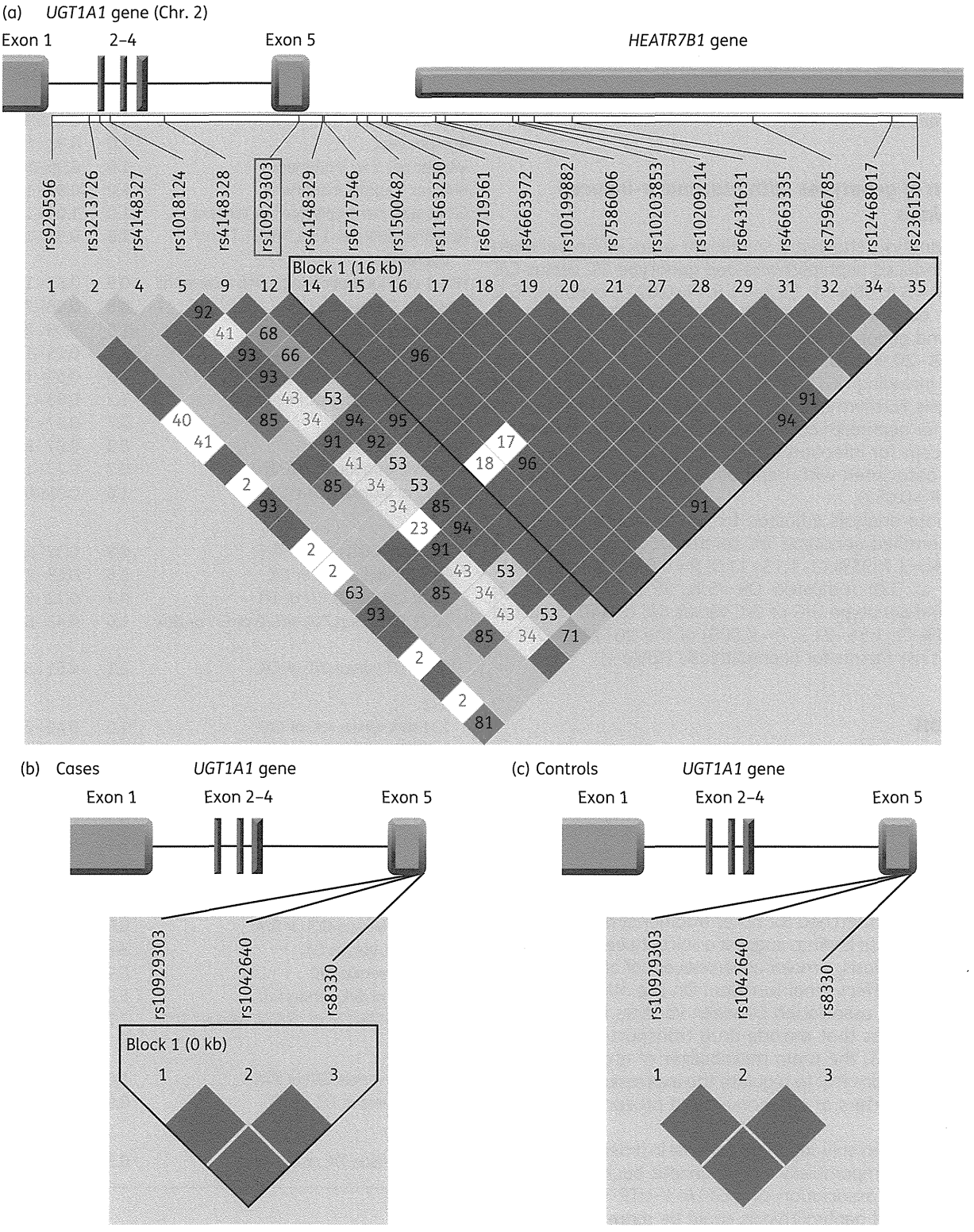

Figure 1. Pairwise linkage disequilibrium analysis of *UGT1A1* and surrounding SNPs. (a) Pairwise linkage disequilibrium analysis of *UGT1A1* and surrounding SNPs using HapMap Japanese samples. SNP c.211 (rs10929303) of the UGT1A-3′-UTR is in tight linkage disequilibrium with the gene next to *UGT1A1* (*HEATR7B1*). Two SNPs at 339 (rs1042640) and 440 (rs8330) of the UGT1A-3′-UTR are not shown in (a), but they are located close to c.211, as shown in (b) and (c). Pairwise linkage disequilibrium analysis of the three risk SNPs in the UGT1A-3′-UTR in (b) 31 cases (patients with atazanavir-induced nephrolithiasis) and (c) 47 controls. The difference between (b) and (c) suggests that the number of risk haplotypes is greater in case patients than in control patients. Estimates of *D′* for SNPs are shown as numbers in the Argyle box. Dark red shading indicates strong linkage disequilibrium (*D′* >0.9). Light blue shading indicates high *D*' values (>0.99) with low statistical significance [LOD (log of the odds) <2].

shows the results of pairwise linkage disequilibrium analysis of *UGT1A1* and SNPs around them derived from HapMap data for the Japanese. On the other hand, there was no difference in the distribution of 16 other SNPs in *ABCB1*, *NR1I2*, *SLCO1B1* and *CYP3A5* between cases and controls. The distribution of UGT1A1*28 was also not different.

Association of genotypes with atazanavir-induced nephrolithiasis

Univariate analysis showed a significant association between atazanavir-induced nephrolithiasis and genotype T/C versus C/C at c.211 (OR=3.8; 95% CI, 1.22–11.6; P=0.022), genotype G/C versus C/C at position 339 (OR=5.9; 95% CI, 1.68–20.9; P=0.006) and genotype G/G or G/C versus C/C at 440 (OR=5.9; 95% CI, 1.68–20.9; P=0.006) of the UGT1A-3′-UTR (Table 3). No other SNPs, including UGT1A1*28, showed any association with nephrolithiasis. Furthermore, basic demographics and established risk factors for nephrolithiasis were not associated with nephrolithiasis, except for infection with hepatitis C virus, which was marginally associated with nephrolithiasis (OR=8.8; 95% CI, 0.98–79.9; P=0.052).

Multivariate analysis adjusted for sex, age and hepatitis C infection identified genotype T/C versus C/C at position c.211 (adjusted OR=3.7; 95% CI, 1.13–11.9; P=0.030), genotype G/C versus C/C at 339 (adjusted OR=5.8; 95% CI, 1.56–21.3; P=0.009) and genotype G/G or G/C versus C/C at 440 (adjusted OR=5.8; 95% CI, 1.56–21.3; P=0.009) of the UGT1A-3′-UTR as independent risk factors for nephrolithiasis (Table 4).

Table 3. Univariate analysis to estimate the association of various factors with atazanavir-induced nephrolithiasis

	OR	95% CI	P value
Male	1.7	0.31–9.51	0.53
Age per year	1.0	0.93–1.03	0.39
Weight per 1 kg increment	1.0	0.95–1.03	0.60
BMI per 1 kg/m^2 increment	1.0	0.83–1.11	0.58
CD4 count per 1 cell/mm^3 increment	1.0	1.00–1.00	0.63
Baseline eGFR per 1 mL/min/1.73 m^2 decrement	1.0	0.98–1.03	0.80
HIV-1 viral load per 1 $\log_{10}$/mL increment	0.9	0.62–1.34	0.64
Hepatitis C infection	8.8	0.98–79.9	0.052
Hepatitis B infection	1.5	0.09–25.5	0.77
Treatment naive	0.7	0.25–1.66	0.37
History of nephrolithiasis	3.3	0.57–19.4	0.18
Uric acid per 1 mg/dL increment	1.2	0.93–1.56	0.16
Hypertension	0.7	0.17–3.17	0.68
Diabetes mellitus	0.8	0.07–8.64	0.82
Co-administration of tenofovir	0.7	0.27–1.92	0.51
History of indinavir use	1.6	0.30–8.34	0.60
ABCB1			
193 A/A versus A/G or G/G	0.8	0.32–1.97	0.61
365 T/T versus T/C or C/C	2.5	0.63–10.0	0.19
1236 C/C versus C/T or T/T	0.7	0.22–2.33	0.57
2677 T/T versus T/A or G/G or G/T or G/A or A/A	1.6	0.43–6.12	0.48
3435 T/T versus T/C or C/C	2.1	0.51–8.40	0.31
NR1I2			
131 A/A versus A/C or C/C	1.0	0.40–2.58	0.97
370 G/G versus G/A or A/A	0.7	0.25–1.84	0.44
522 C/C versus C/T or T/T	0.7	0.27–2.04	0.56
1195 C/C versus C/A or A/A	0.7	0.30–2.27	0.70
1232 C/C versus C/T or T/T	0.7	0.25–1.84	0.44
44477 C/C versus C/T or T/T	1.1	0.42–2.67	0.89
63396 C/C versus C/T or T/T	2.2	0.45–10.5	0.33
UGT1A1			
211 G/G versus G/A or A/A	0.9	0.35–2.29	0.82
c.211 T/C versus C/C	3.8	1.22–11.6	0.022
339 G/C versus C/C	5.9	1.68–20.9	0.006
440 G/G or G/C versus C/C	5.9	1.68–20.9	0.006
UGT1A1 *28/*28 or *28/*1 versus *1/*1	2.2	0.45–10.5	0.33
SLCO1B1			
388 G/G versus G/A or A/A	1.6	0.30–8.34	0.60
521 T/T versus T/C or C/C	0.9	0.36–2.43	0.90
CYP3A5			
14 T/T versus T/C or C/C	0.9	0.38–2.33	0.89

Discussion

To our knowledge, this is the first study that has elucidated the association between genetic polymorphisms in the genes encoding proteins that affect atazanavir exposure and atazanavir-induced nephrolithiasis. The results demonstrated that Japanese HIV-1-infected patients who developed atazanavir-induced nephrolithiasis were ~5-fold more likely to have variants in the UGT1A-3′-UTR, compared with those without nephrolithiasis, who were well-matched for other traditional risk factors for nephrolithiasis. These findings suggest a link between genetic factors and nephrolithiasis, a major adverse event of atazanavir that can significantly affect renal function. On the other hand, the results showed no association between variants in *ABCB1* and *SLCO1B1*, the genes that encode drug transporter protein for atazanavir, *CYP3A5*, the main metabolizer of atazanavir, and *NR1I2*, which encodes PXR to regulate the expression of metabolizers and transporters of atazanavir, and atazanavir-induced nephrolithiasis.

