HIV-1 Transmitted Drug Resistance Mutations in Newly Diagnosed Antiretroviral-Naive Patients in Turkey

M.Sayan[1, 2*], Sargin F[3], Inan D[4], Sevgi DY[5], Celikbas AK[6], Yasar K[7], Kaptan F[8], Kutlu SS[9], Fisgin NT[10], Inci A[11], Ceran N[12], Karaoglan I[13], Çagatay A[14], Celen MK[15], Tekin KS[16], Ceylan B[17], Yıldırmak T[18], Akalın H[19], Korten V[20], Willke A[21].

[1]University of Kocaeli, Faculty of Medicine,Clinical Laboratory, PCR Unit, Kocaeli - Turkey. [2] University of Near East, Research Center of Experimental Health Sciences, Nicosia, Northern Cyprus. [3]Medeniyet University, Goztepe Educational and Research Hospital, Clinic of Infectious Diseases, Istanbul - Turkey. [4]University of Akdeniz, Faculty of Medicine, Department of Infectious Diseases, Antalya - Turkey. [5]Sisli Etfal, Educational and Research Hospital, Clinic of Infectious Diseases, Istanbul -Turkey. [6]Ankara Numune, Educational and Research Hospital, Clinic of Infectious Diseases, Ankara -Turkey. [7]Bakırkoy Dr. Sadi Konuk, Educational and Research Hospital, Clinic of Infectious Diseases, Istanbul -Turkey. [8]University of Katip Celebi, Educational and Research Hospital, Department of Infectious Diseases, İzmir -Turkey. [9]University of Pamukkale, Faculty of Medicine, Department of Infectious Diseases, Denizli - Turkey. [10]University of 19 Mayıs, Faculty of Medicine, Department of Infectious Diseases, Samsun - Turkey. [11]Istanbul Kanuni Sultan Süleyman, Educational and Research Hospital, Clinic of Infectious Diseases, Istanbul - Turkey. [12]Haydarpasa Numune, Educational and Research Hospital, Clinic of Infectious Diseases, Istanbul -Turkey. [13]University of Gaziantep, Faculty of Medicine, Department of Infectious Diseases, Gaziantep - Turkey. [14]University of Istanbul, Faculty of Medicine, Department of Infectious Diseases, İstanbul - Turkey. [15]University of Dicle, Faculty of Medicine, Department of Infectious Diseases, Diyarbakır - Turkey. [16]University of Harran, Faculty of Medicine, Department of Infectious Diseases, Urfa - Turkey. [17]University of Bezm-i Alem, Faculty of Medicine, Department of Infectious Diseases - Turkey. [18]Istanbul Okmeydani, Educational and Research Hospital, Clinic of Infectious Diseases, Istanbul - Turkey. [19]University of Uludag, Faculty of Medicine, Department of Infectious Diseases, Bursa - Turkey. [20]University of Marmara, Faculty of Medicine, Department of Infectious Diseases, İstanbul - Turkey. [21]University of Kocaeli, Faculty of Medicine, Department of Infectious Diseases, Kocaeli - Turkey.

[*]**Corresponding author**: University Hospital of Kocaeli, Clinical Laboratory, PCR Unit, Eski Istanbul Yolu, Umuttepe Campus. 41380 Izmit - Kocaeli, Turkey. e-mail: sayanmurat@hotmail.com. Tel: ++90 0262 303 8571, Fax: ++90 0262 303 8085.

Summary

HIV-1 replication is rapid and highly error-prone. Transmission of a drug-resistant HIV-1 strain is possible and occurs within the HIV-1 infected population. In this study, we aimed to determine the prevalence of transmitted drug resistance mutation (TDRM) in 1306 newly diagnosed untreated HIV-1 infected patients from 21 cities across six regions of Turkey between 2010- 2015. TDRM was identified according to the criteria provided by the World Health Organization's 2009 list of surveillance drug resistance mutation.

