Indian J Med Res 141, January 2015, pp 119-121

Correspondence

Unexpected diagnostic findings in some HIV positive individuals in Bangladesh

Sir,

Both immunological and virological assays are used for diagnosis and management of HIV infection in Bangladesh. The enzyme-linked immunosorbent assay (ELISA) and rapid test kits (RTKs) followed by confirmatory tests such as Western blot/Line immunoassay (WB/LIA) are the principal HIV/AIDS diagnostic assays used. In 2011, the department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, which is the National HIV Reference Laboratory, started monitoring CD4 and CD8 T-lymphocyte count and HIV plasma viral load (VL) as part of an ongoing research among people living with HIV (PLHIV). During this study a few subjects failed to give conclusive results. Among them, three were under regular follow up as long as they remained asymptomatic. In 2011, when their general conditions deteriorated, they were referred to the reference laboratory for CD4 T-lymphocyte count and for VL testing. Prior written consent was obtained from all the patients and the study was approved by the institutional ethics committee. CD4 cell counts performed by flowcytometry (FACSCount, BD Biosciences, USA) were 80, 311 and 181 cells / µl of whole blood for patients 1, 2 and 3, respectively (Table). The VL was estimated in plasma separated from the same samples by real-time PCR (Applied Biosystems 7300, USA) using RoboGene® HIV-1 RNA Quantification kit (Analytik Jena AG, Life Science, Germany). For VL estimation, plasma was separated and preserved at -20° C within 4 h of collection. HIV RNA was isolated using INSTANT Virus RNA kit (Analytik Jena AG, Life Science, Germany). In all three samples the VL was surprisingly found undetectable which raised the suspicion about the diagnosis of HIV infection. To reconfirm the status of patient, the samples were tested again for HIV antibody in plasma by ELISA (Bioelisa HIV 1+2, Spain) and RTKs *i.e.* Uni-gold (Trinity Biotech, Ireland), Determine HIV 1/2 Rapid test kit (Abbot Laboratories, USA), Capillus HIV-1/HIV-2 (Trinity Biotech, Ireland) and SD Bioline HIV 1/2 (Standard Diagnostics Inc, Korea). The results of ELISA for all the samples were positive. Interestingly, a faint band of antibody against HIV-2 was detected by SD Bioline HIV 1/2. For confirmation of HIV infection, Line immunoassay (LIA; Inno-LIA HIV I/II score, innogenetics, Belgium) was performed where bands for antibodies against HIV-1 non envelope peptides p17, p31, p24, HIV-1 specific envelope peptides gp41, and gp120 along with faint bands against sgp36 and sgp105 of HIV-2 specific envelope peptides were observed with different degrees of intensity (Figure). Since their CD4 T cell count was <350 cells/µl, the first line of antiretroviral therapy (ART) was started according to the National ART guidelines[1]. After six months of ART, there was notable improvement in the clinical condition of the patients with increase in their CD4 T-lymphocyte count. On re-testing, no variations on serology and virological test results were observed on the second sample. In addition to these three patients, there were three more samples (patients-4, 5, 6) where bands against HIV-2 along with the bands specific for HIV-1 peptides were present (Figure). In these three cases, the intensity against sgp36 of HIV-2 was more prominent than the three previously described cases (Figure). In patient 4, one band against sgp 105 of HIV-2 was detected with high intensity (+3). Due to lack of samples, VL, CD4 count and other tests could not be performed on these patients.

The standard diagnosis of HIV infection relies on positive antibody test results either by ELISA or three RTK tests followed by confirmation using LIA / WB. In LIA, two of three antigens, *i.e.* HIV core antigen-p24, envelope glycoproteins- gp41 and gp120 in case

