Prevalence of single, double, and triple infections of HIV, HCV and HBV among the MSM community in Pakistan

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Patterns of HIV infection among native and refugee Afghans

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The current study was conducted to explore the origins of the HIV epidemics among the Afghan refugees in Pakistan and the native Afghans in Afghanistan. Phylogenetic analysis of HIV gag gene from 40 samples showed diverse HIV variants, originating from a number of countries. Intermixing of diverse HIV variants among Afghans may give rise to seeding of infections with rare HIV strains which may pose serious challenges for the treatment and control of infection.

Transmigration of infected populations can result in transmission of new HIV-1 variants into the host population. Additionally, intermixing of the pre-existing and newly transmitted HIV variant can give rise to novel circulating recombinant forms (CRFs) and subtypes. Due to nearly 30 years of conflict in Afghanistan, many Afghans have been forced into migration, mostly to Pakistan and Iran [1,2]. In our earlier studies, we have observed a 6% prevalence of HIV among Afghan refugees in Pakistan, with drug use being a common high-risk behavior [3]. Our previous studies on high-risk groups of injecting drug users (IDUs) [4] and men who have sex with men (MSM) (unpublished) have revealed the occurrence of HIV subtype A along with subtype G, CRF01_AE, and CRF02_AG to be circulating within Pakistan. In Afghanistan, a novel recombinant CRF35_AD has been reported as the main genetic variant in this population [5]. The current study was conducted to characterize and compare the HIV epidemics present among the Afghan refugees in Pakistan and the native Afghans in Kabul, Afghanistan.

Samples were obtained through ‘convenience sampling’ of patients attending antenatal clinics and free health camps organized by the Infection Control Society, Pakistan, for the screening of viral infections. After initial screening of 556 samples, a total of 29 HIV-positive samples from the Afghan refugees in Pakistan, and 11 from native Afghans in Kabul, Afghanistan, were included in this study. Samples from Pakistan were collected from Afghan refugee populations residing in Karachi. To investigate the transmission patterns of HIV among Afghans and other high-risk groups, HIV gag sequences from 26 IDUs and 47 MSM from Pakistan were also included. The study was approved by the Ethical Review Committee, Aga Khan University, Karachi, Pakistan, and Ministry of Public Health, Afghanistan. DNA was extracted from the blood samples using QIAamp DNA Blood Mini Kit from Qiagen (Hilden, Germany). PCR amplification of complete gag gene of HIV-1 was performed and the product was partially sequenced using the primer GSP1 (5’-CCATCAATGAGGAAGCTGC-, nt 1400–1418, HXB2) as described elsewhere [6]. Alignments were obtained by the Clustal X program (1.83). Mega 4.0 was used to determine phylogenetic relationships of the aligned sequences using Neighbor-joining and maximum parsimony methods. In order to analyze the recombinant strains, National Center for Biotechnology Information (NCBI) genotyping tool with HIV-1 2009 ref-set was utilized from the NCBI website (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi).

Afghan refugees reported the following high-risk behaviors: use of inhaled and injected drugs, having multiple sex partners, homosexuality, contact with sex workers and travel abroad. Phylogenetic analysis revealed that, within the Afghan refugee population, the majority, that is, 14 out of 29 (48.2%), were subtype A; whereas six (20.6%), five (17.2%), and four (13.7%) were, respectively, CRF02_AG, CRF01_AE, and CRF35_AD. In the native Afghan samples, three (27.2%) were subtype G, two (18.1%) subtype A, one (9.09%) subtype B, three (27.2%) CRF35_AD, one (9.09%) CRF03_AB, and one was (9.09%) CRF43_02G (data not shown). The phylogenetic origins of some of these strains, namely, B, AB, and CRF35_AD, were found in, respectively, Netherlands, Russia, and Afghanistan, consistent with the travel history of the study participant or his/her sex partner (data not shown).