HIV-1 TDRM prevalence was 10.1% (133/1306) in Turkey. Primary drug resistance mutations (K65R, M184V) and thymidine analogue-associated mutations (TAMs) were evaluated together as nucleos(t)ide RT inhibitors (NRTIs) mutations. NRTIs TDRM was found in 8.1% (107/1306). However, TAMs were divided into three categories and M41L, L210W, T215Y mutations were found as TAM1 in 97 (7.4%) of patients, D67N, K70R, K219E/Q/N/R, T215F, T215C/D/S mutations were detected for TAM2 in 52 (3.9%) of patients and, M41L + K219N, M41L + T215C/D/S mutations were detected for TAM1 + TAM2 profile in 22 (1.7%) of patients, respectively. Non-nucleoside RT inhibitor-associated with TDRM was detected in 3.3% (44/1306) of patients (L100I, K101E/P, K103N/S, V179F, Y188H/L/M, Y181I/C, G190A/E/S) and TDRM to protease inhibitors was detected in 2.3% (30/1306) of patients (M46L, I50V, I54V, Q58E, L76V, V82A/C/L/T, N83D, I84V, L90M). In conclusion, long-term and large-scale monitoring on regional levels of HIV-1 TDRM informs treatment guidelines and provides feedback on the success of HIV-1 prevention and treatment efforts.

Key words: HIV-1, drug resistance, sequence analysis, transmitted, prevalence

Introduction

Today, treatment of HIV-1 infection is based on combination of three or more targeted drugs and is called highly active antiretroviral therapy (HAART). A combination of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) and third agent – which may be selected from non-nucleoside reverse transcriptase inhibitor (NNRTI), one of several ritonavir-boosted protease inhibitors (PIs), or that the new class of integrase strand transfer inhibitor (INSTI) – is currently recommended for the first-line therapy (1).

A major cause of antiretroviral resistance mutations in newly diagnosed HIV-1 infected patient is transmission of this strain from another other HIV-1 infected individual (2). The turnover of the HIV-1 population is rapid (approximately 1 day) and error-prone (mutation rate ca. 3×10^{-5} mutations/base/replication cycle), resulting in large and genetically diverse population *in vivo* in which resistance may emerge (3). Analysis of the kinetics of emergence of drug resistance *in vivo* suggests that many single nucleotide mutations conferring drug resistance may be present prior to the start of HAART (4). In 2004, the European HIV Drug Resistance Guidelines Panel presented recommendations for the use of initial HIV-1 drug resistance testing managing treatment for HIV-1 infection (5). However, all current guidelines recommend HIV-1 drug resistance testing for all HIV-1-infected patients prior to therapy initiation (1, 6, 7).

The World Health Organization (WHO) is making the global surveillance of the transmitted HIV-1 drug resistance. Transmitted HIV-1 drug resistance is classified in the three categories according to this surveillance: low prevalence (<5%), moderate prevalence (5% - 15%) and

high prevalence (>15%) (8). In a population, genotypic resistance testing is considered cost-effective for HIV-1 infection when level of transmitted drug resistance is >5% (9).

According to the official HIV/AIDS annually surveillance data of the Turkey Ministry of Health, 1767 patients were newly diagnosed with HIV-1 in 2014. In the period between 1985 and 2014 there were only 9379 cumulative HIV/AIDS cases in Turkey, and so by the end of 2014, the cumulative increase in HIV-1 patients was 38% (10). On the other hand, according to the IMS Health Turkey there are 4117 HIV-1 infected patients under antiretroviral therapy (ART) (11). However, there is limited knowledge of transmitted drug resistance mutations (TDRMs) of HIV-1 strains in Turkish patients. In a single study with 117 newly diagnosed HIV-1 infected Turkish cases, the TDRMs prevalence was 7.6% (12).

The objective of this study is accurately determine and to understand the circulation of TDRM of HIV-1 in newly diagnosed, untreated patients from a cohort of consisting of individuals from cities in all regions of Turkey.

