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PREVALENCE AND PATTERNS OF TRANSMITTED DRUG RESISTANCE IN HIV-INFECTED ADULT PATIENTS INITIATING ANTIRETROVIRAL THERAPY IN HANOI, VIETNAM

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SUMMARY

As antiretroviral therapy (ART) coverage for HIV-infected patients in Vietnam continues to increase, data on the prevalence and patterns of transmitted drug resistance (TDR) mutations are important to guide national ART strategies. TDR was evaluated in 345 antiretroviral-naïve patients consecutively initiating first-line ART in the clinical trial of Virological Monitoring in Vietnam (VMVN) at Bach Mai Hospital in Hanoi between April 2011 and October 2013. TDR mutations were identified by Sanger sequencing of HIV pol gene and were defined based on the 2009 World Health Organization surveillance drug resistance mutation (SDRM) list. 330 plasma samples were successfully sequenced in both protease and reverse transcriptase regions of HIV pol gene. 323 samples were subtype CRF01_AE; two were subtype A/CRF01_AE, two were subtype BC, one was subtype C; one was subtype F/C recombinant. SDRMs were identified in 16 (4.8%) patients. Among them 6 (38%) patients carried mutations conferring resistance to nucleoside/tide reverse transcriptase inhibitors (NRTIs) (K70E, V75M, K219N/E, T215S), 5 (31%) to non-nucleoside reverse transcriptase inhibitors (NNRTIs) (K101E, K103N, Y181C, G190A), 4 (25%) to protease inhibitors (PIs) (M46I/L, I54L, L90M), and one to both NRTIs and NNRTIs (L74I, V75M, M184V, T215F, K101E, G190A). The level of TDR remains low despite the rapid scale up of ART in Vietnam over the past 10 years. TDR to PIs was identified in 4 patients for the first time in Ha Noi. As PIs are the main component of 2nd-line therapy and the last resort for patients with drug-resistant virus in Vietnam. The detection of TDR to PIs is of concern and requires further investigation.

Keywords*: Human Immunodeficiency Virus, Acquired Immunodeficiency Syndrome, Antiretroviral therapy, Transmitted drug resistance, Vietnam*

INTRODUCTION

Human Immunodeficiency Virus (HIV) has infected thirty five million people worldwide. Each year approximately 3 million are infected, and nearly 2 million die of Acquired Immunodeficiency Syndrome (AIDS) (UNAIDS 2013). Antiretroviral drugs are available and are effective in suppressing HIV from replication in an infected person and in reducing the probabilities of HIV to be transmitted from one person to another. HIV is a retrovirus that replicates without a proof reading and repair mechanism and is thus error prone. Another feature is its high replication capacity, producing a billion of new virions a day within an infected individual (Perelson *et al.,* 1996). The above characteristics allow HIV to evolve rapidly, capable of becoming resistant to all currently available antiretroviral drugs ever produced on the market. As a

cure for HIV is still a far reach, life-long ART remains the main tool against HIV, and the development of HIV drug resistance (HIVDR) would threaten the sustainability of ART worldwide.

Drug resistant virus can develop *de novo* or be transmitted from persons to persons within a population; this is called primary or transmitted drug resistance (TDR). Drug resistant virus can develop in an individual taking ART; this is called secondary or acquired resistance. Harbouring drug resistant virus (whether by primary or secondary resistance pathway) can lead to treatment failure, disease progression and death (Hogg *et al.,* 2006, Kozal *et al.,* 2007, Poggensee *et al.,* 2007). Antiretroviral drugs have been available for much longer in the United States and Western Europe; as such the prevalence of TDR are higher (up to 24% in some cities) (Sagir *et al.,* 2007, Vercauteren *et al.,* 2008, Jain *et al.,* 2010). In these countries, drug resistance testing prior to initiation of ART is therefore routinely performed and has been shown to be cost effective (Sax *et al.,* 2005). The cost of HIV genotype testing is still prohibitively expensive for routine clinical care in the developing countries. Patients in these settings do not receive HIV drug resistance testing prior to ART initiation. TDR that goes undetected in settings where ART is being scaled up can have a major impact on the global control of HIV. Increased surveillance of TDR in resource-limited settings is therefore essential and is recommended by the World Health Organization (WHO) (Bennett *et al.,* 2008).

