CCR5 Chemokine Receptor Polymorphism in Patients With HIV-1 From Western India

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To the Editors:

AIDS is a set of symptoms and infections resulting from the damage to the human immune system caused by HIV. The rate of disease progression depends on viral characteristics and host factors. Entry of HIV-1 into target cells requires both CD4 and chemokine receptors. A 32-nucleotide deletion (Δ32) within the β-chemokine CCR5 gene has been described in HIV-1 subjects who remain uninfected. We investigated the frequency of Δ32 deletion in CCR5 among HIV-1-infected patients and normal uninfected healthy individuals belonging to the same ethnic background. A total of 251 samples comprised 161 patients with HIV-1 and 90 normal healthy individuals from the same ethnic group. Genomic DNA from patients and control subjects was extracted and amplified for CCR5 gene segment using polymerase chain reaction. Amplified products were analyzed on 12% acrylamide gel, which yielded an 189-bp fragment for the wild-type allele and 157-bp fragment for the deleted (mutant) allele. Our results revealed patients with HIV-1 were homozygotous for ccr5Δ32 deletion (6.21%) and heterozygous for ccr5Δ32 deletion (0.67%) compared with 6.66% and 0% , respectively, in control subjects (Table 1). Genetic susceptibility to HIV infection has proven to be influenced by chemokine receptor gene polymorphisms culturing on chomosome 3p21. The chemokine receptors CCR2V64I, CCR5-Delta 32, CCR5 m303, CXCR4,(-2459)A CCR5, G190A CCR2,744A, CX3CR1, C838T, and SDF1-3A have been implicated in HIV. Homozygocity for the CCR5 (delta32/delta32) gene is associated with strong resistance against HIV infection. Hetrozygocity for CCR5 (delta32/wt) is associated with protection of HIV disease progression. Global survey for this genotype has revealed that 13% of whites have this mutant allele, whereas in Africans, Eurasians, and Indians, the allele ranges from 5% to 10%.[1] The CCR5 delta is a heterozygous deletion in Slovakian patients with HIV-1 (15.4%)[2] and heterozygous deletion in white patients with HIV (17.5%) and in Polish patients with HIV-1 (16.1%).[1] The CCR5 delta32 homozygous deletion is reported in a single German seroconverter,[3] not identified in North Indians,[4] but identified (6.21%) in our present investigation from Western Indians. Studies revealed that a second mutation in CCR5 that is a single base pair mutation, m303, introduces a stop codon that prevents cell surface expression of a functional CCR5 receptor. A recent study reported the CCR5Delta32 (2.8%) and CCR5m303 (0%) allele polymorphisms among Bahraini population.[5] Considering the ethnicity of the Indian subcontinent, CCR5 gene studies in patients with HIV-1 from other parts of India will give us more information. Our study shows that the CCR5 allele frequency varies in different ethnic groups and 7.84% of the patients with HIV-1 have not been protected although they have the presence of the homo- or heterozygous ccr532bp deletion.

TABLE 1. CCR5 Polymorphism in Patients With HIV-1 and Control Subjects

	Patients With HIV-1 (N = 161)		Control Subjects (N = 90)	
CCR5	N+	Percent	N+	Percent
wt/wt	150	93.16	84	93.33
wt/Δ32	1	0.62	0	0
Δ32/Δ32	10	6.21	6	6.66

REFERENCES

1. Parczewski M, Leszezszyn-Pynka M, Kaczmarczyk M, et al. Sequence variants of chemokine receptor genes and susceptibility to HIV-1 infection *J Appl Genet*. 2009;50:159–166.
2. Takacova M, Nogava P, Habekova M, et al. Prevalence of 32 bp deletion in the gene for human immunodeficiency virus1 co-receptor ccr5 in Slovak population. *Acta Virol*. 2008; 52:261–264.
3. Oh DY, Jessen H, Kucherer C, et al. CCR5Delta32 genotypes in German HIV-1 seroconverter cohort and report of HIV-1 infection in a CCR5delta 32 homozygous individual. *PLoS One*. 2008;3:e2747.
4. Kaur G, Singh P, Rapthap CC, et al. Polymorphism in the CCR5 gene promoter and HIV-1 infection in North Indians. *Hum Immunol*. 2007;68:454–461.
5. Salem AH, Farid E, Fadel R, et al. Distribution of four HIV type 1- resistance polymorphisms (CCR5-Delta32, CCR5-m3o3, CCR2-64I and SDF-3′A) in Bahraini population. *AIDS Res Hum Retroviruses*. 2009;25:973–977.

Determination of Kidney Function Before Tenofovir Initiation: Four-Fold Difference in Need of Tenofovir Dose Reduction Depending on Method Used

To the Editors:

We read with interest the article by Bygrave et al,[1] entitled "Implementing a tenofovir (TDF)-based first-line regimen in rural Lesotho: clinical outcomes and toxicities after two years". Given the recent guideline of the World Health Organization (WHO) to roll-out TDF-based first-line treatment in resource-constrained settings, this study provides important operational data. In this antiretroviral treatment (ART) program,

renal function was assessed before ART initiation. TDF was systematically withheld from patients with kidney dysfunction, defined as a creatinine clearance of <50 mL/minute. In contrast to this study, current WHO guidelines recommend TDF for patients with moderate kidney dysfunction, but at reduced dose.[2] Importantly, with any strategy chosen (systematic avoidance or dose reduction), adequate determination of kidney function will be needed for safe and effective use of TDF in resource-constrained settings. Relating to this, we would like to raise a concern with important practical implications for ART programs starting to implement TDF-based first-line treatment.

