HIV & AIDS Review 15 (2016) 42–46

Contents lists available at ScienceDirect

HIV & AIDS Review

journal homepage: www.elsevier.com/locate/hivar

Mutational analysis of HIV-1 viral protein U at Ser52 and Ser56 among the HIV-1 infected patients of Manipur

Adhikarimayum Lakhikumar Sharma [a], Thiyam Ramsing Singh [a], Khuraijam Ranjana Devi [b], Lisam Shanjukumar Singh [a,*]

[a] Department of Biotechnology, Manipur University, Canchipur 795 003, Manipur, India
[b] Department of Microbiology, Regional Institute of Medical Sciences, Lamphel 795 001, Manipur, India

ARTICLE INFO

Article history:
Received 29 January 2015
Received in revised form 30 June 2015
Accepted 16 July 2015
Available online 21 August 2015

Keywords:
HIV
vpu
ser52
ser56
Manipur
AIDS

ABSTRACT

Background: Phosphorylation by Casein kinase 2 at two conserved seryl residues, Ser52 and Ser56 at the cytoplasmic domain of HIV-1 Viral protein U (Vpu) are essential for inducing CD4 degradation. This study is conducted to investigate the variability of HIV-1 viral protein unique particularly at amino-acid position Ser52 and Ser56 among the HIV infected individuals in the northeastern region of India.
Materials and methods: Blood samples from 90 HIV-1 infected patients of Manipur have been studied by viral amplification and sequencing at *vpu* gene of HIV-1. For analysis of amino acid variation at Ser52 and Ser56, nucleic acid sequences were translated into amino acid and aligned with reference strain of HIV-1. Phylogenetic tree was also inferred among the studied samples.
Results: The results revealed that 93% of HIV-1 infected individuals harbored virus with conserved serine at both amino acid positions 52 and 56 of vpu which were known to have the ability to induce CD4 degradation while 1% harbored viral mutation at both Ser52 and Ser56, replaced by asparagine which has been predicted to may have lost the ability to induce CD4 degradation. Moreover, 5% and 1% of HIV-1 infected individuals were found to be infected with mutated virus at ser52 and ser56 of vpu respectively.
Conclusion: The overall finding of this study reveals that seryl residue of vpu at Ser52 and Ser56 was highly conserved among the HIV-1 infected individuals of the study population.

1. Introduction

According to the UNAIDS HIV report 2012, India is the third highest HIV prevalent country in the world. Among the states of India, Manipur is one of the highest HIV prevalence in the country [1]. HIV outbreaks in Manipur, a northeastern part of Indian and located near the "Golden Triangle" (Myanmar, Laos, and Thailand) have been associated with drug-trafficking routes. Manipur shares a 352-km porous border with Myanmar and is also an important gateway of India to "Golden triangle" of Southeast Asia, the second largest opium producer in the world [2]. Injecting opium (locally call as "number four") is the major drug used among the youths in Manipur and other northeastern states of India [3–6]. HIV transmission in Manipur is through the HIV contaminated intravenous-injection equipment (like syringe, needle) among the intravenous drug users (IDUs). Since the first detection of HIV-1 infection from an IDU of Manipur in 1990 [7], Manipur remains the highest prevalence of HIV-1 in the country. The HIV-1 epidemic in this region was largely due to the use of HIV infected syringes and needles by IDUs but now HIV is transmitted to general public through HIV infected IDUs' spouses and children.

The HIV pathogenesis is different from other primate lentiviruses [8–10]. It has a *vpu* gene which retains exclusive features and differentiates from HIV-2 in terms of pathogenicity and virulent [10]. Vpu (16-kd, 81-amino acid) is a type I integral membrane protein consisting of a short N-terminal domain, a single transmembrane a-helix domain, two cytosolic α-helices separated by a flexible connector loop, and a short C-terminal tail [11,12]. Vpu contains two highly conserved seryl residues (Ser52 and Ser56), located in the cytoplasmic domain, which are phosphorylated by the ubiquitous protein kinase CK-2. This viral protein U has two different biological functions; (a) cytoplasmic domain (CTD) in degradation of CD4 in the endoplasmic reticulum

Abbreviations: IDUs, intravenous drug users; vpu, viral protein U; ORF, open reading frame; FSW, female sex workers.

* Corresponding author at: Department of Biotechnology, Manipur University, Canchipur 795 003, Imphal, Manipur, India. Tel.: +91 385 2435233; fax: +91 385 2435145.
E-mail addresses: lakhee.sharma@gmail.com (A. Lakhikumar Sharma), ramsing1thiyam@gmail.com (T. Ramsing Singh), ranjanakhu@rediffmail.com (K. Ranjana Devi), shanju.lisam@gmail.com (L. Shanjukumar Singh).

http://dx.doi.org/10.1016/j.hivar.2015.07.002
1730-1270/

and (b) transmembrane domain (TMD) in enhancement of virion release from the plasma membrane of HIV-1-infected cells [13]. Moreover, *vpu* locus is considered as one of the most variable regions in HIV-1 genome. According to reports, mutational analysis of Vpu demonstrated that phosphorylation of Vpu at two conserved seryl residues, Ser52 and Ser56 in its cytoplasmic domain is essential for its ability to induce CD4 degradation of host cells.

In this study, the variability of HIV-1 *vpu* has been investigated particularly at amino-acid position Ser52 and Ser56 among the HIV infected individuals in the Manipur, northeastern region of India.