The first case of HIV infection was identified in Vietnam in 1990. HIV has since spread to all 64 provinces of the country. New cases more than doubled in numbers from 112,000 in 2000 to 256,000 in 2014 (Viet Nam MOH 2014). The epidemic is still considered to be in its concentrated phase, meaning HIV is concentrated in intravenous drug users (IDU), female sex workers (FSW) and men who have sex with men (MSM) (UNGASS 2010). However the increasing numbers of women infected with HIV means that the epidemic is beginning to spread to the general population (National Committee for AIDS, Drugs, and Prostitution Prevention and Control, 2012). HIV-1 CRF01_AE subtype remains the predominant subtype seen in Vietnam over the years with >95% prevalence (Hemelaar *et al.,* 2006, Nouhin *et al.,* 2011, Thao *et al.,* 2012). ART was first introduced in Vietnam by private donations and through the black markets in the beginning of 2000s. During this time treatment interruption was common due to unstable drug supply and lack of national treatment guidelines. The Vietnam national ART program began in 2005 with international donations, primarily the US President's Emergency Plan for AIDS Relief (PEFFAR) and the Global Fund to Fight AIDS, Tuberculosis and Malaria. As of September 2014, 88,800 adults and children were on ART (MOH 2014) and ART coverage for patients who meet treatment criteria increased from 1% to 53% in adults and to 83% in children from 2003 to 2011 (UNGASS 2012). Both the history of treatment interruptions and the rapid rise in ART coverage are expected to be accompanied by the emergence of acquired and TDR in Vietnam.

Data on TDR in Vietnam is limited and have shown prevalence ranging from <5% in low-risk populations (e.g. women attending antenatal care centers and individuals diagnosed from volunteer counselling and testing centers (VCT) (Nguyen *et al.,* 2008, Ayouba *et al.,* 2009) to levels of 5-10% in a more general population of patients from the HIV outpatient clinics in Vietnam (Lan *et al.,* 2003, Phan *et al.,* 2010, Dean *et al.,* 2011). These data reflect the TDR situation in Vietnam from 2003 to 2009. More recent data is needed and in this study, we investigate the prevalence and patterns of TDR in patients initiating ART at Bach Mai Hospital in Ha Noi, Vietnam from 2011 to 2013.

MATERIALS & METHODS

Study population

Consecutive baseline plasma samples from patients participating in the Virological Monitoring in Vietnam (VMVN) clinical trial were analysed in this study. VMVN is a randomized trial comparing the impact of routine viral load monitoring versus standard clinical and immunological monitoring on clinical outcome of patients initiating first-line ART in Hanoi, Vietnam over three years of follow up. This trial was registered at Clinicaltrial.gov, number: NCT01317498. HIV-infected patients aged 18 years or older who were not currently taking ART, met Vietnam Ministry of Health (MOH) criteria to initiate ART (CD4 $\leq$350 cells/mm^3, and/or WHO Clinical Stage III or IV), were eligible to enter the trial (Viet Nam MOH 2011, WHO 2010). Patients who had been on ART in the past but stopped for whatever the reason for more than 3 months were

still considered eligible to enter the trial as long as they did not have evidence of treatment failure to first-line ART in the past. For this drug resistance sub-study, only patients who were ART-naive were included in the analysis of TDR. The VMVN clinical trial and this sub-study were approved by the Scientific and Ethical Committees of Bach Mai Hospital and the Beth Israel Deaconess Medical Center.

Specimen collection

5 ml of blood was collected at study entry. Plasma were obtained, stored at -80°C, and these samples were subsequently transported on dry ice to the Oxford University Clinical Research Unit (OUCRU) in Ho Chi Minh City for drug resistance analysis.

Extraction and amplification of plasma HIV-1 viral RNA

HIV-1 RNA was extracted from 200 µl of plasma using a either MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche, Vedbaek, Denmark) or QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany). HIV-1 *pol* gene, which encodes reverse transcriptase and protease, was amplified by reverse transcription PCR and nested PCR according to an in-house assay described initially by Bezemer with some modifications by Thao VP (Thao, Le *et al.,* 2012). MMLV-RT enzyme was substituted by SuperScriptIII RT (Invitrogen, Carlsbad, CA) during reverse transcription; HiFidelity Tag (Qiagen, Center Mainz, Germany) was used for PCR amplification; and 30 instead of 25 cycles were used for second-round PCR. Primers specific for subtype AE were modified from subtype B primer set based on subtype AE consensus sequences.

Reverse transcription PCR

Reverse transcription was performed using primer 3'RT-outAE (TCCACTTGTCCATGCATA GCTTC). 10 µl of RNA was reverse-transcribed in a total volume of 20 µl with 2 mM of dNTP, 0.1 mM of primer, 1x First Strand Buffer, 5 mM of DTT, 16U of RNAse-out (Invitrogen, Carlsbad, CA), and 60U of SuperScriptIII RT. The reaction was incubated at 37^{o}C for 2 hours followed by 95^{o}C for 5 minutes to inactivate the enzyme.

Nested PCR

The *pol* gene was amplified with nested PCR. The entire 20 µl of cDNA was added to the first PCR reaction. The primers used for the first PCR reaction were 5'Prot-IAE (AGGCTAATTTTTA GGGAAAATTTGGCCTTCC) and 3'ET-21AE (AGCTGGCTACTATTTCCTTTGCTACTAYAGG TG). The reaction volume was 50ul, containing 1xPCR buffer, 1 µM of each primer, and 3.33U of HifidelityTaq. The first PCR involved in initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 94°C for 15s, annealing at 55°C for 60s, and polymerisation at 72°C for 4 minutes; and final elongation at 72°C for 10 minutes.