Although complicated tests exist to measure the glomerular filtration rate (GFR) directly, equations to estimate kidney function have been developed for routine clinical practice.[3] Traditionally, the Cockroft-Gault equation, requiring patient age, sex, weight and serum creatinine, has been most often used. More recently, the modification of diet in renal disease (MDRD) Study equation, requiring age, sex and race—for the abbreviated version—has gained importance.[3] Current WHO guidelines recommend to use the Cockroft-Gault equation and to increase TDF dosing interval to 48 hours for those with a creatinine clearance of 30–50 mL/minute, and to 72–96 hours in case of clearance of 10–30 mL/minute.[2] In case of severe dysfunction (clearance <10 mL/min), TDF use should be avoided. Both of the equation methods were developed based on Western populations. Data on its validity in resource-constrained settings, and specifically in HIV-patients, are lacking. We compared the prevalence of kidney dysfunction and the proportion of patients needing TDF dose reduction using these 2 equations in a large ART cohort in Cambodia.

In 2003, we started providing ART in a tertiary hospital in Phnom Penh, Cambodia.[4] From the onset, clinical and laboratory data were prospectively captured, after obtaining informed consent. Treatment was initiated in line with WHO guidelines. Baseline investigations included creatinine determination. We conducted a retrospective analysis of all antiretroviral-naive adult patients initiating ART between March 2003 and December 2010.

A total of 2625 patients were included, with a median age of 35 [interquartile range (IQR) 30–41] years. Fifty-two percent were female, the median baseline CD4 cell count was 87 (IQR 25–206) cells per microliter. At baseline, a median creatinine of 71 (IQR 60–82) μmol/L and 87 (IQR 71–107) μmol/L was documented for females and males, respectively (normal range <80 and 97 μmol/L for females and males). The estimated median baseline GFR was 74 mL/min with the Cockcroft-Gault equation and clearly higher (90 mL/min/1.73 m^2) using the MDRD Study equation (Table 1). Whereas TDF dose reduction was indicated for 10.1% of patients based on the Cockcroft-Gault equation, this was only 2.3% using the MDRD calculation. Severe kidney dysfunction, with TDF contraindicated, was rare (0.04% with both methods). To take into account differences in body habitus between individuals or populations, some have recommended to use Cockroft-Gault estimates standardized for body surface area (BSA), as is done for the MDRD Study equation.[5] Median BSA in our patient population was 1.47 (IQR 1.36–1.48) m^2, clearly lower than the "standard" of 1.73 m^2. Compared with the unadjusted equation, the BSA-adjusted Cockroft-Gault version provided clearly higher GFR estimates that were very similar to the MDRD Study equation.

Depending on the method used, a 4-fold difference in need of TDF dose reduction was observed when applying WHO dosing recommendations. This could lead to either overdosing—with potentially increased toxicity—or underdosing—facilitating the emergence of drug resistance—in a substantial proportion of patients. Future research is needed to determine the preferential method to be used for safe and effective use of this pivotal drug in resource-constrained settings. Standardisation of the Cockcroft-Gault equation for BSA should be explored. Meanwhile, avoidance of TDF for those with kidney dysfunction, as in the study by Bygrave et al,[1] might be an alternative careful strategy.

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TABLE 1. Estimated Glomerular Filtration Rate and WHO-Recommended TDF Dosing Interval Using Different Equations

	CG*	MDRD†‡	CG-BSA Adjusted‡
Median GFR (IQR), mL/min	74 (61–88)	90 (75–108)	87 (73–102)
GFR 30–50 mL/min (TDF 300 mg/48 hrs)§	9.4%	2.2%	2.3%
GFR 10–30 mL/min (TDF 300 mg/72-96 hrs)§	0.65%	0.15%	0.12%
GFR <10 mL/min (TDF avoidance)§	0.04%	0.04%	0.04%

*(140-age) × body weight (kg)/72 × serum creatinine (mg/dL) − (× 0.85 for females).
†MDRD: in mL/min per 1.73 m^2: 186.3 × [serum creatinine (mg/dL)] [exp (-1.154)] × Age [exp (-0.203)] × (0.742 if female) × (1.21 if black).
‡In mL/min per 1.73 m^2.
§WHO guidelines (2010).
CG, CockroftGault.