Material and Methods

Patient population

The present study was conducted between March 2010 and March 2015, and it included 1306 HIV-1 infected patients, who were newly diagnosed in infectious disease departments of 21 cities from all the regions of Turkey. Clinic and laboratory characteristics of the patients are shown in Table 1. The study was approved by the local ethics committee (Clinical Research Ethics Committee of Kocaeli University), and a written informed consent was obtained from each patient. All of the patients were categorized as HIV carrier according to European AIDS Clinical Society (EACS) Guidelines (1). According to records of the Turkey Ministry of Health, the study patients were newly diagnosed and ART-naïve. The U.S. Centers for Disease Control and Prevention (CDC) classification system was used to determine the HIV infection staging of patients (13). Blood samples with K2EDTA were separated by centrifugation immediately, aliquoted and then kept at -80°C until testing. Anti-HIV-1/2 antibody was tested using commercially available microparticle enzyme immunoassay kits (Axsym; Abbott Laboratories, Abbott Park, IL, USA and Elecsys, Roche Diagnostics, Mannheim, Germany). All of the samples which were anti-HIV positive by ELISA at least two times were confirmed by Western blot test (DIA PRO, HIV-1 LIA, Diagnostic Bioprobes Srl, Milano, Italy) in the Istanbul Veneral Diseases Hospital in Turkey. To maintain subject confidentiality, a unique identification number was assigned to each specimen.

HIV-1 RNA detection

HIV-1 RNA was detected and quantified by a commercial real-time PCR assay – QIAsypmhony + Rotorgene Q/artus HIV-1 QS-RGQ v1 (Qiagen GmBH, Hilden, Germany) COBAS Ampliprep/COBAS TaqMan HIV-1 Test (Roche Molecular Systems, Inc. Pleasanton, CA, USA) and Abbott M2000 SP/Abbott RealTime HIV-1 Amplification Kit (Abbott Molecular Inc. Des Plaines, IL, USA).

PCR amplification and sequence analysis

Genotypic resistance test was performed by population sequencing of the viral protease and part of reverse transcriptase (RT) using an in-house method. Specific primer pairs were designed according to the ANRS (National AIDS Research Agency) drug resistance interpretation algorithm (www.hivfrenchresistance.org). The PCR condition was applied as following: RT (codons 41-238): outer primers (798 bp); MJ3: 5'- agtaggacctacacctgtca -3'

(2480 to 2499) and MJ4: 5'- ctgttagtgctttggttcctct -3' (3399 to 3420), inner primers (573 bp) A(35): 5'-ttggttgcactttaaattttcccattagtcctatt-3' (2530 to 2558) and NE1(35): 5'-cctactaacttctgtatgtcattgacagtccagct-3' (3300 to 3334). Sequencing primer; A(20): 5'-attttcccattagtcctatt-3'. Protease (codons 23-90): outer primers: 5' prot 1: 5'-taatttttagggaagatctggccttcc-3' (2082 to 2109) and 3' prot 1: 5'-gcaaatactggagtattgtatggattttcagg-3' (2703 to 2734), inner (amplification: 507 bp fragment) and sequencing primers 5' prot 2: 5'-tcagagcagaccagagccaacagcccca-3' (2136 to 2163) and 3' prot 2: 5'-aatgcttttatttttttcttctgtcaatggc-3' (2621 to 2650). HIV-1 cDNA synthesis was made with First Strand cDNA Synthesis Kit (Thermo Scientific Inc, Fermentas, Lithuania) included M-MuLV revers transcriptase enzyme. The PCR condition: 95°C for 10 min, and then 45 cycles consisting of 95°C for 45 s, 55°C for 45 s, and 72°C for 45 s. (12). All PCR products were purified using the Highly Pure PCR Product Purification Kit (Roche Diagnostics GmbH, Mannheim, Germany) and directly sequenced with the ABI PRISM 310 Genetic Analyzer equipment using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). The following thermal protocol was used for the cycle sequencing: 35 cycles consisting of 95°C for 20 s, 50°C for 25 s, and finally 60°C for 2 min. The sequences obtained with electropherogram were assembled using Vector NTI v5.1 (InforMax, Invitrogen, Life Science Software, Frederick, MD, USA)

Drug resistance mutation detection

Drug resistance mutation was analyzed by the Stanford HIV Drug Resistance Database (www.hivdb.stanford.edu), and TDRM was defined according to the mutation list published for surveillance of TDRM (SDRM) as recommended by the WHO. The WHO SDRM list included only consensus nonpolymorphic drug-resistance mutations at 43 positions in HIV-1 protease and RT. Selected mutations were defined as those occurring at a prevalence ≤0.5% in ART-naive individuals in subtypes for which >1,000 sequences were available (14). However, thymidine analogue-associated mutations (TAMs) were evaluated, for the first time in Turkish patients, in two distinct genotypic profiles; TAM1, TAM2, and in addition TAM1 + TAM2 profiles.