A nested PCR contained 5 µl from first amplicon, 1xPCR buffer, 1 µM of each primer, 0.5 mM $MgSO_4$, and 1.25U of HifidelityTaq. Primers for second PCR were 5'Prot-II (TCAGAGCAGACC AGAGCCAACAG) and 3'RT-20AE (CTGCCAGTT CTAATTCTGCTTC). The cycling conditions were 95^{o}C for 5 minutes, followed by 30 cycles of 94^{o}C for 15 s, 55^{o}C for 1 minute, and 72^{o}C for 2 minutes, and then followed with a final extension at 72^{o}C for 10 minutes.

Five microliters of the PCR products containing 1194 base-pairs of the complete 297 nucleotides protease (PR) region and the first 897 nucleotides in the reverse transcriptase (RT) region of *pol* was checked by agarose gel electrophoresis.

Genotyping and drug resistance determination

Forty-five microliters of amplification products were purified using QIA quick PCR purification kits (Qiagen, Hilden, Germany) and were subjected to direct sequencing with 6 primers 5' PR5 (AGCCAACAGCCCCACCAG), 3' PR2 (CTTTTGGGCCATCCATTC), 5' RT-19new (CACCTGTCAACATAATTGGAAG), 3' B-RTrev (GGTGATCCTTTCCATCCC), 5' B-RT (GGGATGGAAAGGATCACC), 3' RT-20AE (CTGCCAGTT CTAATTCTGCTTC) using the ABI 3130xl Genetic Analyser (Applied Biosystems, Foster City, CA). The gene sequences were analysed using SeqScape (Applied Biosystems, Foster City, CA). The consensus sequence pNL4-3 for HIV-1 subtype B (GenBank accession number M19921) was used as the reference sequence for assessing nucleotide changes in the sample sequences. HIV drug resistance mutations in ART naïve patients were determined according to the 2009 WHO drug resistance surveillance mutations (SDRMs) list (Bennett et al., 2009). HIV subtyping using RT and

PR genes were done by the Stanford database system (http://sierra2.stanford.edu/sierra/servlet/JSierra).

RESULTS

Study population

Between April 2011 and October 2013, 370 patients met the entry criteria and were enrolled in the VMVN trial. Plasma samples from 345 patients who were ART-naïve were analysed for this drug resistance sub-study. Among them 330 samples were successfully sequenced in both PR and RT regions. The characteristics of the 330 patients at study enrolment are listed in table 1.

Table 1. The characteristics of 330 antiretroviral-naïve HIV-infected individuals starting antiretroviral therapy in Ha Noi, Vietnam.

Characteristics	Study population (n=330)
Male gender, n (%)	217 (66%)
Age, median year (IQR)	37 (32-42)
CD4 cell counts, median cell/mm3 (IQR)	114 (32-258)
Viral load, median log10copies/ml (IQR)	5.15 (4.67-5.55)
Transmission routes	
Heterosexual route	260 (79%)
Injection drug use	59 (18%)
Others	11 (3%)

HIV-1 phylogenetic analysis

Phylogenetic analysis of 330 HIV-1 *pol* sequences revealed that 323 (98%) sequences were subtype CRF01_AE, two (0.6%) were subtype A and CRF01_AE recombinant, two (0.6%) were subtype B and C recombinant, one (0.3%) was subtype B and CRF01_AE recombinant, one (0.3%) was subtype F and C recombinant, and one (0.3 %) was subtype C.

Prevalence and patterns of SDRMs

According to the WHO SDRM list, mutations that confer resistance to nucleoside/tide reverse transcriptase inhibitors (NRTIs) were detected in 6/330 (1.7%) patients and to non-nucleotide reverse transcriptase inhibitors (NNRTIs) in 5/330 (1.5%) patients. One patient (0.3%) had a viral strain that carried resistance mutations to both NRTIs and NNRTIs. Resistance to protease inhibitors (PIs) was detected in 4/330 (1.2%) patients. We did not identify any strain that harboured both PI resistance and NRTI or NNRTI resistance mutations. Hence, the overall prevalence of TDR in this cohort in Ha Noi is 16/330 or 4.8%.