This study enrolled only Japanese patients in order to examine a population with comparatively similar genetic backgrounds. It is possible that the association of UGT1A-3′-UTR variants with atazanavir-induced nephrolithiasis could be more significant in people of African or European origin than Japanese or East Asians, considering that the allele frequencies of these variants are higher in these populations according to the HapMap data [e.g. minor allele frequency at position 440 (rs8330): Africans 50%, Europeans 23.3%, Japanese 15.9%, Chinese 15.6%] (www.hapmap.org). Similar studies are needed in these populations to confirm that the association between UGT1A-3′-UTR variants and atazanavir-induced nephrolithiasis is reproducible.

The mechanism by which SNPs in the UGT1A-3′-UTR are associated with the development of nephrolithiasis in patients on an atazanavir-containing regimen is unknown. However, Court

Table 4. Multivariate analysis to estimate the association of SNPs of the UGT1A-3′-UTR with atazanavir-induced nephrolithiasis

UGT1A-3′-UTR	Adjusted OR	95% CI	*P* value
Genotype T/C versus C/C at position c.211	3.7	1.13–11.9	0.030
Genotype G/C versus C/C at position 339	5.8	1.56–21.3	0.009
Genotype G/G or G/C versus C/C at position 440	5.8	1.56–21.3	0.009

Each SNP was tested in the model separately.
Each variable was adjusted for sex, age and hepatitis C infection.

et al.[32] reported that these SNPs are associated with inter-individual variability in acetaminophen (paracetamol) glucuronidation in the human liver, and provide protection against acute liver failure by acetaminophen overdose, probably through more extensive detoxification of acetaminophen via glucuronidation. Because the biotransformation pathways of atazanavir or its metabolites also include glucuronidation,[12] the UGT1A-3′-UTR variants could alter atazanavir metabolism and pharmacokinetics, resulting in increased atazanavir concentration in the blood and increased excretion in urine, facilitating nephrolithiasis formation. Unfortunately, serum and urine concentrations of atazanavir were not measured in the present study. It is also notable that the *UGT1* subfamily has a unique gene structure; the *UGT1* gene has 13 exon 1s from *UGT1A1* to *UGT1A13P*, and exons 2–5, which are common in all mRNAs expressed from the gene.[36] The UGT1A-3′-UTR is located in exon 5, which is commonly present in the UGT1 subfamily (Figure 1), and thus the variants in the UGT1A-3′-UTR might influence not only UGT1A1 but also other UGT1 isoforms that take part in glucuronidation of various substrates,[36] and they might affect atazanavir metabolism and pharmacokinetics as well. Figure 1 also shows that the identified SNPs in the UGT1 3′-UTR are in tight linkage disequilibrium with the gene next to them (*HEATR7B1*), suggesting that the latter could also affect atazanavir metabolism/transportation. To our knowledge, however, there is no information on the role of *HEATR7B1* in drug metabolism/transportation, and the above conjecture remains to be investigated.

In this study, the median serum total bilirubin level in the case patients was higher than that in the control group. Rockwood *et al.*[8] reported a close relationship between hyperbilirubinaemia and the development of atazanavir-induced renal stones. However, no such relationship was found in our previous cohort study.[6] In two pharmacokinetics studies, Rodríguez-Nóvoa *et al.*[20,29] reported that serum bilirubin level correlated with plasma atazanavir concentration, and one can speculate that high bilirubin levels might reflect higher atazanavir concentrations, which result in precipitation of atazanavir in urine and renal stone formation. However, these results are still preliminary and further studies are needed to determine the true relationship between serum bilirubin level and atazanavir-related nephrolithiasis.

Several limitations of this study need to be acknowledged. First, and importantly, although this study identified association between the UGT1A-3′-UTR variants and atazanavir-induced nephrolithiasis, the number of enrolled patients was small in this case–control study; the results need to be interpreted with caution. The results could provide the basis for an exploratory hypothesis and further larger studies are needed to confirm such an association. Second, not all polymorphisms in genes of the targeted proteins were examined. Thus, we might have missed other important SNPs associated with or affecting the metabolism or transportation of atazanavir. There might be other, unknown proteins that take part in the metabolism or transportation of atazanavir that also contribute to susceptibility to atazanavir-induced nephrolithiasis. Third, because renal stone formation occurs as a composite of various factors and the components of nephrolithiasis were not analysed in the study, it is difficult to exclude the effects of classic risk factors for renal stone formation, apart from the genetic factors identified in the present study. However, the two study samples were well matched in terms of risk factors, such as BMI, serum uric acid and history of indinavir use.[4,5,24–26] Furthermore, the susceptibility to nephrolithiasis in patients on an atazanavir/ritonavir-containing regimen is well established; the incidence of nephrolithiasis is 10- to 20-fold higher in patients on atazanavir/ritonavir-containing ART than in patients on other protease inhibitor-containing ART regimens.[6,7] Fourth, because functional data are not yet available, clinical or biochemical studies to confirm the results obtained here are certainly needed. We did not measure atazanavir concentration in blood or urine.

In conclusion, in a setting where other predisposing factors for nephrolithiasis were well matched, the present study demonstrates that the Japanese HIV-1-infected patients who developed atazanavir-induced nephrolithiasis were ~5-fold more likely to have variants in the UGT1A-3′-UTR compared with those without nephrolithiasis. Further studies are warranted to confirm this association and to elucidate how these SNPs might influence the metabolism and excretion of atazanavir and the formation of nephrolithiasis.

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Transparency declarations

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Pharmaceutical, Co., Roche Diagnostics K.K. and ViiV Healthcare, Co. All other authors: none to declare.

References

1 Panel on Antiretroviral Guidelines for Adults and Adolescents. *Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents*. American Department of Health and Human Services. http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf.

2 Chang HR, Pella PM. Atazanavir urolithiasis. *N Engl J Med* 2006; **355**: 2158–9.

3 Anderson PL, Lichtenstein KA, Gerig N *et al.* Atazanavir-containing renal calculi in an HIV-infected patient. *AIDS* 2007; **21**: 1060–2.

4 Chan-Tack KM, Truffa MM, Struble KA *et al.* Atazanavir-associated nephrolithiasis: cases from the US Food and Drug Administration's Adverse Event Reporting System. *AIDS* 2007; **21**: 1215–8.

5 Couzigou C, Daudon M, Meynard JL *et al.* Urolithiasis in HIV-positive patients treated with atazanavir. *Clin Infect Dis* 2007; **45**: e105–8.

6 Hamada Y, Nishijima T, Watanabe K *et al.* High incidence of renal stones among HIV-infected patients on ritonavir-boosted atazanavir than in those receiving other protease inhibitor-containing antiretroviral therapy. *Clin Infect Dis* 2012; **55**: 1262–9.

7 Nishijima T, Hamada Y, Watanabe K *et al.* Ritonavir-boosted darunavir is rarely associated with nephrolithiasis compared with ritonavir-boosted atazanavir in HIV-infected patients. *PLoS One* 2013; **8**: e77268.

8 Rockwood N, Mandalia S, Bower M *et al.* Ritonavir-boosted atazanavir exposure is associated with an increased rate of renal stones compared with efavirenz, ritonavir-boosted lopinavir and ritonavir-boosted darunavir. *AIDS* 2011; **25**: 1671–3.

9 Rule AD, Bergstralh EJ, Melton LJ 3rd *et al.* Kidney stones and the risk for chronic kidney disease. *Clin J Am Soc Nephrol* 2009; **4**: 804–11.

10 Jungers P, Joly D, Barbey F *et al.* ESRD caused by nephrolithiasis: prevalence, mechanisms, and prevention. *Am J Kidney Dis* 2004; **44**: 799–805.

11 Alexander RT, Hemmelgarn BR, Wiebe N *et al.* Kidney stones and kidney function loss: a cohort study. *BMJ* 2012; **345**: e5287.

12 Reyataz (atazanavir sulfate): full prescription information (package insert). Princeton, NJ: Bristol-Myers Squibb, 2012.

13 Crixivan (indinavir sulfate): full prescription information (package insert). Whitehouse Station, NJ: Merck & Co., Inc., 2012.

14 Wempe MF, Anderson PL. Atazanavir metabolism according to CYP3A5 status: an in vitro-in vivo assessment. *Drug Metab Dispos* 2011; **39**: 522–7.

15 Kile DA, MaWhinney S, Aquilante CL *et al.* A population pharmacokinetic-pharmacogenetic analysis of atazanavir. *AIDS Res Hum Retroviruses* 2012; **28**: 1227–34.

16 Lehmann JM, McKee DD, Watson MA *et al.* The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest* 1998; **102**: 1016–23.

17 Geick A, Eichelbaum M, Burk O. Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem* 2001; **276**: 14581–7.

18 Siccardi M, D'Avolio A, Baietto L *et al.* Association of a single-nucleotide polymorphism in the pregnane X receptor (PXR 63396C→T) with reduced concentrations of unboosted atazanavir. *Clin Infect Dis* 2008; **47**: 1222–5.

19 Kis O, Zastre JA, Hoque MT *et al.* Role of drug efflux and uptake transporters in atazanavir intestinal permeability and drug-drug interactions. *Pharm Res* 2013; **30**: 1050–64.

20 Rodríguez-Nóvoa S, Barreiro P, Rendón A *et al.* Plasma levels of atazanavir and the risk of hyperbilirubinemia are predicted by the 3435C→T polymorphism at the multidrug resistance gene 1. *Clin Infect Dis* 2006; **42**: 291–5.

21 Tozzi V. Pharmacogenetics of antiretrovirals. *Antiviral Res* 2010; **85**: 190–200.

22 Ma Q, Brazeau D, Zingman BS *et al.* Multidrug resistance 1 polymorphisms and trough concentrations of atazanavir and lopinavir in patients with HIV. *Pharmacogenomics* 2007; **8**: 227–35.

23 Hartkoorn RC, Kwan WS, Shallcross V *et al.* HIV protease inhibitors are substrates for OATP1A2, OATP1B1 and OATP1B3 and lopinavir plasma concentrations are influenced by SLCO1B1 polymorphisms. *Pharmacogenet Genomics* 2010; **20**: 112–20.

24 Parmar MS. Kidney stones. *BMJ* 2004; **328**: 1420–4.

25 Chan-Tack KM, Edozien A. Ritonavir-boosted atazanavir may be efficacious in HIV-infected patients concurrently receiving omeprazole. *Clin Infect Dis* 2006; **42**: 1344.

