA polymerase (VK 25 RD) formula for inhibiting and or preventing the disease. This pilot study was done on five naive patients who were all positive for HIV antibodies, never treated with anti retroviral medications. Those patients were registered and under surveillance by Human immune deficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) Control Department at the Egyptian Ministry of Health (MOH). Their immunological data revealed a viral load of more than 1,000 copies/ml by human immune deficiency virus-ribonucleic acid-polymerase chain reaction (HIV-RNA-PCR), and antibody positive to HIV-1 and CD4+ T-cell values less than 250 cells/µl. All of the patients showed the same clinical symptoms of HIV/AIDS and wrote consent of acceptance to take this combination therapy in the form of subcutaneous injection of 0.1 cc twice daily for 24 weeks. At the end of therapy, all of the patient's viral loads had reached under the detectable limits (less than 16 copies/ml); also there were significant increases of their CD4 cells count over 500 cells/µl. According to these findings, this therapeutic modality was promising for treating HIV-1 disease and human immunodeficiency syndrome.

Key words: (VK 25 RD) Reverse transcriptase (RT), DNA polymerase mixture formula, acquired immune deficiency syndrome (AIDS), Human immune deficiency virus (HIV).

INTRODUCTION

In Egypt where this study is conducted, the prevalence of newly diagnosed human immune deficiency virus (HIV) positive patients is high, therefore Egypt belongs to one of the regions of the world with rising HIV epidemics. The virus is transmitted mostly sexually in 71% of the cases, with heterosexual contacts comprising almost half of all detected cases. Intravenous drug use and blood transfusion each accounts for 9% of the detected cases, while the percentage of the transmission through renal dialysis and unknown modes comprise the rest.

HIV is a retrovirus that infects the human immune system, 'reverse transcriptase enzyme' is an essential

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part of the virus that reads the sequences of viral RNA nucleic acids that have entered the host cell and transcribes the sequences into complementary DNA sequences. HIV-1 reverse transcriptase (RT) is composed of an extended, asymmetric heterodimer of two related chains, a51 kD subunit (p51) of 440 amino acids and a 66 kD subunit (p66) of 560 amino acids (Misra and Knox, 1999). The p66 subunit shows an overall struc-tural similarity to the polymerase domain of *Escherichia coli* DNA Pol I and other polymerases. Without reverse transcriptase, the viral genome could not incorporate into the host cells, and therefore could not reproduce. This concept leads to the consideration of the pharmacological inhibitors of DNA polymerase for the treatment of cancer.

DNA polymerase enzyme is a vital enzyme for the regulation of multiple physiological cellular functions such as DNA repair, gene transcriptions, cell cycle progression, cell death, chromatin function, and genomic stability. Overconsumption of polymerase will result in cellular energetic depletion, mitochondrial dysfunction, and ultimately cellular necrosis (Abdelkarim et al., 2001; Aldinucci et al., 2007; Akiyama et al., 2001). This enzyme is essential for the process of viral replication and the production of a new viron which also leads us to considering it as a useful tool for the treatment of cancer.

Now the most successful available treatment today is reverse-transcriptase inhibitors [for example azidothymidine (AZT)] and protease inhibitors (Baeten, 2011; Thompson et al., 2010). These treatments interfere with enzymes that are needed for HIV to make copies of itself, a key step in the virus's attack on the cells of the immune system. CD4+ T-cells are one of our immune system components that are necessary to stimulate the activation of other immune cells that attack infectious particles (antigens) in the body. When these cells come under attack by HIV, the immune systems can no longer function effectively, and the body is incapable of combating the HIV.

Accordingly, the question that arises is why is our immune system not able to stop the consumption of the immune cells while the virus can? And why does the viral replication process have a continuous dynamic action with no signs of immune suppression? The answer depends on our vision of the HIV strategy. The HIV stimulates the cytotoxic CD8+ T-cells to act on CD4+ T-cell, the latter cell will try to inhibit this mechanism and such a phenomenon could be considered as a defensive mechanism but as long as this process continues and the inhibitors mechanism is gradually depleted, it will lead to a profound destruction of all CD4+ T-cell paving the way to the spread of the viral invasion. Therefore, we assume that the failure of immune cells to stop the viral replication mainly comes from the mystery stored in the functions of RT enzyme that makes our immune cells compatible with its orders.

METHODOLOGY

Previous studies have proven that RT enzyme works like many other DNA polymerase, where the goal of HIV-1 RT enzyme is to convert the single stranded RNA genome into double-stranded DNA (Jaeger et al., 1998; Lenvin, 1997). Thus we assumed that introducing the reverse transcriptase and DNA polymerase enzyme in a combined form (available in experimental laboratories as Taq DNA polymerase, AMV Reverse Transcriptase, Promega Corporation Manufactured-date; 22, October, 2009, expiry date; 31 July, 2011) to the host subcutaneously will stimulate the immune system to produce neutralizing antibodies to directly inhibit these proteins that the virus is using for its cellular entry which will interfere ultimately with its replication process, considering this as a natural inhibitors to HIV and sparing patients infected with HIV from all serious side effects arising from conventional antiviral drugs available on the market.