The patterns of SDRM are shown in table 2. Patients 12, 13 carried the NRTI mutation V75M which confers resistance to stavudine (d4T). Patient 8 carried the NRTI mutation K219E which confers resistance to zidovudine (AZT). AZT and d4T were the most common NRTI drugs used in Vietnam from 2003 to 2013. Patient 9 carried the NRTI mutation K70E which confers resistance to tenofovir (TDF), abacavir (ABC) and didanosine (ddI); all these drugs had been used either in the first-or second-line ART regimens, except for ddI which came off the national guidelines in 2011. Patient 4 carried the NRTI mutation K219N, an accessory mutation usually occurring in combination with other thymidine analog mutations (TAMs) that are selected by d4T and AZT. Patient 11 carried the revertant NRTI mutation T215S, which does not confer resistance to NRTIs but its presence suggests that the patient had harbored the major mutation T215Y/F in the past. Six patients carried at least one major NNRTI mutation (Y181C, G190A, K101E); each confers high-level resistance to nevirapine (NVP) and/or efavirenz (EFV). These have been the most common NNRTIs used in Vietnam and in other developing countries. Patient 7 had multi-drug class resistance mutations (NRTI mutations: L74I, V75M, M184V, T215F and NNRTI mutations: K101E, G190A). This patient carried a viral strain that confers resistance to all

drugs used in the standard first-line ART regimens in Vietnam. Four major PI resistance mutations were detected in four different patients. Each of these mutations, M46I/L, L90M and I54L, reduces susceptibility to PIs, including lopinavir/ritonavir (LPV/r) which is the standard PI used in the second-line ART regimens in Vietnam.

DISCUSSION

In this study we report a TDR prevalence of 4.8% in a cohort of 330 ART-naïve HIV-infected adults initiating ART in Ha Noi from 2011 to 2013. The detected NRTI and NNRTI mutations confer resistance to the standard first-line ART regimens used in Vietnam. Major PI mutations were detected in four patients (1.2%). These are associated with high-level resistance to many PIs, including LPV/r, the key drug in the second-line ART regimens in Vietnam.

TDR prevalence of 4.8% found in this study is consistent with studies published previously from Vietnam (Dean, Ta Thi *et al.,* 2011, Bontell *et al.,* 2012, Duc *et al.,* 2012). When comparing TDR prevalence among studies, it is important to take into account differences in the study populations, study time periods, geographic locations, study sample sizes, clinical and laboratory methods, and finally drug resistance algorithms used. Published studies in Vietnam using the WHO's recommended threshold survey method, which sampled TDR in low-HIV-risk exposure populations such as women who attend antenatal clinics and people who come to VCTs for HIV testing, reported TDR prevalence of <5% (Nguyen, Duc *et al.,* 2008, Ayouba, Lien *et al.,* 2009). While studies that sampled TDR from patient populations attending HIV clinics reported higher TDR prevalence of 5-10% (Lan, Recordon-Pinson *et al.,* 2003, Phan, Ishizaki *et al.,* 2010, Dean, Ta Thi *et al.,* 2011, Thao, Le *et al.,* 2012). The Vietnam HIV epidemic is still concentrated in the high-HIV-exposure populations; therefore the WHO algorithm which samples from antenatal clinics and VCTs may under-estimate levels of TDR in Vietnam. However studying TDR in chronically-infected patients in HIV clinics are not without disadvantages. First, we rely on patients' self-reported history of ART use. This is not optimal, as some patients might decide to withhold their history of previous ART use for perceived fear that they might not qualify for ART. This can result in overestimation of TDR. On the other hand, TDR might be underestimated in chronically-infected patient populations using standard population sequencing. In the absence of antiretroviral selection pressure TDR strains will overtime revert to minority resistant variants or wild-type virus, and may be missed by the standard population sequencing method which only reliably detects resistant variants present in more than 20% of the viral quasispecies. Our study is not immune to these methodological limitations. The detected TDR prevalence of 4.8% suggests that TDR levels remains relatively stable despite the rapid scale up of ART in Vietnam over the past 10 years.

The observed RT mutations in our study conferred resistance to antiretroviral drugs in the standard first-line ART regimens in Vietnam. One patient has a L74I mutation which confers resistance to abacavir (ABC) and didanosine (ddI). L74I has a moderate fitness cost to the virus, meaning a virus that carries such a mutation has lower fitness compared to a wild type virus in replication. In the absence of drugs that select for such a mutation, the resistant virus will be outcompeted by wild type virus overtime (Martinez-Picado and Martinez 2008). The fitness costs of carrying the L74I and the M184V mutations (M184V is a mutation with high fitness cost in itself) are additive (Martinez-Picado and Martinez 2008). The fact that the L74I was found in patient 7 (Table 2) together with the M184V and multiple other NRTI and NNRTI mutations suggests that: i) the patient may have been recently infected by a person who was highly ART-experienced and had been on ddI or ABC in the past, or ii) the patient was not honest about his previous ART exposure. The latter means that the patient may have recently been on ART and has acquired resistance rather than being infected with a multi-drug resistant strain. A throughout review of the patient treatment history might assist in the differentiation of the two possibilities.