REFERENCES

1. Bygrave H, Ford N, van Cutsem G, et al. Implementing a tenofovir-based first-line regimen in rural lesotho: clinical outcomes and toxicities after two years. *J Acquir Immune Defic Syndr*. 2011;56:e75–e78.
2. World Health Organization.*Antiretroviral Therapy for HIV Infection in Adults and Adolescents: Recommendations for a Public Health Approach: 2010 Revision*. Geneva, Switserland: World Health Organization; 2010.
3. Stevens LA, Coresh J, Greene T, et al. Assessing kidney function–measured and estimated glomerular filtration rate. *N Engl J Med*. 2006;354: 2473–2483.
4. Thai S, Koole O, Un P, et al.Five-year experience with scaling-up access to antiretroviral treatment

in an HIV care programme in Cambodia. 2009; 14:1048–1058.
5. Rostoker G, Andrivet P, Pham I, et al. A modified Cockcroft-Gault formula taking into account the body surface area gives a more accurate estimation of the glomerular filtration rate. *J Nephrol*. 2007;20: 576–585.

Effect of Expanded HIV Testing Programs on the Status of Newly Diagnosed HIV-Infected Patients in Two Veterans Health Administration Facilities: 1999–2009

To the Editor:

INTRODUCTION

Earlier identification of HIV-infected patients reduces both mortality and the cost of treatment by keeping patients out of the hospital and encourages reduction of risk behaviors thus preventing further transmission.[1,2] Unfortunately, despite the promulgation of national and societal guidelines that recommend routinely offering HIV testing, an estimated 21% of the 1.1 million HIV-infected persons in the United States are unaware of their status.[3]

We have previously developed and implemented a multimodal intervention that more than doubled HIV testing rates in individuals receiving care at 2 Veterans Health Administration (VHA) medical facilities.[4] These results were robust with dramatic increases in test rates occurring across patient-level, provider-level, and subfacility level factors. Furthermore, the gains from this intervention were sustained after responsibility for maintenance of the program was largely transferred from the research implementation team to local staff.[5] Finally, the rate of positive tests remained constant in the year before and after implementation of the intervention and well within the range at which the cost of HIV testing is less than $50,000 per quality-adjusted life year.[4,6]

We now compare the characteristics of patients newly diagnosed with HIV infection before and after implementation of this intervention and provide a long-term perspective on the effectiveness of the program. In addition, we compare our results from those of another VHA facility that implemented only the computerized decision support element of this program.

METHODS

At site A, in July 2005, a multicomponent intervention that consisted of an electronic clinical reminder that prompted providers to offer HIV testing to persons with identifiable risk factors for HIV infection, streamlined HIV counseling processes, provider education and clinic-level feedback on HIV testing rates was implemented to promote HIV-testing.[4,5] At site B, the electronic clinical reminder component of this program was put in place in January 2008. At both sites written informed consent, which had been required for HIV testing, was replaced by verbal consent in August of 2009. Subsequently, the electronic clinical reminder was modified to recommend once per lifetime HIV testing in all adults regardless of the presence or absence of identified risk factors. This modification was implemented at site A in December 2010 and at site B in June 2010.

The results of HIV diagnostic tests and laboratory results were extracted from the electronic medical records at each site. For patients receiving care at both sites, other data elements were obtained via retrospective review of electronic medical records. At site B, in addition, all outside medical records were reviewed. Care was taken to discriminate between newly diagnosed patients and patients with confirmatory testing for diagnoses made at other facilities. Patients who had previously tested positive for HIV infection were excluded from all analyses.

The study was approved by the Institutional Review Boards at the VA Greater Los Angeles Healthcare System and at Emory University.

RESULTS

At site A, 9697 tests were done in the pre-intervention period (1763 tests per year) versus 31,116 tests in the postintervention period (5657 tests per year; Table 1). As opposed to the earlier period, in the postintervention period, at the time of diagnosis, patients were less often hospitalized (12.5 vs. 35.4%, $P = 0.001$) and less frequently had CDC category B or C conditions[7] (12.5% vs. 25.6%, $P = 0.018$) or fewer than 200 CD4 cells/μL (29% vs. 46%, $P = 0.029$). Although there was little change in the mean age at diagnosis, the proportion of newly diagnosed persons 60 years of age and older showed a nonsignificant increase from 7.5% to 15.3% ($P = 0.12$).

Similar results were found at site B, where 5432 tests were done in the pre-intervention period (1810 tests per year) versus 24,371 tests in the postintervention period (8124 tests per year; Table 1). At site B, postintervention patients were less likely to have CDC category B or C conditions (18.9% vs. 32.8%, $P = 0.016$), but similar number were hospitalized at the time of diagnosis in the 2 periods. The proportion of patients with fewer than 200 CD4+ cells per microliter at the time of diagnosis decreased from 43% to 29% ($P = 0.036$), and the proportion of patients 60 years of age and older was unchanged (20% vs. 22%).

DISCUSSION

Previous studies by our group demonstrated sustainable increases over a 2-year period of time in the rates of HIV testing after implementation of a multimodal program consisting of computerized decision support for HIV testing (ie, an electronic clinical reminder), provider education, feedback reports, and organizational changes to increase HIV testing.[4,5] The current findings establish the long-term sustainability of this intervention at site A (ie, after 2007 when prior analyses ceased[5]) and demonstrate similar increases in HIV testing in site B, a facility that implemented the electronic clinical reminder without other program elements.

One of the goals of expanded HIV testing programs is to identify patients

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