Next, we explored potential links between the HIV infection in Afghan refugees and that in previously studied Pakistani cohorts of IDUs [7] and MSM (unpublished). Several overlapping clusters of MSM and Afghan refugees were observed, indicating a transmission link between these two cohorts (Fig. 1a). A few of the native Afghan samples also showed close phylogenetic association between the HIV sequences from Afghan refugees and MSM (Fig. 1a). A number of MSM and Afghans in these clusters admitted to drug abuse (marked by black dots in the tree), implicating needle-sharing as the likely mode of HIV transmission among these groups. The phylogenetic analysis in Fig. 1a shows that whereas Afghan HIV sequences closely associate with the MSM sequences, they remain discrete from the IDU cluster. We speculate that the route of HIV...
Fig. 1. Transmission of HIV-1 among high-risk groups in Pakistan and relationship among Iran, Pakistan and Afghanistan. (a) Sequences from our study population of refugee prefix AR and native prefix AFG Afghans were analyzed and compared with IDU (prefix PK–IDU) and MSM (prefix M-) sequences from Pakistan. Those participants who reported involvement in injecting drug use behavior are marked with black dots. Black squares and circles indicate bootstrap values of, respectively, >85 and >50. (b) Phylogenetic relationship of HIV-1 strains among MSM and Afghan refugees, AR in Pakistan compared to strains from Afghanistan, AFG and Iran prefix IR. Iran sequences were obtained from Los Alamos HIV Database. MSMs, Afghan refugees and Afghanistan sequences were obtained from our own collection of HIV-1 sequences. All sequences are 284 bp in length and span the p24 region of gag gene. Black squares and circles indicate bootstrap values of, respectively, >85 and >50.
Fig. 1. (Continued).
transmission among these communities is likely to be: IDUs → MSM → Afghan refugees. Finally, looking at our analysis of high-risk groups within Pakistan, we observed that MSM sequences were also clustering nearby Afghanistan (AFG) and refugee (AR) sequences (Fig. 1a). This cluster of AFG and AR sequences was subtyped as CRF35-AD in this study. The same phylogenetic analysis also showed that MSM sequences, M-366, M-109, M-205, M-428 and M-429, had viral strains homologous to the Afghanistan and refugee subtype AD sequences. It may therefore be speculated that the HIV strain CRF35_AD, which is highly prevalent in Afghanistan, is crossing borders into Pakistan’s high-risk populations.

To explore potential links between the HIV epidemics in Pakistan and Iran, MSM and Afghan refugee sequences representing Pakistani population were aligned with HIV sequences from Kabul, Afghanistan and 11 previously reported Iranian sequences from IDUs in the city of Mashhad, Iran [8], downloaded from the Los Alamos Laboratory Database. Pakistan and Iran are two countries hosting the largest Afghan refugee populations in the world [9]. In our analysis of Iran, Pakistan and Afghanistan HIV-1 sequences, we observed a close phylogenetic relationship between the Iranian cohort of IDUs and our Kabul (labeled AFG) sequences (Fig. 1b). Our phylogenetic tree showed AFG-22 and AFG-26 embedded within a large cluster of Iranian sequences and AFG-19 grouping with two Iranian strains. Incidentally, both the participants AFG-22 and AFG-19 reported to injecting drugs and travelling to Iran; participant behavior and history supporting the association depicted in the phylogenetic analysis.

Our findings have revealed associations between our study populations of native and refugees Afghans and the high-risk groups in Pakistan and Iran. Frequent travel, past displacement and current repatriation of millions of Afghans has now put the Afghan population at risk of infection with novel, possibly drug-resistant HIV viral strains. Treatment for such diverse subtypes and CRFs may prove challenging for the development of effective vaccines and antiretroviral therapies towards such unique viruses.

Acknowledgements

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References


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Secondary hyperparathyroidism in HIV patients: is there any responsibility of highly active antiretroviral therapy?

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Secondary hyperparathyroidism may develop in the presence of hypovitaminosis D in order to maintain calcium homeostasis. We conducted a cross-sectional analysis in a cohort of 371 patients,
identifying secondary hyperparathyroidism in 65 patients. This high prevalence (17.5%) was in part justified by the high prevalence of hypovitaminosis D (77.4%) in the whole sample, but we also identified an independent association with the use of tenofovir.

Comorbidities and long-term toxicities heavily affect the quality of life of HIV patients. They can experience a premature aging due to chronic inflammation as well as bone metabolism disorders [1]. International literature reports an increased rate of osteopenia and osteoporosis in HIV patients [2] compared with control groups, identifying a complex pathogenetic interaction involving viral infection, common risk factors and antiretroviral therapy. The clinically relevant consequence of such a disease is an increased rate of pathological fracture among patients [3].

HIV patients on highly active antiretroviral therapy (HAART) have a high frequency of vitamin D deficiency (VDD), suggesting a role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) through the inhibition of a central step of vitamin D metabolism [4]. VDD may lead to secondary hyperparathyroidism, increased bone turnover and reduced bone mineralization.

Secondary hyperparathyroidism develops in order to normalize low levels of serum calcium [5]. This may happen when calcium deficiency (low diet input or excessive urine loss), VDD (malabsorption, deficitary intake, rickets) or phosphorus metabolism disorders take place.

