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Molecular epidemiology of HIV-1 strains in the south-east and east of Turkey

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ABSTRACT

Objective: To detect the subtype characterization and drug-resistant mutations in HIV-1 strains after the refugee movement from Syria to Turkey between 2011 and 2014 in south east border lines.

Methods: A total of 65 patients were included in this study, of which 57 (88%) patients were antiretroviral therapy-naive patients. HIV-1 RNA was detected and quantified by real-time PCR assay. HIV-1 subtypes and circulating recombinant forms (CRFs) were identified by phylogenetic analysis (neighbor-joining method), and drug-resistant mutations were analyzed.

Results: Three major HIV groups were indicated. Two of these groups were located in subtype B. The other group showed heterogeneity. Subtype B (48/65, 73.8%), followed by CRFs (12/65, 18.5%) was the most common strain. Subtype of CRFs consisted of CRF01_AE (9/65, 13.8%) and CRF02_AG (3/65, 4.6%). Subtype C (1/65, 1.5%), sub-subtypes A1 (2/65, 3.1%) and F1 (2/65, 3.1%) were also detected with low prevalence. The rate of overall primary antiretroviral resistance was 4.9% (3/61). Drug-resistant rate for non-nucleoside reverse transcriptase inhibitors was 4.9%. The thymidine analogue mutation rate was 13.1% (8/61).

Conclusions: HIV molecular epidemiology studies are necessary to determine transmission patterns and spread. Subtype B and CRF01_AE, CRF02_AG are the most prevalent strains in the south-east of Turkey. However, subtype C, sub-subtypes A1 and F1 are of low prevalence but persist in the south-east of Turkey. In the near future, changing of HIV epidemiology will be possible in Turkey due to migration movement in border lines and resistance testing will play an important role in HIV management.

1. Introduction

HIV is a significant public health problem. HIV has two major genotypes including HIV-1 and HIV-2. HIV-1, the most prevalent genotype worldwide, is characterised by a high genetic variability and divided into three groups. Group M is responsible for the HIV pandemic. Group M has several subtypes including A, B, C, D, F, G, H, J, and a number of mosaic strains known as circulating recombinant forms (CRFs) [1–3]. Subtype A is the predominant variant in the Central and Eastern Africa; subtype B is common in the Western and Central Europe, in America, Australia, and Southeastern Asia; subtype C is in Southern Africa and India [4,5]. In addition, CRF01_AE is the most common CRF in Southeastern Asia while CRF02_AG is frequent in Western and Central Africa [4–7]. World Health Organization (WHO) reported that since the beginning of the epidemic, almost 78 million people have been infected with HIV and about 39 million people have died of HIV at the end of 2013, worldwide. According to this report, about 0.8% of adults are living with HIV. Especially Sub-Saharan Africa remains the most severely affected, with nearly 1 in every 20 adults living with HIV [7]. HIV-1 incidence has decreased by estimated 33% since 2001 but remains high with approximately 2.3 million new infections in 2012. In the United States, approximately 50 000 new HIV infections were encountered each year [8].

Zidovudine became the first approved drug for treatment of HIV disease in 1897. Since then, approximately 30 drugs have been approved for HIV therapy. The treatment of HIV/AIDS normally includes the combination therapy of multiple antiretrovirals. There are several classes of antiretroviral agents that function on different

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stages of the HIV life-cycle, such as nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors, entry/fusion inhibitors, C–C-chemokine receptor type 5 antagonists, and integrase inhibitors [9]. A combination therapy of drugs that act on different viral targets is known as highly active antiretroviral therapy (ART) [10]. Recently, because of antiretroviral resistance, antiretroviral failure may occur in patients with HIV [8,11]. Viral mutations are the major cause of therapy failure in patients with HIV. South-east and east regions of Turkey are big areas with high population. In addition, in recent years, there are frequent movements of refugees in borders of south-east and east of Turkey. There were insufficient data available regarding the subtype distribution and antiretroviral resistance mutations in HIV-1 strains in these regions of Turkey. Therefore, we aimed to detect the subtype distribution in 65 HIV-1 patients from south-east and east regions of Turkey and to determine the primary antiretroviral drug-resistant mutations in ART-naive HIV-1 patients.