26 Ando R, Nagaya T, Suzuki S *et al.* Kidney stone formation is positively associated with conventional risk factors for coronary heart disease in Japanese men. *J Urol* 2013; **189**: 1340–6.

27 Taylor EN, Stampfer MJ, Curhan GC. Diabetes mellitus and the risk of nephrolithiasis. *Kidney Int* 2005; **68**: 1230–5.

28 Levey AS, Coresh J, Greene T *et al.* Using standardized serum creatinine values in the Modification of Diet in Renal Disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006; **145**: 247–54.

29 Rodríguez-Nóvoa S, Martín-Carbonero L, Barreiro P *et al.* Genetic factors influencing atazanavir plasma concentrations and the risk of severe hyperbilirubinemia. *AIDS* 2007; **21**: 41–6.

30 Park WB, Choe PG, Song KH *et al.* Genetic factors influencing severe atazanavir-associated hyperbilirubinemia in a population with low UDP-glucuronosyltransferase 1A1*28 allele frequency. *Clin Infect Dis* 2010; **51**: 101–6.

31 Takeuchi K, Kobayashi Y, Tamaki S *et al.* Genetic polymorphisms of bilirubin uridine diphosphate-glucuronosyltransferase gene in Japanese patients with Crigler-Najjar syndrome or Gilbert's syndrome as well as in healthy Japanese subjects. *J Gastroenterol Hepatol* 2004; **19**: 1023–8.

32 Court MH, Freytsis M, Wang X *et al.* The UDP-glucuronosyltransferase (UGT) 1A polymorphism c.2042C>G (rs8330) is associated with increased human liver acetaminophen glucuronidation, increased UGT1A exon 5a/5b splice variant mRNA ratio, and decreased risk of unintentional acetaminophen-induced acute liver failure. *J Pharmacol Exp Ther* 2013; **345**: 297–307.

33 Hirakawa M, Tanaka T, Hashimoto Y *et al.* JSNP: a database of common gene variations in the Japanese population. *Nucleic Acids Res* 2002; **30**: 158–62.

34 Rotger M, Taffe P, Bleiber G *et al.* Gilbert syndrome and the development of antiretroviral therapy-associated hyperbilirubinemia. *J Infect Dis* 2005; **192**: 1381–6.

35 Beutler E, Gelbart T, Demina A. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: a balanced polymorphism for regulation of bilirubin metabolism? *Proc Natl Acad Sci USA* 1998; **95**: 8170–4.

36 Maruo Y, Iwai M, Mori A *et al.* Polymorphism of UDP-glucuronosyltransferase and drug metabolism. *Curr Drug Metab* 2005; **6**: 91–9.

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Short Report: Asymptomatic Intestinal Amebiasis in Japanese HIV-1–Infected Individuals

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Abstract. Seventy-one asymptomatic human immunodeficiency virus-1 (HIV-1) -infected individuals who underwent colonoscopy for detection of diseases other than amebiasis were included in this study. Ulcerative lesions caused by *Entamoeba histolytica* were identified by colonoscopy and biopsy in 11.3% (8 of 71) of individuals. Stool microscopic examination hardly identified *Entamoeba*, whereas serum antibody against *E. histolytica* was often elevated in patients with subclinical intestinal amebiasis. Human leukocyte antigen (HLA) class II allele against *E. histolytica* infection (DQB1*06:01) was frequently identified in these patients. This study emphasizes the endemic nature of *E. histolytica* infection in our cohort and the difficulties in epidemiological control.

INTRODUCTION

Invasive amebiasis caused by *Entamoeba histolytica* is the second most common cause of parasite infection-related mortality worldwide, accounting for 40,600 to 73,800 deaths annually.[1] Recent studies indicated that invasive amebiasis is prevalent in not only developing countries, where food or water is contaminated with stool, but also, East Asian developed countries, including Japan, as a sexually transmitted infection.[2–5] We reported previously high seropositivity for *E. histolytica* among asymptomatic human immunodeficiency virus-1 (HIV-1)-infected individuals in Japan and showed relatively high incidence of invasive amebiasis in that population, probably because of exacerbation of subclinical infection.[6] Other groups also reported that serum antibody against *E. histolytica* can be elevated, even in asymptomatic-infected individuals, and that seroconversion was seen in the absence of any symptoms in longitudinal follow-up in endemic areas.[7] These results indicate that subclinical infection of *E. histolytica* is frequent in high-risk populations, making it difficult to control *E. histolytica* endemicity.

Evidence suggests that human leukocyte antigen (HLA) type plays a role in amebiasis. For example, Duggal and others[8] reported previously that HLA DQB1*0601 seemed to provide protection against *E. histolytica* infection in Bangladeshi children.

This cross-sectional study was designed to determine the prevalence of ulcerative lesions associated with *E. histolytica* infection in asymptomatic HIV-1–infected individuals in Japan. We also examined the pathogenesis of subclinical intestinal amebiasis and the role of HLA genotypes.

MATERIALS AND METHODS

Ethics statement. The study was approved by the Human Research Ethics Committee of our hospital, the National Center for Global Health and Medicine in Tokyo. The study was conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all participants. No children were included in the study.

Study design and participants. This cross-sectional study included HIV-infected patients who underwent colonoscopy between June of 2010 and June of 2013. One week before colonoscopy, each patient filled out a questionnaire about lower gastrointestinal symptoms based on the Gastrointestinal Symptom Rating Scale (GSRS) rating on a seven-graded Likert scale.[9] Asymptomate for lower gastrointestinal diseases was defined as GSRS scores of one or two for three questions on the diarrhea syndrome domain (diarrhea, loose stools, and urgent need to defecate) and one question on bloody stool.[10] Serum antibody testing against *E. histolytica* was performed in all participants on the day of colonoscopy. Serum antibody was tested by indirect fluorescent antibody assay using whole *E. histolytica* antigen according to the protocol described in the instruction sheet of the approved kit (bioMerieux, SA). Seropositivity was defined as positive response in a serum sample diluted at 1:100 ($\times 100$), and anti-Eh titer was determined by the highest dilution for the positive response. HLA type was determined by standard sequence-based genotyping (HLA Laboratory, Kyoto, Japan). The diagnosis of subclinical intestinal infection of *E. histolytica* was established on confirmation of one or two of the following two criteria: (1) identification of amebic trophozoites in biopsy specimens from gross ulcerative lesions obtained during colonoscopy and/or (2) no pathogens identified in biopsy specimens of gross ulcerative lesion, which were compatible with amebic ulcer,[11] but ulcerative lesion resolved completely after metronidazole monotherapy as confirmed by colonoscopy.

Statistical analysis. The patients' characteristics and serum positivities for anti-*E. histolytica* antibody were compared using χ^2 or Mann–Whitney U test for qualitative or quantitative variables, respectively. Statistical significance was defined as two-sided P value < 0.05. All statistical analyses were performed using The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL).

RESULTS

Study population. In total, 380 HIV-1–infected individuals were enrolled during the study period, and 71 patients met the criteria of no symptoms for lower gastrointestinal diseases according to the GSRS. The most common reason for colonoscopy was colorectal cancer screening ($N = 48$), whereas

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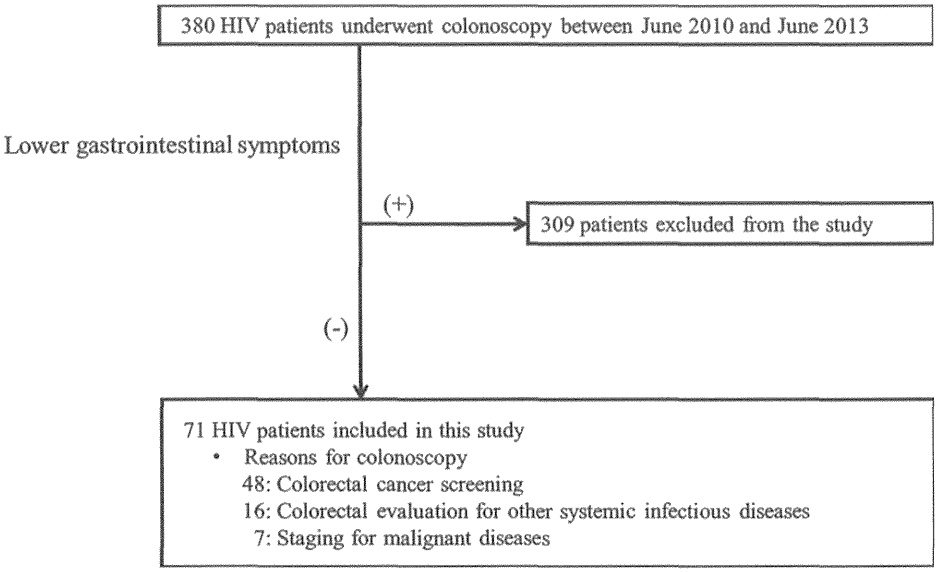

FIGURE 1. Flow diagram of the patient recruitment process. Lower abdominal symptoms were collected based on the GSRS rating on a seven-graded Likert scale at 1 week before colonoscopy.

the other 23 patients underwent colonoscopy for evaluation of progression of malignancies or infections (e.g., malignant lymphoma, Kaposi's sarcoma, tuberculosis, and cytomegalovirus) (Figure 1).

Frequency of intestinal amebic infection among asymptomatic HIV-1–infected individuals. Amebic colitis was confirmed in eight (11.3%) cases. Gross ulcerative lesions were identified by colonoscopy in all eight cases. Amebic trophozoite was identified in the biopsy specimens of five cases (Figure 2). Although amebic trophozoites were not identified in the biopsy specimens of the other three cases, their sera were positive for antibody against *E. histolytica*. In all patients, the ulcerative lesions resolved completely after metronidazole monotherapy.

Clinical features and presentation of patients with and without intestinal amebic infection. As shown in Table 1, patients with amebic intestinal ulcerative lesions tended to be younger, be male homosexuals, have low CD4 counts, and have high HIV-RNA levels, although these differences were not statistically significant. Multiple ulcerative lesions were found in four cases (50%), and the most frequently involved location was the cecum (five cases; 62.5%). Serum antibody against *E. histolytica* was positive in 7 of 8 (87.5%) patients with amebic intestinal ulcerative lesions compared with positivity in only 11 of 63 (17.5%) patients without amebic ulcerative lesions (Table 2).