HIV-1 subtyping

HIV-1 subtype was determined by use of the HIVdb-Stanford University and geno2pheno (http://coreceptor.bioinf.mpi-inf.mpg.de) subtyping tools. The information was then compared to the consensus subtype B reference sequence, and the differences were used as query parameters to interrogate the HIV database as rapid computer-assisted virtual phenotyping.

The submitted nucleotide sequences were assigned GenBank accession numbers from KT284379 - KT285684.

Statistical analysis

Differences between two proportions were measured using Pearson's χ^2 test or Fisher's exact test. $P \leq 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS for Windows statistical software (SPSS Inc., Chicago, Ill., USA).

Results

The average of TDRM prevalence was detected 10.1% (133/1306) in newly diagnosed HIV-1 infected patients in Turkey. The frequencies of the primary drug resistance for NRTI, NNRTI and PI were 0.6% (8/1306), 3.3% (44/1306) and 2.3% (30/1306), respectively. However, most

of the NRTI TDRMs were TAMs and evaluated into three categories as TAM1, TAM2, and TAM1 + TAM2 in the NRTI class (Table 2). The frequency of the TDRM for NRTI + TAMs was 8.1% (107/1306). K65R and M184V for the NRTI class, L100I, K101E, K101P, K103N, K103S, V179F, Y188H, Y188L, Y188M, Y181I, Y181C, G190A, G190E and G190S for the NNRTI class and M46L, I50V, I54V, Q58E, L76V, V82A, V82C, V82L, V82T, N83D, I84V and L90M for the PI class were found as primary drug resistance mutations. Some HIV-1 TDRM was discovered for the first time in Turkish patients – there are L100I, V179F and Y188M for NNRTI, and I50V, Q58E, V82A/C for the PI drug class. However, the differences between NRTI, NNRTI, and PI drug class resistance prevalence were not significant (Fisher's exact test, $p \geq 0.05$). In addition, the TDRM prevalence between present and our recently unpublished (in 2014, n=774 and sampling from newly diagnosed, treatment naïve HIV-1 infected Turkish patients) data was not significant (Fisher's exact test, $p \geq 0.05$).

The prevalence of TAM1, TAM2, and TAM1 + TAM2 profiles and the particular mutations present were as follows: 7.4% (97/1306) and M41L, L210W, T215Y for TAM1, 3.9% (52/1306) and D67N, K70R, K219E/Q/N/R, T215F, T215C/D/S for TAM2, and 0.7% (10/1306) and M41L + K219N, M41L + T215C/D/S for TAM1 + TAM2 (Table 2). Differences in the prevalence of TAM1 vs TAM2, TAM1 vs TAM1 + TAM2, TAM2 vs TAM1 + TAM2 were significant (Fisher's exact test, p=0.01, p=0.02 and p=0.02, respectively).

HIV-1 subtyping results are shown in three categories. According to the results, subtype B was dominant, found in 68% (885/1306) of study patients, while non-subtype B and circulating recombinant form (CRF) subtypes were found in 10% (136/1306) and 22% (285/1306), respectively. Difference in the prevalence of HIV-1 subtypes was not significant (Fisher's exact test, $p \geq 0.05$). The non-subtype B category included five subtypes (C, D, F, G and K) and three sub-subtypes (A1, F1 and F2). However, seven CRF subtypes (CRF01_AE, CRF02_AG, CRF03_AB, CRF07_BC, CRF08_BC, CRF12_BF, CRF14_BG) and three cpx subtypes (CRF06_cpx, CRF11_cpx, CRF13_cpx) were found (Table 1).