Two recently published papers have shown an independent and direct relation between parathormone (PTH) and tenofovir (TDF) use, suggesting a possible new trigger for secondary hyperparathyroidism in HIV patients on HAART [6,7].

We investigated the prevalence and factors associated with secondary hyperparathyroidism in HAART-experienced patients considering the impact on bone metabolism through dual-energy X-ray absorptiometry (DXA) and biochemical markers of bone turnover.

We enrolled all consecutive HIV-infected HAART-experienced patients, aged at least 18 years, attending an outpatient clinic in Milan between 20 April 2007 and 21 July 2010 who agreed to enter this observational study.

We performed a cross-sectional analysis considering immunovirological parameters, serum levels of 25-OH vitamin D, PTH, ionized calcium, osteocalcin, serum C-terminal telopeptide of type I collagen (CTX), HAART treatment and bone mineral density (BMD) at lumbar spine and right femur with DXA. Data about CTX were available for 194 patients (cut-off value 571 pg/ml for women and 584 pg/ml for men). Patients with primary hyperparathyroidism (defined by PTH >65 pg/ml and serum ionized calcium >1.30 mg/dl) were excluded from the study. We also excluded patients with impaired renal function (glomerular filtration rate <60 ml/min evaluated with the Cockroft–Gault formula).

In the univariate analysis, the t-test or the Mann–Whitney U-test were used to compare continuous variables as appropriate. For categorical variable, we used chi-square or Fisher’s exact tests. Pearson’s or Spearman’s correlation coefficient was used to evaluate the correlation between normally and not normally distributed variables. To account for the effects of several factors simultaneously, we used unconditional multiple logistic regression, with maximum likelihood fitting, to obtain odds ratios (ORs) and their corresponding 95% confidence intervals (CIs), as estimates of the association between hyperparathyroidism and the variables of interest.

Three hundred and seventy-one patients without primary hyperparathyroidism were included. Their mean age was 47 years (range 29–75) and men comprised 57.1%. Secondary hyperparathyroidism (>65 pg/ml) was detected in 65 patients (17.5%) and VDD (<30 ng/dl) was detected in 287 (77.4%) patients. Two hundred and fourteen (57.7%) patients were on TDF treatment, 232 (62.5%) on protease inhibitors and 113 (30.5%) on NNRTI treatment. VDD affected the 74.3% of patients on TDF and 81.5% of patients on other treatments (P = 0.10). BMD was 0.990 and 0.992 g/cm² (P = 0.93) in TDF-treated patients vs. others. In patients with normal values of vitamin D, BMD was 1.007 vs. 0.986 g/cm² in patients with hypovitaminosis (P = 0.34).

At the univariate analysis, we found a significant direct relationship between secondary hyperparathyroidism and age, use of TDF, season and BMI. No association was found with protease inhibitor and NNRTI use. An inverse correlation emerged between values of PTH and vitamin D (r = −0.35, P < 0.0001). Biochemical markers of bone turnover were significantly higher in patients with secondary hyperparathyroidism. When age, sex, BMI, season (winter/spring vs. summer/fall) and VDD were included in the multivariate model, we confirmed the association between secondary hyperparathyroidism and TDF use (OR 3.2, 95% CI 1.6–6.3), age (by 5 years, OR 1.5, 95% CI 1.2–1.8), female sex (OR 2.6, 95% CI 1.4–5.0), BMI above 25 (OR 2.9, 95% CI 1.6–3.5), season (winter/spring, OR 2.4, 95% CI 1.2–4.6), 25-OH vitamin D below 30 ng/ml (OR 3.2, 95% CI 1.2–8.8), increased levels of CTX (OR 3.0, 95% CI 1.2–7.7) and osteocalcin (by 5 ng/ml, OR 1.2, 95% CI 1.1–1.4).

Patients with secondary hyperparathyroidism had a lower lumbar spine BMD (0.962 vs. 0.996) and right femur
BMD (0.895 vs. 0.929); these differences were not statistically significant in the univariate and multivariate models (Table 1).

The main result of our study was the observed association between TDF use and secondary hyperparathyroidism. As expected, our analysis confirms the association between PTH and vitamin D. Even though the influence of the high prevalence of VDD (77.4%) on PTH is evident, an independent effect of TDF use emerged. The mechanism underlying this association is not clear but if we assume that VDD and TDF use affect simultaneously the PTH level, the lower BMD in patients with secondary hyperparathyroidism currently on TDF suggests a careful approach in clinical practice. The lack of statistical significance may imply a complex interaction on calcium and phosphorus metabolism between hormonal axes and iatrogenic effects. Whatever the primary cause may be, it seems that this double interaction may affect bone metabolism through an activation of bone turnover, supported by the association between serum osteocalcin, CTX and secondary hyperparathyroidism.