From the limited data on fecal occult blood testing (FOB) and stool microscopic examination before treatment in cases with amebic ulcerative lesions, FOB was positive in two of three cases (66.7%), and the cyst form, not trophozoite form, *Entoamoeba* was found in only one of four cases (25%).

HLA class II allele frequencies in patients with and without subclinical intestinal amebiasis. HLA data were available for 57 patients (7 of 8 patients with amebiasis and 50 of 63 patients without amebiasis) in our study. We investigated the relation between HLA alleles identified in more than five patients (frequency > 10%) and subclinical intestinal amebiasis. HLA DQB1*06:01 allele was significantly more frequent in patients with subclinical intestinal amebiasis than those without it (Table 3). All the HLA DQB1*06:01 holders were heterozygotes. The frequency of the HLA DRB1*15:02 allele was also significantly higher in patients with subclinical intestinal amebiasis ($P = 0.05$); 7 of 10 patients with HLA DQB1*06:01 also held HLA DRB1*15:02. No colonic amebic ulceration was detected in DQB1*06:01 (–)/DRB1*15:02 (+) patients. Thus, DQB1*06:01 seemed to be the primary HLA allele associated with subclinical intestinal amebiasis in the study population.

DISCUSSION

The pathogenesis of amebiasis remains unclear, including the incubation period after cyst ingestion and the mechanism of spontaneous remission. We reported previously high seroprevalence of *E. histolytica* (21.3%) in HIV-1–infected individuals and that the majority of these patients (78.3%) had no history of invasive amebiasis. In that study, the patients were considered to be at high risk for developing symptomatic amebic infection in longitudinal follow-up (about 20% within the first 1 year of the follow-up period).[6] Based on those results, we speculated the presence of subclinical intestinal amebiasis in patients positive for antibody against *E. histolytica* in the serum resulting in high frequency of symptomatic amebic diseases thereafter, although we did not identify the lesions of *E. histolytica* in these individuals in that study. However, Okamoto and others[12] reported that intestinal ulcerative lesions of *E. histolytica* were rare based on colonoscopic examination in the general population in Japan with positive FOB (0.1%; 4 of 5,193). Our group reported previously that patients with cecal amebic ulcers were sometimes asymptomatic.[11] In this regard, however, the clinical significance of *E. histolytica* infection in asymptomatic individuals had not been fully assessed. In this study, we identified gross amebic ulcers by colonoscopy in 11.2% of asymptomatic HIV-1–infected individuals.

Detection of intestinal amebiasis in asymptomatic individuals is important for not only treatment but also, epidemiological control, especially in endemic areas, because individuals

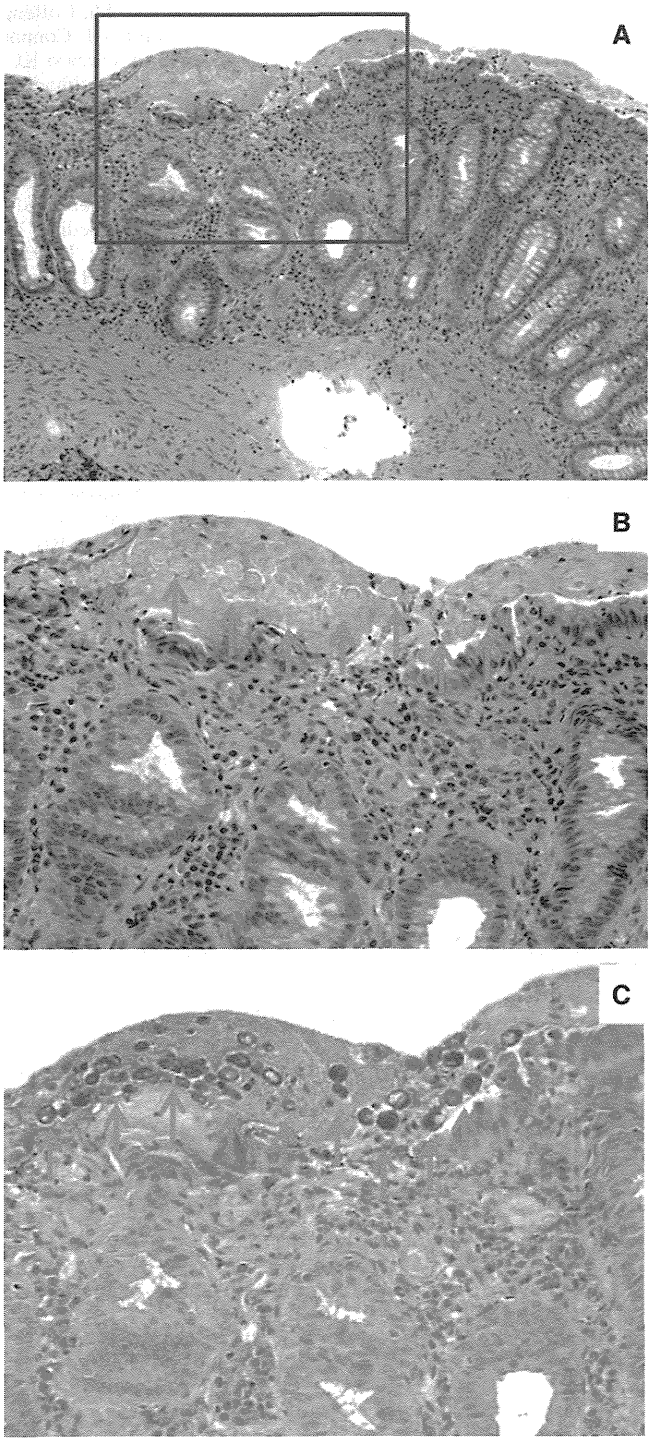

FIGURE 2. Histopathological findings in subclinical intestinal amebiasis. Colonic tissue section was obtained during colonoscopy from a representative asymptomatic patient. *E. histolytica* on the surface of large-intestinal mucosa was clearly stained with periodic acid-Schiff (PAS) staining (green arrows). (**A**) Hematoxylin-eosin staining, ×100. (**B**) Higher magnification of the boxed area in **A**. Hematoxylin-eosin staining, ×400. (**C**) PAS staining, ×400.

with intestinal amebic ulcers can act as a reservoir for *E. histolytica*. However, it is sometimes difficult to identify amebiasis in these individuals, because they lack typical abdominal symptoms related to amebiasis, such as tenesmus, diarrhea, and dysentery. Moreover, our results showed that stool microscopic examination hardly identified amebiasis in these individuals. FOB is more sensitive than stool microscopic examination. However, FOB was positive in 72.7% (16 of 22) of patients free of amebic ulceration. Serum antibody against *E. histolytica* might be a sensitive marker of amebic ulcer in asymptomatic individuals. However, low titers of serum antibody were frequently found in individuals without amebic ulcer. The optimal cutoff value of antibody titer for amebic ulcer is still unclear (for cutoff titer of ×100, sensitivity is 87.5%, and specificity is 82.5%, whereas for cutoff titer ×400, sensitivity is 75.0%, and specificity is 95.2%) (Table 2).

Interestingly, our analysis showed high frequency of HLA DQB1*06:01 heterozygote in patients with subclinical intestinal amebiasis. This allele was reported previously to provide protection against *E. histolytica* infection in Bangladeshi patients.[8] One possible explanation is that ulcerative lesions could occur asymptomatically in patients with HLA DQB1*06:01 and that their immune system could prevent the development of invasive disease from *E. histolytica*, resulting in the high frequency of subclinical intestinal amebiasis observed in our cross-sectional analysis. Genetic differences between Bangladeshi and Japanese patients should also be considered. HLA DQB1*06:01 and DRB1*15:01 were the most common haplotypes in Bangladesh, although they were not identified in our patients.

TABLE 1
Characteristics of patients with and without subclinical intestinal amebiasis

	Amebiasis	No amebiasis	*P* value
n	8	63	
Age (years), median (range)	39 (27–62)	51 (26–81)	0.07
Male sex (%)	8/8 (100%)	56/63 (88.9%)	1.00
Men who have sex with men (%)	8/8 (100%)	44/63 (69.8%)	0.10
Past history of amebiasis (%)	0/8 (0%)	7/63 (11.1%)	1.00
CD4/μL, median (range)	301 (70–584)	436 (21–1,697)	0.28
HIV-RNA (LC/mL), median (range)	4.02 (UD–5.41)	UD (UD–5.85)	0.09

LC/mL = log 10 copies per milliliter; UD = undetectable.

TABLE 2
Clinical presentation of patients with and without subclinical intestinal amebiasis

	Amebiasis	No amebiasis	*P* value
n	8	63	
Serum positivity for anti-*E. histolytica* antibody (%)	7/8 (87.5%)	11/63 (17.5%)	< 0.001
< ×100	1	52	
×100	1	5	
×200	0	3	
×400	3	2	
×800	1	1	
×1,600	2	0	
Site of intestinal amebiasis			
Cecum	5		
Ascending	3		
Transverse	1		
Descending	0		
Sigmoid	1		
Rectum	4		

TABLE 3
Frequencies of HLA class II alleles in patients with and without amebiasis

	Patients with amebiasis ($N = 7$)	Patients without amebiasis ($N = 50$)	P value
DRB1			
*04:03	1 (14.3%)	5 (10.0%)	0.56
*04:05	3 (42.9%)	16 (32.0%)	0.68
*04:06	1 (14.3%)	5 (10.0%)	0.56
*09:01	1 (14.3%)	17 (34.0%)	0.41
*11:01	0 (0.0%)	6 (12.0%)	1.00
*13:02	0 (0.0%)	7 (14.0%)	0.58
*15:01	1 (14.3%)	7 (14.0%)	1.00
*15:02	3 (42.9%)	5 (10.0%)	0.050
DQB1			
*03:01	1 (14.3%)	11 (22.0%)	1.00
*03:02	2 (28.6%)	12 (24.0%)	1.00
*03:03	1 (14.3%)	20 (40.0%)	0.24
*04:01	3 (42.9%)	16 (32.0%)	0.68
*05:02	1 (14.3%)	3 (6.0%)	0.42
*05:03	0 (0.0%)	6 (12.0%)	1.00
*06:01	5 (71.4%)	5 (10.0%)	0.001
*06:02	1 (14.3%)	7 (14.0%)	1.00
*06:04	0 (0.0%)	7 (14.0%)	0.58

Data are numbers and frequencies of patients harboring each HLA allele. HLA data were available in 57 patients. HLA alleles identified in more than five patients (> 10%) were considered.