Discussion

In the last few years, HIV-1 TDRM have been analyzed all around the world. Previous studies have found the risk of transmitted HIV-1 drug resistant to be of 5 - 18% in the USA and Europe, 13.8% in Asia, and 2.2 - 24% in Africa (15 - 18). According to the our unpublished study in 2014, the prevalence of HIV-1 TDRMs was found 6.7% in 774 newly diagnosed treatment naïve Turkish patients (19). Although new TDRM of HIV-1 was found here, the prevalence was higher than our previously published and/or unpublished reports. But, the prevalence of HIV-1 TDRM still moderate in Turkey. This moderate view on the TDRM frequency may make the necessary analysis costs in the management of ART naïve Turkish patients. The low median $CD4^+$ T-cell count of the study suggested a lot of the patients may be late presenter. This may be affected the level and TDRM type in Turkey. However, our results still show that the genotypic resistance testing must remain an integral part of the management of HIV-1 infection in Turkey.

There are some similarities in surveillance strategies between our study and The SPREAD Programme of Europe: both studies used population sequencing technique, accumulated surveillance data for long term, and sampled randomly from multiple areas and large patient size for newly diagnosed of HIV-1 infection. The average prevalence of TDRM in the SPREAD Programme was 8.4% and the frequency of the NRTI, NNRTI and PI resistance

were 4.7%, 2.3%, and 2.9%, respectively (20). Turkey showed higher average prevalence than SPREAD Programme. This higher TDRM prevalence and the discrepancy between the NRTI resistance between Turkey and Europe may be associated with TAMs (18). Furthermore, the surveillance periods in both studies are different. Our study was performed in 2010 – 2015, but SPREAD programme was performed in 2002 - 2006. On the other hand, on the backbone treatment using mostly tenofovir + emtricitabine (~81%, unpublished data) in Turkey and lower circulation or decreasing of K65R and M184V mutations frequency is important for the first-line therapy in HIV-1 infected patients. The lower prevalence of NRTI backbone treatment mutations and higher prevalence of TAMs may be associated with the increase of HIV-1 infection in MSM patients in the last two years (in 2012, 23% of patients were MSM, compared to 43% in this study) (12).

A significant proportion of the patients with TDRMs have revertant TAMs which transmitted but do not reduce drug susceptibility. The largest proportion of TAMs included firstly by position of 215C/D/F/S/Y and secondly D67N, K219Q or M41L mutations (21- 23). TAMs were shown in Table 2, but have been evaluated separately in TAM1, TAM2 and TAM1 + TAM2 genotypic profiles. Our analysis clearly shows TAM1 profile mutations are significantly more prevalent than TAM2 or TAM1 + TAM2 in Turkey ($p \geq 0.05$). This is may be due to M41L, frequently occurred as a single resistance mutation in untreated patients. However, according to new findings, detection of a single M41L mutation at baseline did not influence the development of resistance *in vitro* or virological outcome on tenofovir-containing regimen in patients (24). In addition, codon 215 is known to atypical or partial revertant amino acid position which occur approximately 3% of newly diagnosed patients in the USA (25, 26). In Turkey, T215F and T215C/D/S mutations were categorized in TAM2 and in our study TAM2 proportion was determined 3.9% (Table 2). However, there are no data available to date on frequency and type of TAM1 and TAM2 profiles in HIV-1 infected patients in Turkey, and this study provides them for the first time.

The molecular evidence of the present study indicates that the subtype B is the most prevalent subtype among newly diagnosed HIV-1 infected patients in Turkey. However, trends in HIV-1 circulation in Turkey seems substantially heterogeneous with five non-subtype B, three non-sub-subtype B, seven CRF subtypes and three cpx subtypes of HIV-1 in spite of subtype B dominancy. This heterogenity may be associated with demographically changes in Turkey due to it's specific geographic location: an increase the number of refugees, especially from Syria (nearly 1.7 million since the 2011 crisis) and several countries from Africa, an increase in asylum-seekers (nearly 100,000 as of January 2015, originating mainly from Iraq, Afghanistan, the Islamic Republic of Iran, and Somalia), ongoing human trafficking (84% suspected to be for sexual exploitation), and movements of tourists (27 - 29). Subtype diversity of HIV-1 is a major challenge in the development of a global control strategy for HIV-1 circulation.