Table. Characteristics of three HIV infected patients

Features			Patient 1	Patient 2	Patient 3
Demography	Age (yr)		35	38	22
	Sex		Female	Male	Female
	Mode of infection		Heterosexual	Heterosexual	Heterosexual
	H/O visit to India		Yes	Yes	No*
	ART initiated		Yes	Yes	Yes
Serological screening	Uni-gold		+	+	+
	Determine HIV 1/2		+	+	+
	Capillus HIV-1/HIV-2		+	+	+
	SD Bioline HIV-1		+	+	+
	HIV-2		+	+	+
	ELISA		+	+	+
INNO-LIA	p24		3+	3+	3+
	p17		3+	3+	3+
	p19		3+	3+	3+
	HIV-1	gp120	3+	1+	3+
		gp 41	3+	3+	3+
	HIV-2	sgp36	1+	1+	1+
		sgp105	1+	1+	1+
Real-time PCR	HIV-1		UD	UD	UD
	HIV-2		NT	NT	NT
CD4 T-lymphocyte count (cells/µl)	Before ART		80	311	181
	After ART		448	368	310
Clinical improvements			Yes	Yes	Yes

NT, not tested; UD, undetected; ART, antiretroviral therapy
*History of husband's HIV disease unavailable

of HIV-1 or p24, sgp36 and sgp105 in case of HIV-2 must be present. In all these six samples of PLHIV, in addition to bands against HIV-1 antigens, faint bands against HIV-2 antigens were also observed.

The diagnosis of dual HIV-1/HIV-2 infections is complicated because in most of the cases HIV-1 RNA is more commonly detected than HIV-2 RNA[2-4]. Therefore, if these cases were due to HIV 1 and 2 dual infections, then amplification of HIV-1 RNA was expected. Although the antibodies against different *gag* gene products *i.e.* p17 (MAtrix) and p24 (CApsid) antigens of HIV were highly reactive in LIA in patients 1, 2 and 3, the *gag* gene of HIV-1 which was the amplification target of the real time PCR kit used in the present study was not amplified. If we assume that these faint bands are cross-reactive antibodies against HIV-2 antigen on LIA, then RNA of HIV-1 should have been present. On the other hand, cross-reactivity between anti-HIV-2 antibodies and envelope glycoproteins of HIV-1 is not uncommon. Cross-reactivity with the immunodominant regions of HIV-1 envelope proteins has been previously reported in HIV-2 infected patients from Mali and the Ivory Coast[5]. Another probable reason of undetected plasma VL in these patients may be infection with HIV-1 species other than group M (*i.e.* groups N, O). The real-time PCR assay kit used for measurement of VL in this study detected only HIV-RNA of group M type. Thus, the probability of presence of HIV-1 group N or O or HIV-2 among the studied patients could not be excluded. Among the elite controllers, who are characterized by stable absolute CD4 T-lymphocyte with undetectable plasma VL[6], decreasing trend of CD4 T-lymphocyte is not uncommon[7]. These three studied HIV positive cases showed similar trend[8] but the appearance of sgp36, sgp105 in LIA, which is characteristics of HIV-2, was against this possibility. The CDC guidelines (2012)[9]

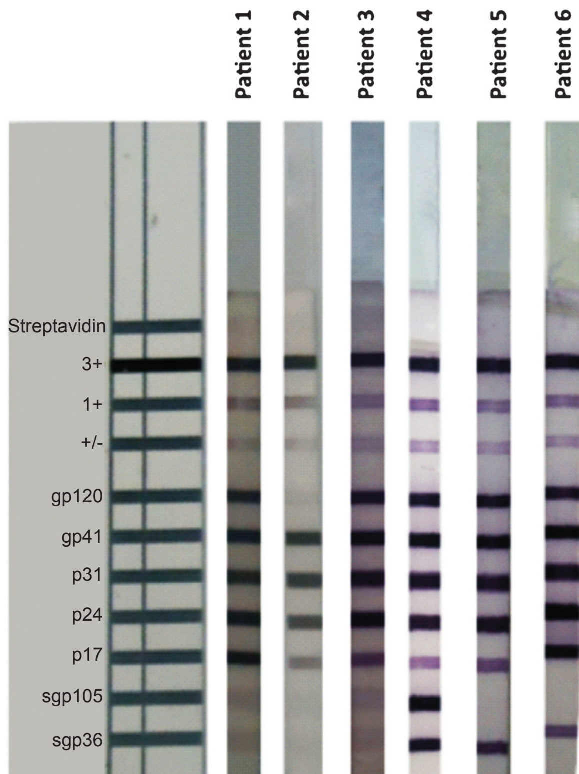

Fig. Band patterns showing presence of antibodies to HIV peptides and proteins (HIV-1 specific envelope peptides gp120, gp41, and non envelope peptides p31, p24, and p17; HIV-2 specific envelope peptides sgp105, and sgp36) in line immunoassay (LIA) among the HIV infected patients. A reading card is placed on the left side of the LIA strips to rate the band reactivity. Low and high reactive bands are present on the LIA strips of patients 1, 2, 3 and patients 4, 5, 6, respectively.