Additional studies are needed to examine the effects of host genetic factors on *E. histolytica* infection and the development of invasive disease. Interestingly, not only HLA but also, mutation of the leptin receptor were reported to be associated with amebic infection.[13]

In conclusion, intestinal amebic ulcerative lesions were frequently found in asymptomatic HIV-1–infected Japanese individuals who could otherwise act as reservoirs for new infection in other high-risk populations. Additional studies of subclinical infection are needed to control the *E. histolytica* endemicity.

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REFERENCES

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Barker-Collo S, Bartels DH, Bell ML, Benjamin EJ, Bennett D, Bhalla K, Bikbov B, Bin Abdulhak A, Birbeck G, Blyth F, Bolliger I, Boufous S, Bucello C, Burch M, Burney P, Carapetis J, Chen H, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahodwala N, De Leo D, Degenhardt L, Delossantos A, Denenberg J, Des Jarlais DC, Dharmaratne SD, Dorsey ER, Driscoll T, Duber H, Ebel B, Erwin PJ, Espindola P, Ezzati M, Feigin V, Flaxman AD, Forouzanfar MH, Fowkes FG, Franklin R, Fransen M, Freeman MK, Gabriel SE, Gakidou E, Gaspari F, Gillum RF, Gonzalez-Medina D, Halasa YA, Haring D, Harrison JE, Havmoeller R, Hay RJ, Hoen B, Hotez PJ, Hoy D, Jacobsen KH, James SL, Jasrasaria R, Jayaraman S, Johns N, Karthikeyan G, Kassebaum N, Keren A, Khoo JP, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Lipnick M, Lipshultz SE, Ohno SL, Mabweijano J, MacIntyre MF, Mallinger L, March L, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGrath J, Mensah GA, Merriman TR, Michaud C, Miller M, Miller TR, Mock C, Mocumbi AO, Mokdad AA, Moran A, Mulholland K, Nair MN, Naldi L, Narayan KM, Nasseri K, Norman P, O'Donnell M, Omer SB, Ortblad K, Osborne R, Ozgediz D, Pahari B, Pandian JD, Rivero AP, Padilla RP, Perez-Ruiz F, Perico N, Phillips D, Pierce K, Pope CA 3rd, Porrini E, Pourmalek F, Raju M, Ranganathan D, Rehm JT, Rein DB, Remuzzi G, Rivara FP, Roberts T, De León FR, Rosenfeld LC, Rushton L, Sacco RL, Salomon JA, Sampson U, Sanman E, Schwebel DC, Segui-Gomez M, Shepard DS, Singh D, Singleton J, Sliwa K, Smith E, Steer A, Taylor JA, Thomas B, Tleyjeh IM, Towbin JA, Truelsen T, Undurraga EA, Venketasubramanian N, Vijayakumar L, Vos T, Wagner GR, Wang M, Wang W, Watt K, Weinstock MA, Weintraub R, Wilkinson JD, Woolf AD, Wulf S, Yeh PH, Yip P, Zabetian A, Zheng ZJ, Lopez AD, Murray CJ, AlMazroa MA, Memish ZA, 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet 15:* 2095–2128.
2. Hung CC, Chang SY, Ji DD, 2012. *Entamoeba histolytica* infection in men who have sex with men. *Lancet Infect Dis 12:* 729–736.
3. Park WB, Choe PG, Jo JH, Kim SH, Bang JH, Kim HB, Kim NJ, Oh MD, 2007. Amebic liver abscess in HIV-infected patients, Republic of Korea. *Emerg Infect Dis 13:* 516–517.
4. Nagata N, Shimbo T, Akiyama J, Nakashima R, Nishimura S, Yada T, Watanabe K, Oka S, Uemura N, 2012. Risk factors for intestinal invasive amebiasis in Japan, 2003–2009. *Emerg Infect Dis 18:* 717–724.
5. Watanabe K, Gatanaga H, Escueta-de Cadiz A, Tanuma J, Nozaki T, Oka S, 2011. Amebiasis in HIV-1-infected Japanese men: clinical features and response to therapy. *PLoS Negl Trop Dis 5:* e1318.
6. Watanabe K, Aoki T, Nagata N, Tanuma J, Kikuchi Y, Oka S, Gatanaga H, 2014. Clinical significance of high anti-entamoeba histolytica antibody titer in asymptomatic HIV-1-infected individuals. *J Infect Dis 209:* 1801–1807.
7. Hung CC, Ji DD, Sun HY, Lee YT, Hsu SY, Chang SY, Wu CH, Chan YH, Hsiao CF, Liu WC, Colebunders R, 2008. Increased risk for *Entamoeba histolytica* infection and invasive amebiasis in HIV seropositive men who have sex with men in Taiwan. *PLoS Negl Trop Dis 2:* e175.
8. Duggal P, Haque R, Roy S, Mondal D, Sack RB, Farr BM, Beaty TH, Petri WA Jr, 2004. Influence of human leukocyte antigen class II alleles on susceptibility to *Entamoeba histolytica* infection in Bangladeshi children. *J Infect Dis 189:* 520–526.
9. Svedlund J, Sjodin I, Dotevall G, 1988. GSRS–a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci 33:* 129–134.
10. Hamada Y, Nagata N, Nishijima T, Shimbo T, Asayama N, Kishida Y, Sekine K, Tanaka S, Aoki T, Watanabe K, Akiyama J, Igari T, Mizokami M, Uemura N, Oka S, 2014. Impact of HIV infection on colorectal tumors: a prospective colonoscopic study of Asian patients. *J Acquir Immune Defic Syndr 65:* 312–317.

11. Nagata N, Shimbo T, Akiyama J, Nakashima R, Niikura R, Nishimura S, Yada T, Watanabe K, Oka S, Uemura N, 2012. Predictive value of endoscopic findings in the diagnosis of active intestinal amebiasis. *Endoscopy 44:* 425–428.
12. Okamoto M, Kawabe T, Ohata K, Togo G, Hada T, Katamoto T, Tanno M, Matsumura M, Yamaji Y, Watabe H, Ikenoue T, Yoshida H, Omata M, 2005. Amebic colitis in asymptomatic subjects with positive fecal occult blood test results: clinical features different from symptomatic cases. *Am J Trop Med Hyg 73:* 934–935.
13. Duggal P, Guo X, Haque R, Peterson KM, Ricklefs S, Mondal D, Alam F, Noor Z, Verkerke HP, Marie C, Leduc CA, Chua SC Jr, Myers MG Jr, Leibel RL, Houpt E, Gilchrist CA, Sher A, Porcella SF, Petri WA Jr, 2011. A mutation in the leptin receptor is associated with *Entamoeba histolytica* infection in children. *J Clin Invest 121:* 1191–1198.

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Brain Magnetic Resonance Imaging Screening Is Not Useful for HIV-1-Infected Patients Without Neurological Symptoms

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Abstract

We investigated the diagnostic usefulness of brain magnetic resonance imaging (MRI) screening in HIV-1-infected patients without neurological symptoms in detecting intracranial diseases at early stages. In this retrospective analysis, the study patients were HIV-1-infected patients who underwent brain MRI scan in clinical practice between 2001 and 2013. We excluded patients with MRI for (1) follow-up examination for prediagnosed intracranial diseases, (2) cancer staging, (3) screening mycobacterium/bacteria/fungi disease proliferation in the brain, and (4) evaluation for meningitis/encephalitis. The study patients ($n=485$) were classified into two groups: those who underwent brain MRI scan without any neurological symptoms/signs (asymptomatic patients, $n=158$) and those who underwent MRI due to such symptoms (symptomatic patients, $n=327$). Asymptomatic patients had lower CD4 counts than symptomatic patients (median 78 versus 241/μl). Intracranial diseases were detected in three (2%) of the asymptomatic patients [two toxoplasmosis and one progressive multifocal leukoencephalopathy (PML)] compared to 58 (19%) of the symptomatic patients (the χ^2 test, $p<0.01$). The latter included toxoplasmosis ($n=10$), PML ($n=7$), cytomegalovirus encephalitis ($n=3$), primary central nervous system lymphoma ($n=3$), cryptococcoma/meningitis ($n=3$), and HIV-associated dementia ($n=17$). Among symptomatic patients, intracranial diseases were common in those with slurred speech (3/6, 50%), seizure (4/10, 40%), eyesight/vision abnormality (5/16, 31%), altered mental status (8/31, 26%), and hemiplegia/numbness (13/50, 26%). For patients with CD4 count $<200/\mu$l, intracranial diseases were detected in only 3 (3%) of 144 asymptomatic patients, compared with 46 (32%) of 113 symptomatic patients ($p<0.01$). Brain MRI screening for HIV-1-infected patients without neurological symptoms is of little value.

Introduction

PATIENTS WITH ADVANCED HIV-1 INFECTION are prone to develop intracranial opportunistic diseases, such as toxoplasma encephalitis, primary central nervous system lymphoma (PCNSL), progressive multifocal leukoencephalopathy (PML), and cytomegalovirus (CMV) encephalitis.[1] Although the introduction of antiretroviral therapy (ART) substantially decreased the incidence of neurological opportunistic infections,[2,3] such diseases have high associated mortality even with appropriate treatment, and recurrences and residual neurological deficits can occur.[4,5] Because delayed diagnosis of these intracranial diseases has a detrimental effect on patients with HIV-1 infection,[5,6] early diagnosis, not to mention prevention, of such diseases is of importance.

Brain magnetic resonance imaging (MRI) is often preferred to computed tomography (CT) in establishing the diagnosis of many of these diseases due to its superior sensitivity to subtle white matter and meningeal disease.[7–10] However, there is no information on the utility of brain MRI screening for HIV-1-infected patients without neurological symptoms/signs in detecting intracranial opportunistic diseases at early stages. This observational study was designed to assess the usefulness of brain MRI screening of such patients with HIV-1 infection.

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Materials and Methods

Study design, setting, and participants

We conducted an observational single-center study to investigate the usefulness of brain MRI screening in HIV-1-infected patients without neurological symptoms who warrant investigation for intracranial diseases. The study was conducted at the AIDS Clinical Center, National Center for Global Health and Medicine (NCGM), Tokyo, the largest referral center for HIV care in Japan.[11] The study patients were those who fulfilled the following inclusion criteria: HIV-1-infected patients who underwent brain MRI scan in clinical practice between June 2001 and August 2013. In addition, the following exclusion criteria were applied: patients who underwent brain MRI for (1) follow-up examination during the study period because of intracranial diseases such as opportunistic infections, stroke, or malignancy, which were diagnosed prior to the referral to our clinic, (2) staging of malignant tumors, (3) screening mycobacterium/bacteria/fungi disease proliferation in the brain in patients who were already diagnosed with mycobacterial diseases or bacteremia or fungemia, and (4) evaluation of meningitis/encephalitis.