In conclusion, moderate HIV-1 TDRM prevalence show that the drug resistance testing must remain an integral part of the management of newly diagnosed HIV-1 infected patients in Turkey. On the other hand, long-term and large-scale monitoring on regional levels of HIV-1 TDRMs informs treatment guidelines and provides feedback on the success of HIV-1 prevention and treatment efforts.

Acknowledgement & Conflict of Interest:

We thank Dr.Alison Lynn Hill (from Harvard University, Cambridge, MA, USA) for English language editing.

Funding: This study was not funded from any organisation.

Competing interests: No conflict of interest to declare.

Ethical approval: KOU KAEK 201345

References

1. European AIDS Clinical Society (EACS) Guidelines. (2013): Version 7.0 - October 2013. www.europeanaidsclinicalsociety.org

2. Zolopa AR: The evolution of HIV treatment guidelines: current state-of-the art of ART. Antiviral Res 2010;85:241-244.

3. Perelson AS, Neumann AU, Markowitz M, *et al*.: HIV-1 dynamics *in vivo*: Virion clearance rate, infected cell life-span, and viral generation time. Science 1996;271:1582-1586.

4. Cortez KJ, Maldarelli F: Clinical management of HIV drug resistance. Viruses 2011;3:347-378.

5. Vandamme AM, Camacho RJ, Silberstein FC, *et al*.: European recommendations for the clinical use of HIV drug resistance testing: 2011 Update. AIDS Rev 2011;13:77-108.

6. British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2012 (Updated November 2013). HIV Medicine 2014;15 (Suppl. 1):1-85.

7. Department of Health and Human Services. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. 13 November, 2014. Available at: http://www.aidsinfo.nih.gov.

8. Bennett DE, Myatt M, Bertagnolio S, *et al*.: Recommendations for surveillance of transmitted HIV drug resistance in countries scaling up antiretroviral treatment. Antiviral Therapy 2008;13(Supplement 2):25-36.

9. Sax P, Islam R, Walensky R, *et al*.: Should resistance testing be performed for treatment-naive HIV-infected patients? A cost-effectiveness analysis. Clin Infect Dis 2005;41:1316-1323.

10. Hacettepe University, HIV/AIDS Treatment and Research Centre. The 2014 Annual Report. http://www.hatam.hacettepe.edu.tr

11. IMS Health Turkey, August 2015. http://www.imshealth.com/portal/site/imshealth

12. Sayan M, Willke A, Ozgunes N, *et al*.: HIV-1 subtypes and primary antiretroviral resistance mutations in antiretroviral therapy naive HIV-1 infected individuals in Turkey. Jpn J Infect Dis 2013;66:306-311.

13. Centers for Disease Control and Prevention. Guidelines for national human immunodeficiency virus case surveillance, including monitoring for human immunodeficiency virus infection and acquired immunodeficiency syndrome. MMWR Recomm Rep Dec 1999;10;48:1-27.

14. Bennett DE, Camacho RJ, Otelea D, Kuritzkes DR, Fleury H, Kiuchi M, *et* al. Drug resistance mutations for surveillance of transmitted HIV-1 drug-resistance: 2009 Update. PLoS ONE 2009;4:e4724.

15. Alteri C, Svicher V, Gori C, *et al*.: Characterization of the patterns of drug-resistance mutations in newly diagnosed HIV-1 infected patients naive to the antiretroviral drugs. BMC Infect Dis 2009;9:1-11.

16. Vercauteren J, Derdelinckx I, Sasse A, *et al*.: 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 2008;24(3):355-362.

17. Price MA, Wallis CL, Lakhi S, *et al*.: Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in East and Southern Africa. AIDS Res Hum Retroviruses 2011;27(1):5-12.

18. Frentz D, van de Vijver D, Abecasis A, *et al*.: Patterns of transmitted HIV drug resistance in Europe vary by risk group. PLoS ONE 2014;9(4):e94495.

19. Sayan M, Sargın F, Inan D, *et al*.: Transmitted antiretroviral drug resistance mutations in newly diagnosed HIV-1 positive patients in Turkey. J Int AIDS Soc 2014;2;17(4 Suppl 3):19750.