Patient 11 has a revertant T215S mutation, which does not reduce NRTI susceptibility but arises from viruses that once contained the mutations T215Y and F (Garcia-Lerma *et al.,* 2001). The mutations T215Y/F cause intermediate to high level resistance to AZT and d4T. These mutations have a fitness cost to the virus (Cong *et al.,* 2007). Thus when AZT/d4T drug pressure is removed, T215F/Y-containing viral strains will reverse-mutate to wild type viruses which confer better survival in the

absence of treatment. The viral strains containing the T215F/Y mutations would have to back mutate in two different nucleotides to return to the wild-type viruses, the process which takes time. This likely explains why T215 revertant mutations (which require only one nucleotide change) are among the most commonly reported transmitted drug resistance mutations (Garcia-Lerma, Nidtha *et al.,* 2001, Wensing *et al.,* 2005, Wheeler *et al.,* 2010). Comparing to the T215F/Y and other TAM mutations, T215 revertant mutations have been shown to persist much longer, consistent with the fitness advantages associated with this resistance pathway (Yerly *et al.,* 1998).

Table 2. The surveillance drug resistance mutations detected in 16 antiretroviral-naïve HIV-1 infected individuals in Ha Noi from 2011-2013.

Subject	Transmission route	CD4 at baseline (cells/μL)	Viral Load (copies/mL)	Drug resistance associated mutations		
				Protease inhibitors (PIs)	Nucleoside/tide reverse transcriptase inhibitors (NRTIs)	Non-nucleotide reverse transcriptase inhibitors (NNRTIs)
1	Heterosexual	215	354,000	L90M		
2	Heterosexual	24	54,200			Y181C, G190A
3	Heterosexual	306	18,400	M46L		
4	Injection drug use	82	99,300		K219N	
5	Heterosexual	22	54,100	I54L		
6	Heterosexual	480	13,200			K101E
7	Other	7	1,540,000		L74I, V75M, M184V, T215F	K101E, G190A
8	Injection drug use	11	615,000		K219E	
9	Heterosexual	144	272,000		K70E	
10	Heterosexual	120	154,000	M46I		
11	Injection drug use	13	12,900		T215S	
12	Heterosexual	20	130,000		V75M	
13	Heterosexual	33	898,000		V75M	
14	Heterosexual	35	189,000			Y181C
15	Heterosexual	303	22,500			K101E
16	Heterosexual	173	42,600			K103N

TDR mutations that confer resistance to PIs have been identified in the past in Northern Vietnam (Phan, Ishizaki *et al.,* 2010) and in southern Vietnam (Lan, Recordon-Pinson *et al.,* 2003) but are quite rare. In this study, PI mutations were found in 4 of 16 patients with TDR mutations. Each of the identified PI mutations M46L, M46I, I54L, and L90M was detected as a single mutation in a different individual. The M46L/I mutations confer low-level resistance to nelfinavir. The I54L mutation confers high-level resistance to fosamprenavir and low-level resistance to indinavir, atazanavir, and lopinavir. The L90M mutation confers high-level resistance to nelfinavir, intermediate level resistance to saquinavir, and low level resistance to most PI drugs except the second-generation PI drugs darunavir and tipranavir. The PI mutation pattern is consistent with the history of PI use in Vietnam. Nelfinavir and indinavir were available in public private settings for patients who developed treatment intolerance to nevirapine before efavirenz and lopinavir became available in Vietnam in 2006. It has been shown that these mutations have low fitness costs and therefore can persist in the viral

populations in the absence of PI therapy (Martinez-Picado *et al.,* 1999). The detection of these as TDR mutations is concerning as LPV/r is the last treatment option for patients who fail first-line therapy with drug resistance HIV in Vietnam. These patients are participants in the VMVN clinical trial. The trial will follow these patients for 3 years and will provide the opportunity to study both the impact and the evolution of these primary resistance mutations during long-term ART.

As mentioned previously an inherent limitation of this study is that TDR might be underestimated in our study population of chronically-HIV-infected patients. The standard Sanger sequencing method used to detect drug resistance may miss minority drug resistance population present in <15-20% of the viral population as resistant viral population revert to wild type in the absence of antiretroviral drug pressure overtime. Newer high throughput sequencing methods that allow detection of resistant viral populations down to 1% will allow more accurate detection of TDR in chronically infected patients.

CONCLUSION

In summary, we report a TDR prevalence of 4.8% in 330 antiretroviral- naive individuals initiating antiretroviral therapy in Ha Noi, Vietnam from 2011 to 2013 using the 2009 WHO SDRM algorithm. The identified drug resistance mutations confer resistance to the NRTIs and/or NNRTIs used in the first-line ART regimens but also to PIs, the main component of second-line ART in Vietnam. TDR level in this study is consistent with TDR prevalence reported in previous studies, suggesting that TDR remains relatively stable despite the rapid ART scale up in Vietnam over the last 10 years.