The study patients ($n=485$) were classified into those who underwent brain MRI scan without any neurological symptoms, such as seizure, altered mental status, hemiplegia/numbness, headache, or fever (asymptomatic patients, $n=158$), and those who underwent MRI due to the abovementioned symptoms, which can suggest a focal brain lesion[5] (symptomatic patients, $n=327$). Asymptomatic patients included those who underwent MRI due to positive antitoxoplasma IgG antibody ($n=38$) and positive serum cryptococcal antigen ($n=1$). At our clinic, patients with a low CD4 cell count (typically less than 200/μl) often underwent brain MRI even though they had no neurological symptoms/signs that would warrant a brain imaging examination to rule out intracranial opportunistic infections or malignancy at early stages.

The study was approved by the Human Research Ethics Committee of NCGM. All patients included in this study provided written informed consent for their clinical and laboratory data to be used and published for research purposes. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Measurements

At our hospital, brain MRI was routinely read by one experienced radiologist and the findings were confirmed by another radiologist. Furthermore, the MRI diagnosis was confirmed by reviewing the medical records and follow-up brain imaging when available. The diagnostic criteria for cryptococcal meningitis, cytomegalovirus encephalitis, and toxoplasmic encephalitis were those adopted by the AIDS Clinical Trials Group (ACTG)-A5164.[12] HIV-associated dementia in this study was diagnosed based on the MRI findings, which included generalized atrophy and prominent white matter changes plus cognitive impairment based on the chart review, and not necessarily required neurocognitive function tests.[8] The reasons for conducting an MRI were also extracted from the medical records. Baseline characteristics and HIV-1-related variables at the time of brain MRI were also extracted from the medical records. They included age, sex, ethnicity, history of AIDS, route of HIV-1 transmission, treatment status for HIV-1 infection (either treatment naive or experienced), CD4 cell count, and HIV viral load. For CD4 count and HIV load, we used data collected closest to and preceding by up to 3 months the day of the brain MRI. In Japan, because the prescription period under the health care system is limited to 3 months, patients need to visit the HIV Clinic at least once every 3 months for prescriptions as well as monitoring CD4 cell count and HIV-1 load.[11]

Statistical analysis

Baseline characteristics were compared between asymptomatic and symptomatic patients using the Student's t-test and χ^2 test (Fisher's exact test) for continuous and categorical variables, respectively. Prevalence of intracranial diseases was calculated among asymptomatic patients and compared to that of symptomatic patients with the χ^2 test. The logistic regression model was used to estimate the associations of lack of neurological symptoms/signs over the presence of such symptoms/signs with the MRI findings of intracranial diseases. The model was adjusted for age, sex, CD4 count, HIV treatment status, and history of AIDS. Subgroup analysis included the prevalence of intracranial diseases in patients with a CD4 count $<200/\mu$l. Statistical significance was defined as two-sided p values <0.05. We used odds ratios (ORs) with 95% confidence intervals (95% CIs). All statistical analyses were performed with The Statistical Package for Social Sciences ver. 21.0 (SPSS, Chicago, IL).

Results

The study included 485 patients who underwent a brain MRI scan in clinical practice, of whom 158 had no neurological symptoms (asymptomatic) and 327 did have such symptoms (symptomatic). Of the total patients, 475 (98%) were Asians, 446 (92%) were males, and 365 (75%) were infected with HIV-1 through homosexual contact (Table 1). The median age of the study patients was 41 [interquartile range (IQR) 34–51]. Asymptomatic patients had a lower CD4 count [median 78/μl, interquartile range (IQR) 21–237, symptomatic: 241/μl, 60–470 ($p<0.01$)] and higher HIV-1 viral load [4.84 $\log_{10}$/ml, IQR 2.97–5.62, symptomatic: 2.95 $\log_{10}$/ml, 1.70–5.11 ($p<0.01$)] than symptomatic patients. Asymptomatic patients were more likely to be treatment naive (68% versus 41%, $p<0.01$) and have a history of AIDS (62% versus 47%, $p<0.01$). There was no significant difference in other baseline characteristics between the two groups (Table 1).

Among the 158 asymptomatic patients, brain MRI screening detected toxoplasmosis ($n=2$) and PML ($n=1$, with CD4 43/μl), i.e., a prevalence of intracranial diseases of 2%. The two patients with toxoplasmosis underwent brain MRI due to positive antitoxoplasma IgG antibody with a titer of 20,480 (CD4 168/μl) and 1,280 (CD4 16/μl) IU/ml. In asymptomatic patients who underwent brain MRI due to positive antitoxoplasma IgG antibody, intracranial diseases were detected in 3 (8%) out of 38 patients (Table 2). On the other hand, brain MRI for symptomatic patients detected 58 intracranial diseases with a prevalence of 19%. The cases included toxoplasmic encephalitis ($n=10$), PML ($n=7$), CMV encephalitis ($n=3$), PCNSL ($n=3$), cryptococcosis/meningitis ($n=3$), herpes simplex virus encephalitis ($n=1$), HIV-associated dementia ($n=17$), acute cerebral infarction ($n=8$), gummatous

TABLE 1. CLINICAL CHARACTERISTICS OF THE STUDY PATIENTS ACCORDING TO NEUROLOGICAL SYMPTOMS

	All patients (n=485)	*Patients without neurological symptoms (n=158)*	*Patients with neurological symptoms (n=327)*	p *value*
Male sex, *n* (%)	446 (92)	146 (92)	300 (92)	0.86
Age[†]	41 (34–51)	42 (33–52)	41 (35–49)	0.95
Asian, *n* (%)	475 (98)	154 (98)	321 (98)	0.74
CD4 cell count (/μl)[a]	178 (41–420)	78 (21–237)	241 (60–470)	<0.01
HIV-1 load (log_{10}/ml)[a]	4.20 (1.70–5.26)	4.84 (2.97–5.61)	2.95 (1.70–5.11)[b]	<0.01
Homosexual contact, *n* (%)	364 (75)	117 (74)	247 (76)	0.74
Treatment naive, *n* (%)	240 (50)	107 (68)	133 (41)	<0.01
History of AIDS, *n* (%)	250 (52)	98 (62)	152 (47)	<0.01

[a]Median (interquartile range).
[b]Data on HIV-1 load are not available for two patients.

syphilis ($n=1$), tuberculoma ($n=1$), metastatic cancer ($n=1$), chronic subdural hematoma ($n=1$), schwannoma ($n=1$), and progressive supranuclear palsy ($n=1$) (Table 2). In asymptomatic patients, intracranial diseases were less likely to be detected by brain MRI, compared to symptomatic patients [by univariate and multivariate analysis (OR=0.1; 95% CI, 0.03–0.29; $p<0.01$) (adjusted OR=0.1; 95% CI, 0.02–0.17; $p<0.01$)]. Patients with higher CD4 counts were also less likely to have intracranial diseases (per 100/μl increment, adjusted OR=0.7; 95% CI, 0.55–0.83; $p<0.01$). Among the symptomatic patients, those who presented with slurred speech, seizure, eyesight/vision abnormality, altered mental status, and hemiplegia/numbness were highly likely to have intracranial diseases, with a prevalence of 50%, 40%, 31%, 26%, and 26%, respectively (Table 3).

Subgroup analysis limited to data of patients with CD4 count of <200/μl showed that the abovementioned three intracranial diseases were detected in 144 asymptomatic patients with a prevalence of 3%, compared to 46 (32%) of 113 symptomatic patients (asymptomatic over symptomatic, OR=0.1; 95% CI, 0.02–0.19; $p<0.01$) (Table 2). Only a few intracranial opportunistic diseases were diagnosed in patients with a CD4 count of ≥200/μl; PCNSL ($n=1$), HIV-associated dementia ($n=4$), acute cerebral infarction ($n=6$), metastatic cancer ($n=1$), and progressive supranuclear palsy ($n=1$).

Discussion

In this observational study of patients who underwent brain MRI screening in clinical practice, only 2% of patients without neurological symptoms/signs that warranted investigation of intracranial diseases were found to have intracranial diseases, whereas a significantly higher prevalence (19%) of intracranial diseases was detected in patients who underwent brain MRI due to such symptoms. Among patients with a CD4 count of <200/μl, who are reported to be at high risk for intracranial diseases,[5,10] the result was similar; 3% and 32% of asymptomatic and symptomatic patients, respectively, were found to have intracranial diseases. On the other hand, high detection rates of intracranial diseases by brain MRI were observed in patients who presented with slurred speech (50%), seizure (40%), eyesight/vision abnormality (31%), altered mental status (26%), and hemiplegia/

TABLE 2. PREVALENCE OF INTRACRANIAL DISEASES DETECTED BY BRAIN MAGNETIC RESONANCE IMAGING ACCORDING TO NEUROLOGICAL SYMPTOMS

Intracranial diseases	*Patients without neurological symptoms (n=158)*	*Patients without neurological symptoms with CD4 <200/μl (n=144)*	*Patients with neurological symptoms (n=327)*	*Patients with neurological symptoms with CD4 <200/μl (n=113)*	*Positive toxoplasma Ab and without neurological symptoms (n=38)*
Toxoplasmosis	2 (1)	2 (2)	10 (3)	10 (7)	2 (1)
PML	1 (1)	1 (1)	7 (2)	7 (5)	1 (1)
HIV-associated dementia			17 (6)	13 (9)	
Malignant lymphoma			4 (1)	3 (2)	
CMV encephalopathy			3 (1)	3 (2)	
Cryptococcoma/meningitis			3 (1)	3 (1)	
HSV encephalopathy			1	1	
Gummatous syphilis			1	1	
Tuberculoma			1	1	
Metastatic cancer			1		
Cerebral infarction			8 (3)	2 (1)	
Others			3 (1)	2 (1)	
Total	3 (2)	3 (3)	59 (19)	46 (32)	3 (8)

Data are numbers (percentages) of patients.
Ab, antibody; PML, progressive multifocal leukoencephalopathy; CMV, cytomegalovirus; HSV, herpes simplex virus.