2. Materials and methods

2.1. Patients

This study was performed between June 2009 and February 2012. A total of 65 HIV-1 patients were included in this study. Sera of 65 HIV-positive patients were collected from provinces including Diyarbakır, Gaziantep, Şanlıurfa, Mardin, Batman, Siirt, Bitlis, and Elazığ located in south-east and east of Turkey.

Of the patients, 57 (88%) were newly diagnosed and ART-naive HIV-1 patients, while 8 were under different ARTs. All patients were categorized as HIV-1 carriers according to European AIDS Clinical Society Guidelines [10].

The U.S. Centers for Disease Control and Prevention classification system was used to stage HIV infections [12]. Blood samples were immediately separated by centrifugation, aliquoted, and then stored at −80 °C. Anti-HIV 1/2 antibody titers were detected by commercially available microparticle enzyme immunoassay kits (Axsym, Abbott Laboratories, Abbott Park, III., USA and Elecsys, Roche Diagnostics GmbH, Mannheim, Germany). In the study, all anti HIV antibody reactive samples were confirmed by ELISA, at least for twice, and were further confirmed by Western blot analysis (DIA PRO, HIV-1 LIA, Diagnostic Bioprobes Srl, Milano, Italy).

Ethic approval was obtained from Ethics Committee, Faculty of Medicine, Dicle University, Diyarbakir, Turkey.

2.2. HIV-1 RNA isolation and real-time PCR

HIV-1 RNA was detected and quantified from serum samples by using commercial real-time PCR assays (COBAS, Ampliprep/COBAS, and TaqMan HIV-1 Test, Roche Molecular Systems, Inc. Pleasanton, Calif., USA and Abbott M2000 SP/Abbott RealTime HIV-1 amplification kit, Abbott Molecular Inc., Des Plaines, III., USA).

2.3. Population-based sequencing of HIV-1 pol

Primer pairs were designed based on the French National Agency for AIDS Research AC11 Resistance Group (www.hivfrenchresistance.org) for analysis of pol sequences (reverse transcriptase and protease regions) of HIV-1. The sequence and location of each primer are given in Table 1.

First strand cDNA synthesis kit (Thermo Scientific Inc., Fermentas, Lithuania) including the Moloney murine leukemia virus reverse transcriptase enzyme was used for HIV-1 cDNA synthesis. Then PCR amplification was carried out in the following conditions: at 95 °C for 10 min, then 45 cycles at 95 °C for 45 s, 55 °C for 45 s, and 72 °C for 45 s. PCR products was purified by the highly pure PCR product purification kit (Roche Diagnostics) and directly sequenced by using the ABI PRISM 310 genetic analyzer with the DYEnamic ET terminator cycle sequencing kit (Amersham Pharmacia Biotech Inc., Piscataway, N.J., USA). Thermal protocol for the cycle sequencing was as follows: 35 cycles at 95 °C for 20 s, 50 °C for 25 s, and 60 °C for 2 min. The sequences obtained from the electropherogram were assembled by using Vector NTI 5.1 software (InforMax, Invitrogen, Life Science Software, Frederick, Md., USA).

2.4. HIV-1 subtyping

Neighbor-joining method was carried out with other sequences from all HIV-1 subtypes from GenBank by using CLC sequence viewer 7.5 software (Qiagen, CLC bio A/S, Aarhus, Denmark). Bootstrap support values (1 000 replicates) were shown at the respective branches. The consensus reference sequences of HIV-1 subtypes were obtained from Los Alamos National Laboratory (www.hiv.lanl.gov) database.

2.5. Determination of antiretroviral drug-resistant mutations

HIV-1 antiretroviral drug-resistant mutations were performed in a total of 61 patients and analyzed according to criteria established by the WHO (last updated in 2009) for surveillance of drug-resistant mutations. WHO criteria for surveillance of drug-resistant mutations included only nonpolymorphic drug-resistant mutations, which were defined as those occurring at a prevalence $\leq$0.5% in ART-naive individuals in subtypes for which >1 000 sequences were available [13]. Antiretroviral drug-resistant mutations were also interpreted by using the Stanford University HIVdb algorithm (www.hivdb.stanford.edu). The information was then compared to the consensus subtype B reference sequence, and the differences were used as query parameters to interrogate the HIV drug resistance database [14].

Table 1
Primers used for amplification and sequencing of reverse transcriptase and protease genes.