As ART continues to be scaled up in Vietnam, it is likely that the prevalence of TDR will increase, and surveillance of TDR continues to be important to inform treatment strategies. Prevention of transmission of drug resistance HIV during ART scale up is a national priority. This highlights the needs to continue to strengthen the HIV care delivery system, the antiretroviral adherence support, and ensuring a continuous supply of antiretroviral drugs in Vietnam.

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REFERENCES

UK Drug Resistance database (2003) HIV drug resistance in the United Kingdom. *Commun Dis Rep CDR Wkly 2003* 13(10): 5.

Ayouba A, Lien TT, Nouhin J, Vergne L, Aghokeng AF, Ngo-Giang-Huong N, Diop H, Kane CT, Valea D, Rouet F, Joulia-Ekaza D, Toni TD, Nerrienet E, Ngole EM, Delaporte E, Costagliola D, Peeters M, Chaix ML (2009) Low prevalence of HIV type 1 drug resistance mutations in untreated, recently infected patients from Burkina Faso, Cote d'Ivoire, Senegal, Thailand, and Vietnam: the ANRS 12134 study. *AIDS Res Hum Retroviruses* 25(11): 1193-1196.

Bennett DE, Camacho RJ, Otelea D, Kuritzkes DR, Fleury H, Kiuchi M, Heneine W, Kantor R, Jordan MR, Schapiro JM, Vandamme AM, Sandstrom P, Boucher CA, van de Vijver D, Rhee SY, Liu TF, Pillay D, Shafer RW (2009) Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 update. *PLoS One* 4(3): e4724.

Bennett DE, Myatt M, Bertagnolio S, Sutherland D, Gilks CF (2008) Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. *Antivir Ther* 13 Suppl 2: 25-36.

Bontell I, Cuong do D, Agneskog E, Diwan V, Larsson M, Sonnerborg A (2012) Transmitted drug resistance and phylogenetic analysis of HIV CRF01_AE in Northern Vietnam. *Infect Genet Evol* 12(2): 448-452.

Cong ME, Heneine W, Garcia-Lerma JG (2007) The fitness cost of mutations associated with human immunodeficiency virus type 1 drug resistance is modulated by mutational interactions. *J Virol* 81(6): 3037-3041.

Dean J, Ta Thi TH, Dunford L, Carr MJ, Nguyen LT, Coughlan S, Connell J, Nguyen HT, Hall WW, Nguyen Thi LA (2011) Prevalence of HIV type 1 antiretroviral drug resistance mutations in Vietnam: a multicenter study. *AIDS Res Hum Retroviruses* 27(7): 797-801.

Duc NB, Hien BT, Wagar N, Tram TH, Giang le T, Yang C, Wolfe MI, Hien NT, Tuan NA (2012) Surveillance of transmitted HIV drug resistance using matched plasma and dried blood spot specimens from voluntary counseling and testing sites in Ho Chi Minh City, Vietnam, 2007-2008. *Clin Infect Dis* 54 Suppl 4: S343-347.

Garcia-Lerma JG, Nidtha S, Blumoff K, Weinstock H, Heneine W (2001) Increased ability for selection of zidovudine resistance in a distinct class of wild-type HIV-1 from drug-naive persons. *Proc Natl Acad Sci USA*

98(24): 13907-13912.

Hemelaar J, Gouws E, Ghys PD, Osmanov S (2006) Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. *AIDS* 20(16): W13-23.

Hogg RS, Bangsberg DR, Lima VD, Alexander C, Bonner S, Yip B, Wood E, Dong WW, Montaner JS, Harrigan PR (2006) Emergence of drug resistance is associated with an increased risk of death among patients first starting HAART. *PLoS Med* 3(9): e356.

Jain V, Liegler T, Vittinghoff E, Hartogensis W, Bacchetti P, Poole L, Loeb L, Pilcher CD, Grant RM, Deeks SG, Hecht FM (2010) Transmitted drug resistance in persons with acute/early HIV-1 in San Francisco, 2002-2009. *PLoS One* 5(12): e15510.

Kozal MJ, Hullsiek KH, Macarthur RD, Berg-Wolf M, Peng G, Xiang Y, Baxter JD, Uy J, Telzak EE, Novak RM, Terry Beirn Community Programs for Clinical Research on A (2007) The Incidence of HIV drug resistance and its impact on progression of HIV disease among antiretroviral-naive participants started on three different antiretroviral therapy strategies. *HIV Clin Trials* 8(6): 357-370.

Lan NT, Recordon-Pinson P, Hung PV, Uyen NT, Lien TT, Tien HT, Garrigue I, Schrive MH, Pellegrin I, Lafon ME, Aboulker JP, Barre-Sinousi F, Fleury HJ (2003) HIV type 1 isolates from 200 untreated individuals in Ho Chi Minh City (Vietnam): ANRS 1257 Study. Large predominance of CRF01_AE and presence of major resistance mutations to antiretroviral drugs. *AIDS Res Hum Retroviruses* 19(10): 925-928.