Patient number 1

Mr Shady, is a 24 year old Egyptian male, HIV positive since February, 2009 with symptoms of early HIV infection such as joint pain, muscle ache, weakness and white spots on the tongue. Physical examination revealed hepatomegaly and splenomegaly, shallow and rapid respirations. Serological testing was positive for hepatitis A antibody, negative for hepatitis B core antibody and hepatitis C (HCV). CD4+ count were 270 cells/µl (normal range 350 to 550 cells/µl), viral load was 92,000 copies/ml, white blood cell (WBC) count 9,600 cells/µl, alanine aminotransferase (ALT) 67 IU/L (normal range 0 to 40 IU/L), aspartate aminotransferase (AST) 60 IU/L (normal range ,0 to 40 IU/L).

Patient number 2

Mr Mouhamed is a 26 year old Egyptian male diagnosed with HIV disease after he had developed a fever of unknown cause, abdominal pain, weight loss and sore throat. His CD4+ cell counts were 315 cells/µl, and a viral load of 105,000 copies/ml. WBC count were 10,800 cells/µl, negative antibodies to HCV, HBV, AST and ALT were within normal range.

Patient number 3

Mrs Aum ommr is a 34 year old female from Saudia Arabia with a history of HIV infection diagnosed in 2004 via sexual contact and history of pulmonary tuberculosis, presented with significant weight loss, chronic diarrhea, joint pain, muscle aches, chills, and lymphadenopathy. Serological testing showed CD4+ cell count of 180 cells/µl, viral load of 447,300 copies/ml , WBC count 4200 cells/µl, ALT 67 IU/L, and AST 42 IU/L. Serology for HCV and HBV were negative, and sputum for tuberculosis were positive in three different samples.

Patient number 4

Mrs Ayaa is a 23 year old HIV positive Egyptian female, presented immediately after she was diagnosed seeking medical treatment. Patient was asymptomatic. Serological testing showed CD4+ cell count of 170 cells/µL, viral load of 4,300 copies , WBC count 5300 cells/µL, ALT and AST were within normal range, serology were negative for HCV, and positive for HBV and Hepatitis B surface

antibody(HB S ab)

Patient number 5

Mr Ahmed a 38 year old HIV positive Egyptian male was diagnosed after returning to Cairo after spending most of his life abroad, the patient presented with right upper quadrant abdominal pain, nausea, anorexia, significant weight loss, weakness, and intermittent diarrhea. Serological testing showed CD4 count of 150 mm^3, viral loads of 315,000 copies, WBC count 3400 cells/µl, negative serology for HCV and HBV, and normal liver enzymes.

Our hypothesis was tested on the patients after written informed consent was signed by each of them to participate in the trial, no significant side effects of the treatment were anticipated except for possible allergic reaction. The duration of the therapy was 24 weeks, every patient was trained to self inject 0.10 cc of the combination (stored in 3 cc vials) twice daily after breakfast and after dinner as a total of 120 injection (total of 4 vials). Patients were followed up weekly in the outpatient clinic for observation only.

RESULTS

After 24 weeks of the beginning of the therapy, the immunological testing of the above patients was repeated, showing surprisingly undetectable viremia (reference range < 16 copies/ml) for all of the patients with significant elevation in CD4+ T-lymphocytes above 500 cells/ml. The most important finding of great immunological value is that HIV antibodies by enzyme linked immunosorbent assay (ELISA) testing were negative also, the patients reported significant improvement of their physical activities and the constitutional symptoms of HIV including malaise, fatigue, weight loss and joint pain.

DISCUSSION

This study introduced a novel biological enzymatic mixture comprising reverse transcriptase and DNA polymerase enzyme (VK 25 RD) for the treatment of HIV/AIDS differing from all remedial methods which depend on treating the viral infection by immune inhibitors for the virus replicating materials reverse transcriptase, DNA. The recent trend of treating HIV/AIDS is to combine at least three drugs from two different classes. These classes include: Nun-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), fusion inhibitors and integrase inhibitor. They generally work by disabling the formation of proteins needed by the virus to copy itself (NNRTIs, PIs and integrase inhibitors) or blocks the HIVs entry into the CD4 cells (fusion inhibitors). The side effects of these drugs are remarkable, their use, whatever the time it takes never leads to complete cure, but it aims to ameliorate the clinical picture, to increase the CD4 cell count and decrease the viral load. Versus the strategy of these drugs which aims to block HIV copying by direct interference through the drug action, our hypothesis aims to enhance the immune system of the patient to build its own neutralizing antibodies against the virus.

This study and its promising results could be a step towards a real solution for the global problem. However, we have to confess that there was a limitation concerning our study due to the small sample size which emerged from the limited infected cases of HIV/AIDS within reach in Cairo. Besides, a restricted budget was available which also challenged us, so we emphasize that a further extended and tedious study is needed to evaluate the benefits and values of the compound.

Conclusion

From the results we found that stimulating the body with this combination therapy produced “broadly neutralizing antibodies” that will be the key for further research to explore the possibilities of developing a prophylaxis (vaccine) and/or curative treatment for HIV infection.

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UPCOMING CONFERENCES

7th IAS Conference on HIV Pathogenesis, Treatment and Prevention, Kuala Lumpur, Malaysia, 30 Jun 2013

6th International Meeting on HIV Persistence, Reservoirs and Eradication Strategies, Miami, USA, 3 Dec 2013

17th International Conference on AIDS and Sexually Transmitted Infections in Africa, Durban, South Africa, 7 Dec 2013

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