Our study has limitations. Firstly, the cross-sectional design of the study does not allow us to analyze changes over time of the variables of interest, in particular HAART exposure. Secondly, we lack data about tubular dysfunction and phosphaturia, which are known to be affected by both PTH and TDF. Thirdly, data about CTX were not available for the whole sample.

Clinical implications of these data could be of great importance, suggesting a periodic monitoring of vitamin D and PTH levels in patients treated with TDF and a prompt correction of VDD detected in patients with secondary hyperparathyroidism, particularly those on TDF treatment.

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References


Table 1. Multivariate analysis according to parathormone status.

<table>
<thead>
<tr>
<th>Parathormone</th>
<th>≤ 65 (n = 306, 82.5%)</th>
<th>&gt; 65 (n = 65, 17.5%)</th>
<th>Total (N = 371)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>181 (59.2)</td>
<td>31 (47.7)</td>
<td>212 (57.1)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>125 (40.8)</td>
<td>34 (52.3)</td>
<td>159 (42.9)</td>
<td></td>
</tr>
<tr>
<td>Age, (years) mean (SD)</td>
<td>46.7 (7.1)</td>
<td>50.1 (9.6)</td>
<td>47.3 (7.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI, (kg/m²) mean (SD)</td>
<td>24.2 (4.6)</td>
<td>26.3 (5.3)</td>
<td>24.6 (4.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>HIV RNA log10, mean (SD)</td>
<td>1.89 (0.57)</td>
<td>1.45 (1.42)</td>
<td>1.93 (0.65)</td>
<td>0.18</td>
</tr>
<tr>
<td>CD4⁺ cell count (cells/µl), mean (SD)</td>
<td>562 (283)</td>
<td>532 (198)</td>
<td>556 (269)</td>
<td>0.80</td>
</tr>
<tr>
<td>Season [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>67 (21.9)</td>
<td>19 (29.2)</td>
<td>86 (23.2)</td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>84 (27.4)</td>
<td>28 (43.1)</td>
<td>112 (30.2)</td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>66 (21.6)</td>
<td>4 (6.2)</td>
<td>70 (18.9)</td>
<td></td>
</tr>
<tr>
<td>Fall</td>
<td>89 (29.1)</td>
<td>14 (21.5)</td>
<td>103 (27.8)</td>
<td>0.004</td>
</tr>
<tr>
<td>25(OH)D &lt;30 ng/ml, n (%)</td>
<td>227 (74.2)</td>
<td>60 (92.3)</td>
<td>287 (77.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>Ostecalcin, mean (SD)</td>
<td>22.9 (9.9)</td>
<td>28.7 (16.9)</td>
<td>24.7 (11.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>CTX (&gt;571 female, &gt;584 male)</td>
<td>32 (22.5)</td>
<td>15 (42.9)</td>
<td>47 (25.7)</td>
<td>0.01</td>
</tr>
<tr>
<td>LS BMD, mean (SD)</td>
<td>0.996 (0.139)</td>
<td>0.962 (0.177)</td>
<td>0.991 (0.145)</td>
<td>0.10</td>
</tr>
<tr>
<td>RF BMD, mean (SD)</td>
<td>0.929 (0.132)</td>
<td>0.895 (0.130)</td>
<td>0.924 (0.132)</td>
<td>0.16</td>
</tr>
<tr>
<td>TDF use, n (%)</td>
<td>165 (53.9)</td>
<td>49 (75.4)</td>
<td>214 (57.7)</td>
<td>0.002</td>
</tr>
<tr>
<td>PI use, n (%)</td>
<td>196 (64.0)</td>
<td>36 (55.4)</td>
<td>232 (62.5)</td>
<td>0.19</td>
</tr>
<tr>
<td>NNRTI use, n (%)</td>
<td>90 (29.4)</td>
<td>23 (35.4)</td>
<td>113 (30.5)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

25(OH)D, 25-hydroxyvitamin D; BMD, bone mineral density; CTX, serum C-terminal telopeptide of type I collagen; LS, lumbar spine; NNRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; RF, right femur; TDF, tenofovir.

*Adjusted in turn for age, sex, BMI, season and hypovitaminosis D as appropriate.


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