Martinez-Picado J, Martinez MA (2008) HIV-1 reverse transcriptase inhibitor resistance mutations and fitness: a view from the clinic and ex vivo. *Virus Res* 134(1-2): 104-123.

Martinez-Picado J, Savara AV, Sutton L, D'Aquila RT (1999) Replicative fitness of protease inhibitor-resistant mutants of human immunode ficiency virus type 1. *J Virol* 73(5): 3744-3752.

Nguyen HT, Duc NB, Shrivastava R, Tran TH, Nguyen TA, Thang PH, McNicholl JM, Leelawiwat W, Chonwattana W, Sidibe K, Fujita M, Luu CM, Kakkar R, Bennett DE, Kaplan J, Cosimi L, Wolfe MI (2008) HIV drug resistance threshold survey using specimens from voluntary counselling and testing sites in Hanoi, Vietnam. *Antivir Ther* 13 Suppl 2: 115-121.

Nouhin J, Donchai T, Hoang KT, Ken S, Kamkorn J, Tran T, Ayouba A, Peeters M, Chaix ML, Lien TX, Nerrienet E, Ngo-Giang-Huong N (2011) Natural polymorphisms of HIV-1 CRF01_AE integrase coding region in ARV-naive individuals in Cambodia, Thailand and Vietnam: an ANRS AC12 working group study. *Infect Genet Evol* 11(1): 38-43.

Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD (1996) HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science* 271(5255): 1582-1586.

Phan TT, Ishizaki A, Phung DC, Bi X, Oka S, Ichimura H (2010) Characterization of HIV type 1 genotypes and drug resistance mutations among drug-naive HIV type 1-infected patients in Northern Vietnam. *AIDS Res Hum Retroviruses* 26(2): 233-235.

Poggensee G, Kucherer C, Werning J, Somogyi S, Bieniek B, Dupke S, Jessen H, Hamouda O, Group HIVSS (2007) Impact of transmission of drug-resistant HIV on the course of infection and the treatment success. Data from the German HIV-1 Seroconverter Study. *HIV Med* 8(8): 511-519.

Sagir A, Oette M, Kaiser R, Daumer M, Fatkenheuer G, Rockstroh JK, Knechten H, Schmutz G, Hower M, Emmelkamp J, Pfister H, Haussinger D (2007) Trends of prevalence of primary HIV drug resistance in Germany. *J Antimicrob Chemother* 60(4): 843-848.

Sax PE, Islam R, Walensky RP, Losina E, Weinstein MC, Goldie SJ, Sadownik SN and Freedberg KA (2005) Should resistance testing be performed for treatment-naive HIV-infected patients? A cost-effectiveness analysis. *Clin Infect Dis* 41(9): 1316-1323.

Thao VP, Le T, Torok EM, Yen NT, Chau TT, Jurriaans S, van Doorn HR, de Jong MD, Farrar JJ, Dunstan SJ (2012) HIV-1 drug resistance in antiretroviral-naive individuals with HIV-1-associated tuberculous meningitis initiating antiretroviral therapy in Vietnam. *Antivir Ther* 17(5): 905-913.

UNAIDS (2013) UNAIDS report on the global AIDS epidemic 2013.

UNGASS (2010) *The Socialist Republic of Viet Nam: Declaration of Commitment on HIV and AIDS adopted at the 26th United Nations General Assembly Special Session in June 2001*.

UNGASS (2012) The Socialist Republic of Viet Nam: Declaration of Commitment on HIV and AIDS *Vietnam AIDS response progress report 2012*.

Vercauteren J, Derdelinckx I, Sasse A, Bogaert M, Ceunen H, De Roo A, De Wit S, Deforche K, Echahidi F, Fransen K, Goffard JC, Goubau P, Goudeseune E, Yombi JC, Lacor P, Liesnard C, Moutschen M, Pierard D, Rens R, Schrooten Y, Vaira D, van den Heuvel A, van der Gucht B, van Ranst M, van Wijngaerden E, Vandercam B, Vekemans M, Verhofstede C, Clumeck N, Vandamme AM, van Laethem K (2008) Prevalence and epidemiology of HIV type 1 drug resistance among newly diagnosed therapy-naive patients in Belgium from 2003 to 2006. *AIDS Res Hum Retroviruses* 24(3): 355-362.

Viet Nam MOH (2011) National Guidelines for Diagnosis and Treatment of HIV/AIDS. *Ministry of Health*.