2. Materials and methods

2.1. Ethics statement

The Institutional Human Ethical Committee (IHEC) of Regional Institute of Medical Science (RIMS) and Manipur University (MU) approved the study and written consent was obtained from all study participants.

2.2. Study participants and samples collection

After obtaining the ethical clearances, HIV-1 infected patients of Manipur, a part of northeastern India were recruited for our studies. Participants were counseled individually and allowed to sign voluntary the informed consent form before the sample collection. HIV positive blood samples were collected from RIMS during the year 2011–2014. Two milliliters (2 ml) of whole blood was drawn from each participant's veins and collected in an EDTA Vacutainer (Becton Dickinson, USA). The clinical parameters of infected individuals (age, sex and CD4 counts) were also recorded.

2.3. DNA extraction

Fresh blood samples were processed to isolate Peripheral Blood Mononuclear Cells (PBMCs). PBMCs were isolated from the blood samples using the Ficol lhypaque (GE healthcare, USA) density gradient centrifugation method as per manufacturer's instructions. Genomic DNA was extracted from the PBMCs through QIAamp DNA blood mini kit (Qiagen GmbH, Germany), according to the manufacturers' protocol. Lysis buffer of 200 μl was added to equal volume of PBMC suspended in phosphate buffer saline (PBS). Cells were allowed to lyse completely with incubation at 56 °C for 10 min. The lysed sample was transferred to the silica-based column, centrifuge at 8000× RPM for 60 s. After washing the bound DNA twice, DNA was eluded with ultrapure water. Isolated genomic DNA was analyzed on an agarose gel (0.8%) to check the integrity prior to viral gene amplification.

2.4. Gene amplification

The complete open reading frames (ORFs) corresponding to the *vpu* locus of HIV-1 genome, (HXB2 position 5861-6352, Genbank Acc No: K03455) was amplified from genomic DNA of HIV infected patients' samples by nested polymerase chain reaction (PCR) technique using high fidelity Taq DNA polymerase (Invitrogen, USA). The primers used in the study are listed in Table 1.

In a total volume of 50 μl reaction volume, about 1.0–5.0 μg of genomic DNA was used for PCR amplification in the presence of 1× PCR Buffer, 250 μM dNTPs, 200 nM of each primer, 2 mM of $MgCl_2$, and 6U of Taq polymerase [14]. The following PCR conditions were used; one cycle of denaturation at 94 °C for 5 min and then add 6U of PCR enzyme as a manual hot-start, followed by 10 cycles of 94 °C for 30 s, 60 °C for 30 s, and 68 °C for 45 s, then 20 cycles of 94 °C for 30 s, 55 °C for 30 s and 68 °C for 45 s and one cycle of final extension at 68 °C for 10 min. PCR conditions for pre-nested and nested PCR were the same except the cycling extension time of 4 min in pre-nested PCR.

2.5. DNA purification and sequencing

The amplicons were analyzed by electrophoresis on a 2.0% agarose gel, stained with ethidium bromide, and evaluated under UV light. Desired amplicons products were excised and purified by using the QIAquick PCR purification kit (Qiagen, Valencia, CA) according to the manufacturer's protocols. Later the purified PCR product was sequenced with an ABI PRISM 3730XL DNA Analyzer using BigDye terminators (Applied Biosystems, Foster City, California, USA).

2.6. Sequence comparison

The sequence chromatograms were viewed by Chromas 2.4.1 (http://www.technelysium.com.au), manually edited and trimmed so as to contain only the complete *vpu* of HIV-1. All the nucleotide sequences obtained were also screened using the basic BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to search for sequence similarities for previously reported sequences in the databases. Nucleotide sequences of vpu regions were compared with the respective sequences of HIV-1 strains isolated from different geographic regions available in the HIV sequence database (http://www.hiv.lanl.gov/content/index). To study the mutational variations exhibited by *vpu* at Ser52 and Ser56 among HIV infected patients of Manipur, the sequences were translated and aligned with translated reference sequence using clustalW in BioEdit software (BioEdit software version 7.1.11). The reference sequences of HIV-1 were obtained from HIV website (http://www.hiv.lanl.gov.com). Moreover, Phylogenetic tree of complete vpu was also inferred among the studied samples and reference sequences of HIV-1 using Maximum Likelihood on the Kimura 2-parameter distance matrix in the MEGA software (Version 6.0).

3. Results

3.1. Clinical and demographic characteristics of study subject

The study samples were intravenous drug users (IDUs) and heterosexually transmitted HIV-1 infected individuals of Manipur. The male and female ratio of the studied samples was 1.5:1. The male participants in the study were infected with HIV through the HIV contaminated intravenous drug injection tools or multiple sex partners, while female participants were the wives of HIV infected

Table 1
List of primers used to amplify complete vpu.

PCR mode	Primer	Position	Sequence (5′–3′)
Pre-nested PCR	MUHIVVF1 (+)	5550–5574	AGARGAYAGATGGAACAAGCCCCAG
	MUHIVVR1 (−)	9157–9181	GTGTGTAGTTCTGCCAATCAGGGAA
Nested PCR	MUHIVVF2 (+)	5861–5884	TGGAAGCATCCRGGAAGTCAGCCT
	MUHIVVR2 (−)	6324–6352	GGTACCCCATAATAGACTGTRACCCACAA