Genes	Primes	
	Forward	Reverse
Reverse transcriptase		
Outer	MJ3:5′-AGTAGGACCTACACCTGTCA-3′ (2480-2499)	MJ4,5′-CTGTTAGTGCTTTGGTTCCTCT-3′ (3399-3420)
Inner	A(35):5′-TTGGTTGCACTTTAAATTTTCCCATTAGTCCTATT-3′ (2530-2558)	NE1(35):5′-CCTACTAACTTCTGTATGTCATTGACAGT CCAGCT-3′ (3300-3334)
Sequences	A(20):5′- ATTTTCCCATTAGTCCTATT-3′	
Protease		
Outer	5′prot1:5′-TAATTTTTTAGGGAAGATCTGGCCTTCC-3′ (2082-2109)	3′prot1:5′-GCAAATACTGGAGTATTGTATGGATTTT CAGG-3′ (2703-2734)
Inner	5′prot2:5′-TCAGAGCAGACCAGAGCCAACAGCCCCA-3′ (2136-2163)	
Sequences	3′prot2:5′-AATGCTTTTATTTTTTCTTCTGTCAATGGC-3′ (2621-2650)	

2.6. Statistical analysis

Differences between two proportions were measured by using Pearson's *Chi*-squared test or Fisher's exact test. $P < 0.05$ was considered as statistically significant. Statistical analyses were performed by using SPSS 13.0.0 Windows statistical software (SPSS Inc., Chicago, III., USA).

3. Results

Among 65 patients, 57 (88%) were newly diagnosed and ART-naive patients, while 8 were under different ARTs. The population-based sequencing of the reverse transcriptase and protease domains of pol gene region of HIV-1 indicated that subtype B (48/65, 73.8%), followed by CRFs (12/65, 18.5%) was the most commonly