state that the HIV-2 serology should be checked in patients who are residents of Africa or HIV-2 endemic areas, share needles or sexual partner with HIV-2, have received transfusion or children of women with suspicion of HIV-2 infection. None of the patients in this study had visited Africa but two of them had history of immigration to India where HIV-2 has been frequently detected[10,11]. Therefore, there could be possibility of exposure to HIV-2 among those patients.

In conclusion, such findings with mixed HIV-1 and HIV-2 banding pattern and undetectable viral load with declining CD4 cell count responding to ART seen in Bangladesh deserve careful observation and need further confirmation.

Acknowledgment

The study was partly supported by a grant from the Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. Authors acknowledge the Ashar Alo Society (AAS) for support and co-operation throughout the study.

S.M. Rashed U Islam,
Saif U. Munshi* &
Shahina Tabassum
Department of Virology
Bangabandhu Sheikh Mujib
Medical University, Dhaka, Bangladesh
**For correspondence*:
saifmunshi@yahoo.com

References

1. National AIDS/STD Programme, Directorate General of Health Services, Ministry of Health and Family Welfare, Bangledesh. *National guidelines of antiretroviral therapy Bangladesh*. Dhaka, Bangladesh: National AIDS STD Programme; 2011.
2. Peeters M, Fransen K, Gershy-Damet GM, Willems B, Koffi K, Coulibaly M, *et al*. Effect of methodology on detection of HIV-1/HIV-2 dual infections in Cote d'Ivoire. *J Virol Methods* 1994; *48* : 23-30.
3. Sarr AD, Hamel DJ, Thior I, Kokkotou E, Sankale JL, Marlink RG, *et al*. HIV-1 and HIV-2 dual infection: lack of HIV-2 provirus correlates with low CD4+ lymphocyte counts. *AIDS* 1998; *12* : 131-7.
4. Ampofo WK, Koyanagi Y, Brandful J, Ishikawa K, Yamamoto N. Seroreactivity clarification and viral load quantitation in HIV-1 and HIV-2 infections in Ghana. *J Med Dent Sci* 1999; *46* : 53-62.
5. Damond F, Apetrei C, Robertson DL, Souquière S, Leprêtre A, Matheron S, *et al*. Variability of human immunodeficiency virus type 2 (hiv-2) infecting patients living in France. *Virology* 2001; *280* : 19-30.
6. Deeks SG, Walker BD. Human immunodeficiency virus controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. *Immunity* 2007; *27* : 406-16.
7. Andrade A, Bailey JR, Xu J, Philp FH, Quinn TC, Williams TM, *et al*. CD4+ T cell depletion in an untreated HIV type 1-infected human leukocyte antigen-B*5801-positive patient with an undetectable viral load. *Clin Infect Dis* 2008; *46* : e 78-82.
8. Pereyra F, Palmer S, Miura T, Block BL, Wiegand A, Rothchild AC, *et al*. Persistent low-level viremia in HIV-1 elite controllers and relationship to immunologic parameters. *J Infect Dis* 2009; *200* : 984-90.
9. Centers for Disease Control (2012). *Revision of the HIV Surveillance Case Definition*. Available from: *http: // www. cdc.gov/hiv/resources/reports/pdf/HIV_Case_Def_Consult_ Summary.pdf*, accessed on September 12, 2012.
10. Sarkar K, Bal B, Mukherjee R, Niyogi SK, Saha MK, Bhattacharya SK. Epidemiology of HIV infection among brothel-based sex workers in Kolkata, India. *J Health Popul Nutr* 2005; *23* : 231-5.
11. Chiara M, Rony Z, Homa M, Bhanumati V, Ladomirska J, Manzi M, *et al*. Characteristics, immunological response & treatment outcomes of HIV-2 compared with HIV-1 & dual infections (HIV 1/2) in Mumbai. *Indian J Med Res* 2010; *132* : 683-9.