Viet Nam MOH (2014) *HIV/AIDS Case Report and Implementation of HIV/AIDS Prevention and Control Programme till September 2014*

Wensing AM, van de Vijver DA, Angarano G, Asjo B, Balotta C, Boeri E, Camacho R, Chaix ML, Costagliola D, De Luca A, Derdelinckx I, Grossman Z, Hamouda O, Hatzakis A, Hemmer R, Hoepelman A, Horban A, Korn K, Kucherer C, Leitner T, Loveday C, MacRae E, Maljkovic I, de Mendoza C, Meyer L, Nielsen C, Op de Coul EL, Ormaasen V, Paraskevis D, Perrin L, Puchhammer-Stockl E, Ruiz L, Salminen M, Schmit JC, Schneider F, Schuurman R, Soriano V, Stanczak G, Stanojevic M, Vandamme AM, Van Laethem K, Violin M, Wilbe K, Yerly S, Zazzi M, Boucher CA, Programme S (2005) Prevalence of drug-resistant HIV-1 variants in untreated individuals in Europe: implications for clinical management. *J Infect Dis* 192(6): 958-966.

Wheeler WH, Ziebell RA, Zabina H, Pieniazek D, Prejean J, Bodnar UR, Mahle KC, Heneine W, Johnson JA, Hall HI, Variant A, Resistant HIVSG (2010) Prevalence of transmitted drug resistance associated mutations and HIV-1 subtypes in new HIV-1 diagnoses, U.S.-2006. *AIDS* 24(8): 1203-1212.

Yerly S, Rakik A, De Loes SK, Hirschel B, Descamps D, Brun-Vezinet F, Perrin L (1998) Switch to unusual amino acids at codon 215 of the human immunodeficiency virus type 1 reverse transcriptase gene in seroconvertors infected with zidovudine-resistant variants. *J Virol* 72(5): 3520-3523.

TỶ LỆ VÀ KIỂU HÌNH KHÁNG THUỐC TRÊN BỆNH NHÂN HIV Ở NGƯỜI TRƯỞNG THÀNH CHƯA ĐIỀU TRỊ ARV TẠI HÀ NỘI, VIỆT NAM

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TÓM TẮT

Tại Việt Nam, do nhu cầu điều trị kháng virus cho bệnh nhân HIV (điều trị ARV) ngày càng gia tăng, những dữ liệu về tần suất và kiểu hình đột biến kháng thuốc trên bệnh nhân chưa điều trị ARV có vai trò quan trọng trong việc định hướng cho các chiến lược điều trị ARV trong nước. Vì vậy, chúng tôi thực hiện nghiên cứu về tỷ lệ kháng thuốc và đột biến kháng thuốc trên 345 bệnh nhân nhiễm HIV chưa được điều trị ARV. Các bệnh nhân này đủ tiêu chuẩn điều trị phác đồ bậc 1 và tham gia vào nghiên cứu thử nghiệm lâm sàng "Theo Dõi Tải Lượng virut tại Việt Nam" (VMVN) tại bệnh viện Bạch Mai, Hà Nội, từ tháng 4 năm 2011 đến tháng 10 năm 2013. Để xác định đột biến kháng thuốc, một phần đoạn gen HIV *pol* được giải trình tự bằng phương pháp Sanger và đột biến kháng thuốc sau đó được xác định dựa vào "Danh sách giám sát đột biến kháng thuốc" của Tổ chức Y tế Thế giới ban hành năm 2009. Vùng protease (PR) và reverse transcriptase (RT) của gen HIV *pol* phân lập từ 330 mẫu huyết tương đã được giải trình tự thành công. 323 mẫu được xác định thuộc thứ type CRF01_AE; 2 mẫu thuộc thứ type A/CRF01_AE, 2 mẫu thuộc thứ type BC, 1 mẫu thuộc thứ type C; 1 mẫu thuộc thứ type F/C. Tổng cộng 16/330 (4.8%) bệnh nhân mang đột biến kháng thuốc. Trong đó, 6 (38%) bệnh nhân có đột biến kháng thuốc ức chế phiên mã ngược tương tự nucleosid NRTIs (K70E, V75M, K219N, K219E, T215S); 5 (31%) bệnh nhân có đột biến kháng thuốc ức chế phiên mã ngược phi nucleosid NNRTIs (K101E, K103N, Y181C, G190A); 4 (25%) bệnh nhân có đột biến kháng thuốc ức chế protease PI (M46I, M46L, I54L, L90M); và 1 bệnh nhân kháng cả 2 thuốc NRTI và NNRTI (L74I, V75M, M184V, T215F, K101E, G190A). Kết quả

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trên cho thấy ở Việt Nam mặc dù tỷ lệ bệnh nhân được điều trị ARV tăng nhanh chóng trong 10 năm qua, tỷ lệ kháng thuốc trên bệnh nhân nhiễm HIV chưa điều trị vẫn duy trì ở mức thấp. Tuy nhiên ,sự xuất hiện những đột biến kháng PIs trên 4 bệnh nhân chưa điều trị ARV là vấn đề đáng quan tâm và cần được nghiên cứu thêm.

Từ khoá: *Virus gây suy giảm hệ miễn dịch ở người, Hội chứng suy giảm miễn dịch, điều trị ARV, kháng thuốc trước điều trị ARV, Việt Nam*