20. SPREAD Programme. Transmission of drug-resistant HIV-1 in Europe remains limited to single classes. AIDS 2008; 22: 625–635

21. Garcia-Lerma JG: Diversity of thymidine analogue resistance genotypes among newly diagnosed HIV-1-infected persons. J Antimicrob Chemother 2005;56:265-269.

22. Weinstock H, Zaidi I, Heneine W, *et al*.: The epidemiology of antiretroviral drug resistance among drug-naive HIV-1 infected persons in 10 U.S. cities. J Infect Dis 2004;189:2174-80.

23. Violin M, Cozzi-Lepri A, Velleca R, *et al*.: Risk of failure in patients with 215 HIV-1 revertants starting their first thymidine analog-containing highly active antiretroviral therapy. AIDS 2004;18:227-235.

24. Pingen M, Nijhuis M, Mudrikova T, *et al*.: Infection with the frequently transmitted HIV-1 M41L variant has no influence on selection of tenofovir resistance. J Antimicrob Chemother 2015;70(2):573-80.

25. Pingen M, Nijhuis M, de Bruijn JA, *et al*.: Evolutionary pathways of transmitted drug-resistant HIV-1. J Antimicrob Chemother 2011;66:1467-1480.

26. Shafer RW, Rhee SY, Pillay D, Miller V, Sandstrom P, Schapiro JM, *et* al. HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. AIDS 2007;21:215-223.

27. UNHCR official web page; http://www.unhcr.org/pages/49e48e0fa7f.html

28. Inan D, Sayan M: Molecular epidemiology of HIV-1 strains in Antalya, Turkey. J Int AIDS Soc 2014;2;17(4 Suppl 3):19684.

29. Trafficking in human beings report of European Commission.https://ec.europa.eu/anti-trafficking/sites/antitrafficking/files/trafficking_in_human_beings_-_eurostat_-_2014_edition.pdf

Table 2. Primary drug resistance mutations in newly diagnosed HIV-1 infected patients in Turkey (between 2010 – 2015, n=1306).

Drug class	**Drug resistance mutation**[*]	**n**	**%**
NRTI	K65R, M184V	8	0.6
TAM1	M41L, L210W, T215Y	97	7.4
TAM2	D67N, K70R, K219E/Q/N/R, T215F, T215C/D/S	52	3.9
TAM1 + TAM2	M41L + K219N, M41L + T215C/D/S	10	0.7
		107	8.1
NNRTI	L100I, K101E/P, K103N/S, V179F, Y188H/L/M, Y181I/C, G190A/E/S	44	3.3
PI	M46L, I50V, I54V, Q58E, L76V, V82A/C/L/T, N83D, I84V, L90M	30	2.3
Total		133	10.1

Abbreviations; NRTI; nucleoside RT inhibitors, NNRTI; non-nucleoside RT inhibitors, PI; protease inhibitors, TAM; thymidine analogue - associated mutation

[*]The number of "n" consisted with each mutation detected patient

[*]Subtype of HIV-1 is not a variable in identification of drug resistance mutation.

Table 1: Demographic characteristics of the patients infected with HIV-1.

Characteristic	**Study group**			
Patient, n	1306			
Gender, M/F (%)	1151/155 (88/12)			
Age, median years (range)	36 (3 - 74)			
$CD4^+$ T-cell count, median mm^3 (range)	361 (4 - 1351)			
HIV-RNA load, median IU/ml (range)	2.59+E6 (6.8+E2 - 3.29+E6)			
HIV-1 subtype, n (%)	Subtype B	885 (68)	-	-
	Non-subtype B	136 (10)	A1 C D F F1 F2 G K	48 (3.6) 21 (1.6) 3 (0.2) 2 (0.1) 24 (1.8) 1 (0.07) 36 (2.7) 1 (0.07)
	Circulating recombinant form (CRF)	285 (22)	CRF01_AE CRF 02_AG CRF 03_AB CRF 06_cpx CRF 07_BC CRF 08_BC CRF 11_cpx CRF 12_BF CRF 13_cpx CRF 14_BG	132 (10.1) 85 (6.5) 13 (1) 3 (0.2) 1 (0.07) 1 (0.07) 3 (0.2) 33 (2.5) 3 (0.2) 11 (0.8)
Sampling, region/city of Turkey	Marmara/Kocaeli, İstanbul, Edirne, Bursa, Sakarya, Bolu Black Sea/Samsun, Artvin, Giresun, Trabzon Southeast Anatolia/Urfa, Diyarbakir, Gaziantep Central Anatolia/Ankara, Kayseri Aegean/İzmir, Denizli, Çanakkale Mediterranean/Antalya, Adana, Mersin			
Acquisition route, n (%)	Heterosexual contact MSM Bisexual contact Blood transfusion Injection drug use Tattoo Dental/medical surgery Breast-feeding Vertical route **Total**			674 (52) 563 (43) 47 (3.6) 8 (0.6) 4 (0.3) 4 (0.3) 2 (0.1) 2 (0.1) 2 (0.1) **1306 (100)**