TABLE 3. PREVALENCE OF INTRACRANIAL DISEASES DETECTED BY BRAIN MAGNETIC RESONANCE IMAGING ACCORDING TO NEUROLOGICAL SYMPTOM CATEGORIES

	Intracranial diseases	*Prevalence of intracranial diseases*
Slurred speech ($n=6$)	Cerebral infarction $n=2$ PML $n=1$	50%
Seizure ($n=10$)	Toxoplasmosis $n=2$ PML $n=1$ HSV encephalitis $n=1$	40%
Eyesight/vision abnormality ($n=16$)	Malignant lymphoma $n=2$ HIV-associated dementia $n=2$ Metastatic cancer $n=1$	31%
Altered mental status ($n=31$)	Toxoplasmosis $n=2$ HIV-associated dementia $n=2$ Cryptococcoma/meningitis $n=2$ PML $n=1$ Tuberculoma $n=1$	26%
Hemiplegia/numbness ($n=50$)	Cerebral infarction $n=5$ Toxoplasmosis $n=3$ PML $n=3$ HIV-associated dementia $n=1$ Other $n=1$	26%
Neurocognitive impairment ($n=62$)	HIV-associated dementia $n=9$ Cerebral infarction $n=1$ CMV encephalitis $n=2$	19%
Fever work-up ($n=12$)	Malignant lymphoma $n=1$ HIV-associated dementia $n=1$	17%
Dizziness/vertigo/tinnitus ($n=45$)	Toxoplasmosis $n=1$ PML $n=1$ Malignant lymphoma $n=1$ HIV-associated dementia $n=1$ CMV encephalitis $n=1$	11%
Abnormal ophthalmologic examination ($n=11$)	HIV-associated dementia $n=1$	9%
Headache ($n=49$)	Toxoplasmosis $n=2$	4%
Syncope ($n=16$)		0%

PML, progressive multifocal leukoencephalopathy; HSV, herpes simplex virus; CMV, cytomegalovirus.

numbness (26%). The present study indicates that brain MRI screening for HIV-1-infected patients without neurological symptoms/signs, even those with a low CD4 count (< 200/μl), is of little value. In contrast, MRI screening is useful for patients with particular neurological symptoms/signs. These findings can help reduce unnecessary brain MRI examinations and can be helpful in clinical decision making.

Interestingly, in both of the two asymptomatic toxoplasmic encephalitis patients who underwent brain MRI screening because of positive antitoxoplasma IgG antibody, the antibody titer was very high (20,480 IU/ml and 1,280). Together with the fact that the prevalence of intracranial diseases in asymptomatic patients with positive antitoxoplasma IgG antibody was higher (8%) than the 2% in the entire group of asymptomatic patients, brain MRI screening for patients without neurological symptoms/signs who presented with high antitoxoplasma antibody may be of value and clinically justifiable.

Our study has certain limitations. First, because brain MRI was performed at the discretion of the treating physician, patient selection bias, especially among those without neurological symptoms/signs, cannot be ruled out. However, we had a large number of study patients, and considering the availability and cost of an MRI scan, the results of the present study are of value and are useful in clinical decision making. Second, because endemic opportunistic infections vary depending on the region[13,14] and the majority of our patients were Asian, the results of the present study might not be applicable to patients in other regions. Third, in this study the diagnosis of HIV-associated dementia was based on the MRI findings plus cognitive impairment based on a chart review, and the patients did not necessarily undergo neurocognitive function tests.[8] This is because the present study included patients from 2001, long before the diagnostic Frascati criteria for an HIV-associated neurocognitive disorder that required neurocognitive function tests were established.[15]

In conclusion, although our results suggest that brain MRI screening is of little value in HIV-1-infected patients without neurological symptoms/signs that warrant investigation on intracranial diseases, it should be performed in HIV-1-infected patients who present with particular neurological symptoms, such as slurred speech and seizure.

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Author Disclosure Statement

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References

1. Panel on Opportunistic Infections in HIV-Infected Adults and Adolescents: Guidelines for the prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: Recommendations from the Centers for Disease Control and Prevention, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf. Accessed February 27, 2014.
2. Garvey L, Winston A, Walsh J, *et al.:* HIV-associated central nervous system diseases in the recent combination antiretroviral therapy era. Eur J Neurol 2011;18:527–534.
3. d'Arminio Monforte A, Cinque P, Mocroft A, *et al.:* Changing incidence of central nervous system diseases in the EuroSIDA cohort. Ann Neurol 2004;55:320–328.
4. Mocroft AJ, Lundgren JD, d'Armino Monforte A, *et al.:* Survival of AIDS patients according to type of AIDS-defining event. The AIDS in Europe Study Group. Int J Epidemiol 1997;26:400–407.
5. Tan IL, Smith BR, von Geldern G, *et al.:* HIV-associated opportunistic infections of the CNS. Lancet Neurol 2012; 11:605–617.
6. Antinori A, Larussa D, Cingolani A, *et al.:* Prevalence, associated factors, and prognostic determinants of AIDS-related toxoplasmic encephalitis in the era of advanced highly active antiretroviral therapy. Clin Infect Dis 2004; 39:1681–1691.
7. Cinque P, Koralnik IJ, Gerevini S, *et al.:* Progressive multifocal leukoencephalopathy in HIV-1 infection. Lancet Infect Dis 2009;9:625–636.
8. Clifford DB and Ances BM: HIV-associated neurocognitive disorder. Lancet Infect Dis 2013;13:976–986.
9. Utsuki S, Oka H, Abe K, *et al.:* Primary central nervous system lymphoma in acquired immune deficiency syndrome mimicking toxoplasmosis. Brain Tumor Pathol 2011;28: 83–87.
10. Graham CB 3rd, Wippold FJ 2nd, Pilgram TK, *et al.:* Screening CT of the brain determined by CD4 count in HIV-positive patients presenting with headache. AJNR Am J Neuroradiol 2000;21:451–454.
11. Nishijima T, Kawasaki Y, Tanaka N, *et al.:* Long-term exposure to tenofovir continuously decrease renal function in HIV-1-infected patients with low body weight. AIDS 2014:1.
12. Zolopa A, Andersen J, Powderly W, *et al.:* Early antiretroviral therapy reduces AIDS progression/death in individuals with acute opportunistic infections: A multicenter randomized strategy trial. PLoS One 2009;4:e5575.
13. McLeod DS, Mortimer RH, Perry-Keene DA, *et al.:* Histoplasmosis in Australia: Report of 16 cases and literature review. Medicine (Baltimore) 2011;90:61–68.
14. Ustianowski AP, Sieu TP, and Day JN: Penicillium marneffei infection in HIV. Curr Opin Infect Dis 2008;21: 31–36.
15. Antinori A, Arendt G, Becker JT, *et al.:* Updated research nosology for HIV-associated neurocognitive disorders. Neurology 2007;69:1789–1799.

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Original article

Low body weight and tenofovir use are risk factors for renal dysfunction in Vietnamese HIV-infected patients. A prospective 18-month observation study

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ABSTRACT

Background: The use of tenofovir has been rapidly increasing in Vietnam. Several studies identified low body weight as a risk factor for tenofovir-induced nephrotoxicity. However, little is known about the impact of tenofovir on renal function in HIV-infected Vietnamese with generally low weight.
Methods: An observational single-center cohort of adult HIV-infected patients on antiretroviral therapy at National Hospital of Tropical Diseases, Hanoi. Patients on tenofovir or with creatinine clearance ≤60 ml/min at baseline were excluded. The incidence of renal dysfunction was compared between patients who switched to tenofovir and those who did not. Renal dysfunction was defined as 25% decline of creatinine clearance from baseline. Time to renal dysfunction was analyzed by the Kaplan–Meier method between the two groups. The Cox hazard model was used to determine risk factors for renal dysfunction in uni- and multivariate analyses.
Results: Of 556 patients enrolled in this study, 403 were non-tenofovir group while 153 were the tenofovir-switched group. Renal dysfunction occurred at a higher rate in the tenofovir-switched group (92.5 per 1000 person-years) than the non-tenofovir group (47.8 per 1000 person-years)($p = 0.023$, Log-rank test). Multivariate analysis confirmed that tenofovir use, low body weight and glucosuria were significant risk factors for renal dysfunction (hazard ratio = 1.980; 95% confidential interval, 1.094–3.582, HR = 1.057; 95%CI, 1.016–1.098, HR = 5.202; 95%CI, 1.245–21.738, respectively).
Conclusions: Tenofovir use, low body weight and glucosuria were significant risk factors for renal dysfunction. We suggest close monitoring of renal function in patients with these risk factors even in resource-limited setting.

Key points

Treatment with TDF and low body weight were significant risk factors for renal dysfunction in Vietnamese HIV-treated patients. Given that the average body weight of Vietnamese is small, close monitoring of renal function in HIV-1-infected patients is important during treatment with TDF.

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1. Introduction

Although renal dysfunction is an important cause of morbidity and mortality in HIV-infected patients [1–7], only limited information is available on renal function in Vietnamese HIV-infected patients. Along with the 2010 WHO guidelines which phased out stavudine and recommended tenofovir (TDF) (URL: http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf), the use of TDF had been increasing in Vietnam in recent years.

TDF-associated nephrotoxicity is well known adverse effect. However, a meta-analysis study that evaluated the safety of TDF concluded that TDF-associated nephrotoxicity can be considered negligible and thus there is no need to restrict TDF use even when regular observation of renal function is not feasible [8]. Other experimental and clinical studies, however; provide a different scenario: one study of rhesus macaques described a dose-dependent nephrotoxic effect for TDF [9] and several studies reported cases of TDF-associated nephrotoxicity in low-body-weight HIV-infected patients [10,11]. Our group also reported that low body weight and use of TDF were significantly associated with chronic kidney dysfunction in Vietnamese HIV-infected patients in a cross-sectional study [12]. Since Vietnamese have a considerably smaller body weight compared with Caucasians, and the use of TDF in Vietnam is increasing throughout the country, the potential risk for TDF-related nephrotoxicity is a concern in Vietnam. This is also true in all countries in the region since the Asian population is, in general, of low body weight. To examine this issue in more detail, we conducted a longitudinal study to evaluate the incidence of renal dysfunction in Vietnamese HIV-infected patients and the risk factors of such morbidity, including use of TDF and low body weight.