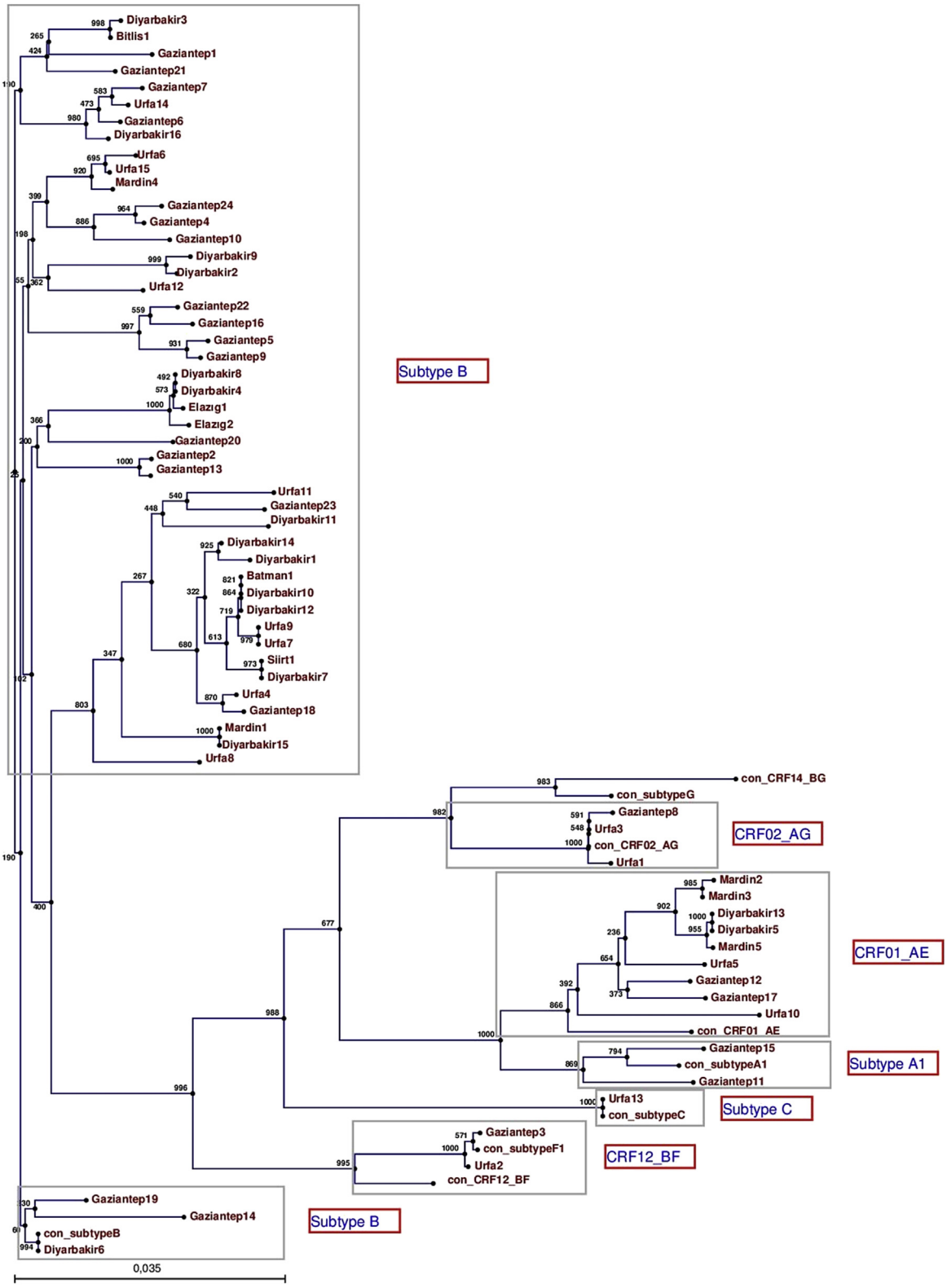

Figure 1. Phylogenetic tree of HIV-1 reverse transcriptase (codon 41-238) and protease (codon 1-99) domains (~667 bp) of pol gene region.

identified strain. Subtype of CRFs consisted of CRF01_AE (9/65, 13.8%) and CRF02_AG (3/65, 4.6%). Subtype C (1/65, 1.5%), subsubtypes A1 (2/65, 3.1%) and F1 (2/65, 3.1%) were also detected with low prevalence (Figure 1).

Three major groups were obtained in phylogenetic analysis. Two of these groups were located in subtype B. The other group showed heterogeneity and consisted of CRF02_AG, CRF01_AE, subtype C, CRF12_BF, and subtype A1 (Figure 1). The patients had antiretroviral resistance mutations to NRTIs (M184V) and NNRTIs (K103N, Y181C, K101E, K103S).

Among 61 patients, the prevalence of overall primary antiretroviral resistance was 4.9% (3/61). All of the patients with antiretroviral resistance have history of treatment with antiretroviral drugs. Resistant rate for NRTIs and NNRTIs were 0% (0/61) and 4.9% (3/61), respectively.

The rate of thymidine analogue mutations (M41L, D67N, K70R, T215l, K219E, K70L, T215N, T215D, A62V, T215N, T215D, V75L, K219Q) was 13% (8/61) in HIV-1 patients.

4. Discussion

Turkey is not an endemic country for HIV infection but according to the HIV/AIDS surveillance data of the Turkey Ministry of Health, 6802 patients were suffered from HIV-1 infection from 1985 through 2013 in Turkey [15]. First-line therapy choice is very important for the management of HIV and it is related to drug resistance caused by HIV mutations and subtypes. In recent years, there are frequent movements of refugees in borders of south-east and east of Turkey. There were insufficient data available regarding subtype distribution and antiretroviral resistant mutations in HIV-1 strains in these regions.

In Turkey, the first study on HIV-1 subtype was reported in 2006. A total of 27 HIV/AIDS patients were examined. HIV-1 subtype B was determined as the most prevalent subtype in Turkey and the distribution of the non-B-subtypes was as follows: four were subtype A, one subtype C, one subtype D, and two subtype F1. Non-B-subtype infections were thought to be mainly transmitted by immigrants from Africa, the Balkans, and the Middle East [16]. After this study, Hemelear described CRFs of HIV-1 in Turkey for the first time and also found that CRF02_AG was prevalent in West and Central Africans, and Middle Easterns/North Africans, CRF01_AE in South-East Asians, East Asians and Central Africans, CRF03_AB in Eastern Europeans and Central Asians, and CRF12_BF in South Americans [17].