Co-infection status, n (%)	Hepatitis B	35 (2.7)
	Syphilis	18 (1.4)
	Tuberculosis	14 (1.1)
	P. jiroveci pneumonia	11 (0.8)
	Hepatitis C	7 (0.5)
	Kaposi sarcoma	7 (0.5)
	Candida esophagitis	7 (0.5)
	HPV infection	4 (0.3)
	Hepatitis D	3 (0.2)
	Herpes zoster	3 (0.2)
	Toxoplasmosis	3 (0.2)
	Condyloma	2 (0.15)
	CMV retinitis	1 (<0.1)
	Cryptococcal meningitis	1 (<0.1)
	PML	1 (<0.1)
	Total	**117 (8.9)**

Abbreviations: M/F; male/female, MSM; men who have sex with men, HPV; Human papilloma virus, CMV; cytomegalovirus, PML; progressive multifocal leukoencephalopathy

The Fisher exact test

Subtip B vs non subtip B

Row1: 885; 68

Row2: 136; 10

The total number of cases= 1099
The smallest value= 10
The smallest marginal= 78

Use of * (starred) statistics is advised

The p for exactly this table= 0.13774

One sided p-values: for p(O>=E):
p(O>=E): 0.6041346 *
p(O>E): 0.4663976
mid-p: 0.5352661
p(O<=E): 0.5336024

Two sided p-values p(O>=E|O<=E):
p= 1 * (sum of small p's)
p= 0.137737 (left+right-exact)
p= 0.862263 (left+right)
p= 0.932795 (double the single sided p)
p(O>E|O<E)= 0.86226 (sum of small p's)
mid-p 0.9311315 (sum of small p's)

The Fisher exact test

Subtip B vs CRF

Row1: 885; 68

Row2: 285; 22

The total number of cases= 1260
The smallest value= 22
The smallest marginal= 90

Use of * (starred) statistics is advised

The p for exactly this table= 0.10112

One sided p-values: for p(O>=E):
p(O>=E): 0.5360523 *
p(O>E): 0.4349321
mid-p: 0.4854922

p(O<=E): 0.5650679

Two sided p-values p(O>=E|O<=E):
p= 1 * (sum of small p's)
p= 0.1011202 (left+right-exact)
p= 0.8988798 (left+right)
p= 0.927895 (double the single sided p)
p(O>E|O<E)= 0.89888 (sum of small p's)
mid-p 0.9494399 (sum of small p's)

The Fisher exact test

Non subtip B vs CRF

Row1: 136; 10

Row2: 285; 22

The total number of cases= 453
The smallest value= 10
The smallest marginal= 32

Use of * (starred) statistics is advised

The p for exactly this table= 0.15505

One sided p-values: for p(O>=E):
p(O>=E): 0.6179361 *
p(O>E): 0.4628886
mid-p: 0.5404124
p(O<=E): 0.5371114

Two sided p-values p(O>=E|O<=E):
p= 1 * (sum of small p's)
p= 0.1550476 (left+right-exact)
p= 0.8449524 (left+right)
p= 0.925777 (double the single sided p)
p(O>E|O<E)= 0.84495 (sum of small p's)
mid-p 0.9224762 (sum of small p's)