A total of 72 HIV-1 patients inhabiting in Istanbul, Turkey were included in a study between 2009 and 2012. Among those patients, 57 were newly diagnosed and ART-naive patients, while 15 were under different ARTs. The population-based sequencing of the reverse transcriptase region of HIV-1 indicated that CRFs (36/72; 50%) were the most commonly identified strains, followed by subtype B (31/72; 43%) among Turkish patients. Subtypes A1 (4.2%) and F1 (2.8%) were also detected with low prevalence. In that study, the recombinant forms of HIV-1 circulated in Istanbul, Turkey were found as follows: CRF02_AG (25%, West Africa, Central Africa and Middle East/North Africa origin), CRF12_BF (12.5%, South America origin), CRF03_AB (9.7%, Eastern Europe and Central Asia origin) and CRF01_AE (2.8%, South-East Asia, East Asia and Central Africa origin) [18].

For the first time, in 2013, a extreme subtype, CRF06_cpx was reported in a HIV-1 positive married couple from Izmir, Turkey [19]. In the same year, subtype B (48%) was found to be the most common subtype in 77 HIV-1 positive patients from Antalya, Turkey, and CRF14_BG (12.9%) was identified for the first time in Antalya in contrast to previous observations in the other reports in Turkey [20]. In a different study, in 117 newly diagnosed HIV-1 positive patients, subtype CRFs (CRF02_AG, CRF01_AE, CRF12_BF and CRF03_AB; 47.0%, 55/117) and subtype B (33.3%, 39/117) were identified as the most common HIV-1 subtypes [21]. Our study indicated that subtype B (73.8%), followed by CRFs (18.5%) were the most prevalent strains. We found that subtype of CRFs consisted of CRF01_AE and CRF02_AG. We found that subtype C, subtypes A1 and F1 were of low prevalence but persisted in south-east of Turkey. Turkey has a migration movement in border lines of south-east and the Ministry of the Interior, Turkey estimated that around one million people between April 2011 and May 2014 migrated to Turkey due to internal turmoil in Syria [22]. We suggested that in the near future, changing of HIV epidemiology was possible in Turkey.

In the study of Sayan *et al.*, the prevalence of overall primary antiretroviral resistance was 7.6% in HIV-1 patients from Turkey and drug-resistant rates for NRTIs, NNRTIs, and protease inhibitors were 4.2%, 1.7%, and 1.7%, respectively [21]. A recent study performed in 774 antiretroviral naive HIV-1 patients from Turkey between 2009 and 2014 reported that the prevalence of overall transmitted drug resistance mutations was 6.7% (52/774). Resistance mutations were found to be 0.7%, 4.1% and 2.1% to NRTIs, NNRTIs and protease inhibitors, respectively. Three patients had NRTIs + NNRTs resistance mutations (M184V + K103N) as multiclass drug resistance. However, thymidine analogue resistance mutations (TAMs) determined two distinct genotypic profiles in the HIV-1 reverse transcriptase: TAM1 (M41L, L210W and T215Y), and TAM2 (D67N, K70R, K219E/Q, and T215F). The prevalence of TAM1 and TAM2 was 7.8% (60/774) and 4.4% (34/774), respectively [23]. In our study, the prevalence of overall primary antiretroviral resistance was 4.9% (3/61) (treatment naive) in HIV-1 patients and drug-resistant rate for NRTIs and NNRTIs were 0% and 4.9%, respectively. The TAMs (M41L, D67N, K70R, T215l, K219E, K70L, T215N, T215D, A62V, T215N, T215D, V75L, K219Q) was 13% in HIV-1 patients. Our study suggested that the prevalence of HIV-1 primary drug-resistant mutations was in substantial rates and resistance testing should be incorporated as an integral part of HIV management, and the choice of a first-line therapy regime in our patients.

In conclusion, subtype B and CRF01_AE, CRF02_AG were the most prevalent strains in south-east of Turkey. Subtype C, subtypes A1 and F1 were of low prevalence but persisted in south-east of Turkey. The movements of refugees in the border lines of Turkey may change the circulating HIV-1 strain pool and the molecular epidemiology researchs on HIV-1 may be more important than ever. It should be mentioned that development of the effective vaccine for HIV is an important public health concern and global monitoring of HIV subtypes will supply data for HIV vaccine development studies. Our study revealed that the resistance testing should be an integral part of the HIV infection management.

Conflict of interest statement

We declare that we have no conflict of interest.

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