2. Patients and methods

2.1. Study design

We performed a prospective observational study of a single-center cohort of Vietnamese HIV-infected patients on antiretroviral therapy (ART) to evaluate the impact of TDF and low body weight on renal function. This cohort was established in 2007 at the National Hospital of Tropical Disease (NHTD) in Hanoi, one of the biggest outpatient clinics for HIV infected-patients in Vietnam. The population of the cohort consists of Vietnamese HIV-infected patients on ART aged more than 17 years referred to NHTD.

To evaluate renal function, serum creatinine had been measured since October 2011, which is the baseline of this study. Entry criteria were patients who were registered in this cohort on October 2011. Patients taking TDF or with serum creatinine clearance (Crcl) of ≤60 ml/min at baseline were excluded. Also excluded from the study were patients whose creatinine was not obtained twice at least. The follow-up period was 18 months (between October 2011 and April 2013). All patients of this cohort received ART at baseline. ART included Zidovudine (AZT)/Lamivudine (3 TC), Stavudine (d4T)/3 TC or TDF/3 TC as nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) in combination with efavirenz (EFV), Nevirapine (NVP) or ritonavir boosted lopinavir (LPV/r). To estimate the incidence of renal dysfunction in this population, patients were divided into those who switched to TDF and those who did not. Laboratory data, including serum creatinine, were measured twice a year (in April and October) in this cohort. The study was approved by the Human Research Ethics Committee of NHTD. Each patient included in this study provided a written informed consent for the clinical and laboratory data to be used for publication. The study was conducted according to the principles expressed in the Declaration of Helsinki.

2.2. Measurements

Clinical and laboratory data included demographic variables (age, sex and weight), serum creatinine (mg/dl, measured by Jaffe method), CD4 cell count (cell/mm^3, measured by flow cytometry), plasma HIV-RNA (copies/ml, measured by the Roche COBAS TaqMan HIV monitor assay), complete history of ART, use of cotrimoxazole, date of HIV diagnosis, and presence of other comorbidities such as hepatitis B and C virus, diabetes mellitus and AIDS defining diseases. Renal dysfunction was defined as 25% decline in Crcl estimated by the Cockcroft–Gault formula, relative to the baseline.

2.3. Statistical analysis

Baseline characteristics were compared between case patients and control patients by the Student's t-test for continuous variables and by either the χ^2 test or Fisher's exact test for categorical variables. The time from baseline to renal dysfunction was analyzed by the Kaplan–Meier method for patients who switched to TDF and those who did not, and the log-rank test was used to determine the statistical significance. Censored cases represented those who died, dropped out, or were referred to other facilities before the end of follow-up period. The Cox proportional hazards regression analysis was used to estimate the impact of TDF use on the incidence of renal dysfunction. The impact of basic demographics, baseline laboratory data, and other medical conditions was also estimated with univariate Cox proportional hazards regression. Variables significantly associated with renal dysfunction in univariate analysis ($p < 0.05$) were entered into multivariate analysis. Statistical significance was defined at two-sided p value < 0.05. We used the hazard ratio (HR) and 95% confidence interval (95%CI) to estimate the association of each variable with renal dysfunction. All analyses were performed in SPSS (version 22.0).

3. Results

At baseline, 793 Vietnamese HIV-infected patients on ART were registered in this study. However, 237 patients were excluded from the study due to existing renal dysfunction at baseline (Crcl < 60 ml/min, $n = 72$), had already been treated with TDF at baseline ($n = 143$), and lack of repeated measurements of Crcl ($n = 22$). Thus, 556 patients who received ART met the study criteria and were included in the study. Of these, 153 patients were switched to TDF during the study period, while 403 patients continued treatment with non-TDF-containing regimen. The criteria for switch to TDF were adverse event caused by ART or induction of treatment for chronic hepatitis B virus infection.

Table 1 compares the baseline demographics and clinical variables of patients of the TDF-switched group and the non-TDF group. The TDF-switched group was significantly more likely to be males, hepatitis B virus S antigen-positive and hepatitis C virus antibody-positive compared to the non-TDF group. The TDF-switched group had marginally significant trend to be older and have diabetes mellitus. Body weight, serum creatinine, CD4 count, HIV RNA viral load, duration of ART, frequency of proteinuria and glucosuria, use of ritonavir boosted lopinavir (LPV/r) and cotrimoxazole, and history of AIDS-defining disease were not significantly different between the two groups. The mean CD4 count was >300/mm^3 and the mean HIV RNA load was <100 copies/ml in both groups.

During the observation period, renal dysfunction, defined as 25% decline in Crcl, was observed in 19 (12.4%) of the TDF-switched group and 27 (6.7%) of the non-TDF group, with an estimated incidence of 92.5 and 47.8 per 1000 person-years, respectively. Fig. 1 depicts the time from the baseline to the development of

Table 1
Baseline characteristic of Vietnamese patients treated with or without TDF.

Variables	Without TDF	With TDF	*P* value
Number of patients (%)	403 (72.5)	153 (27.5)	
Age, years	35.6 ± 7.0	36.9 ± 6.8	0.064
Women, *n* (%)	167 (41.4)	45 (29.4)	0.009
Body weight	55.7 ± 8.3	56.5 ± 8.2	0.284
Serum creatinine, mg/dl	0.93 ± 0.13	0.93 ± 0.12	0.668
CD4+ cell count, cell/μl	394 ± 197	385 ± 166	0.651
Log 10 HIV-RNA level, copies/ml	1.48 ± 0.55	1.42 ± 0.41	0.190
Proteinuria, *n* (%)	48 (11.9)	21 (13.7)	0.522
Glucosuria, *n* (%)	3 (0.7)	2 (1.3)	0.617
HBVAg (+), *n* (%)	22 (5.5)	29 (18.9)	<0.001
HCVAb (+), *n* (%)	153 (38.0)	69 (45.1)	0.014
Duration of ART, years	1.14 ± 1.35	1.20 ± 1.47	0.650
Use of ritonavir boosted lopinavir, *n* (%)	7 (1.7)	5 (3.3)	0.326
Use of cotrimoxazole drug, *n* (%)	136 (33.7)	45 (29.4)	0.330
Prior AIDS defining disease, *n* (%)	36 (8.9)	12 (7.8)	0.683
Diabetes mellitus (+), *n* (%)	31 (7.7)	19 (12.4)	0.082

Data are expressed as mean ± SD.
ART = Antiretroviral therapy; TDF = tenofovir.

renal dysfunction by Kaplan–Meier method in the two groups. The incidence of renal dysfunction was significantly higher in the TDF-switched group, compared with the non-TDF group ($p = 0.023$, Log-rank test). With regard to the time of switch to TDF, 109 (71.5%) patients of the TDF-switched group switched their nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) to TDF within 3 months from the baseline and additional 31 (20.0%) switched between 3 and 6 months. Furthermore, of the 19 patients of the TDF-switched group who developed renal dysfunction, 13 (71.2%) switched to TDF within 3 months from the baseline and additional 5 (23.5%) switched to TDF between 3 and 6 months.

Table 2 shows the results of the Cox proportional hazards regression model. Univariate analysis identified body weight per 1 kg-decrement, use of TDF, and glucosuria as factors significantly associated with renal dysfunction. After adjustment by multivariate analysis, body weight per 1 kg-decrement (HR = 1.057; 95%CI, 1.016–1.098; $p = 0.006$), use of TDF (HR = 1.980; 95%CI, 1.094–3.582; $p = 0.024$), and glucosuria (HR = 5.202; 95%CI, 1.245–21.738; $p = 0.024$) were still associated significantly with renal dysfunction.

We also compared the incidence of renal dysfunction in the TDF-switched group according to body weight. Fig. 2 shows the time from baseline to renal dysfunction in patients with body weight of <55 kg, representing the average weight of this study population, and in those with ≥55 kg of the TDF-switched group by Kaplan–Meier method. Patients of the <55 kg group were significantly more likely to develop renal dysfunction [12/66 cases (18.2%), 145.3/1000 person-year] compared to patients of the ≥55 kg group [7/87 cases (8.0%), 57.0/1000 person-year] ($p = 0.040$, Log-rank test).

Table 2
Risk factors for 25% decline in creatinine clearance estimated by uni- and multivariate analyses.

	Univariate analysis			Multivariate analysis		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Age, per year	1.022	0.984–1.061	0.259			
Women	1.484	0.832–2.646	0.181			
Body weight per 1 kg decrease	1.053	1.013–1.094	0.008	1.057	1.016–1.098	0.006
Serum creatinine >1.1 mg/dl	0.397	0.096–1.636	0.201			
CD4+ cell count per cell/μl	1.001	0.999–1.002	0.227			
HIV-RNA level per log 10 copies/ml	0.887	0.446–1.764	0.733			
Proteinuria	0.474	0.147–1.528	0.211			
Glucosuria	5.372	1.301–22.176	0.020	5.202	1.245–21.738	0.024
HBVAg (+)	1.466	0.622–3.458	0.382			
HCVAb (+)	0.949	0.521–1.728	0.864			
Duration of ART per year	1.151	0.970–1.367	0.108			
Use of tenofovir	1.927	1.071–3.465	0.029	1.980	1.094–3.582	0.024
Use of ritonavir boosted lopinavir	2.024	0.491–8.349	0.329			
Use of cotrimoxazole	0.663	0.337–1.305	0.234			
Prior AIDS defining disease	0.043	0.000–4.144	0.177			
Diabetes mellitus (+)	0.952	0.341–2.654	0.925			

HR = hazard ratio; CI = confidence interval; ART = antiretroviral therapy.

The mean serum creatinine was higher in the TDF-switched group compared with the non-TDF group, and the difference in the mean serum creatinine between the two groups increased from 0 mg/dl at baseline, to 0.4 mg/dl at 6 month, 0.5 mg/dl at 12 months and 0.6 mg/dl at 18 months from the baseline.

4. Discussion

In this 18-month prospective study of a single-center cohort, we evaluated the impact of TDF on renal function in Vietnamese HIV-infected patients with low body weight of approximately 55 kg. The Kaplan–Meier curve showed that the cumulative incidence of renal dysfunction was significantly higher among the patients who switched to TDF than among those who did not ($p = 0.023$). Cox proportional hazards regression model identified the use of TDF, low body weight and glucosuria as significant high risk factors for renal dysfunction. In sub-analysis of the TDF-switched group, we confirmed that the cumulative incidence of renal dysfunction was significantly higher in patients with body weight <55 kg compared to those weighing ≥55 kg.

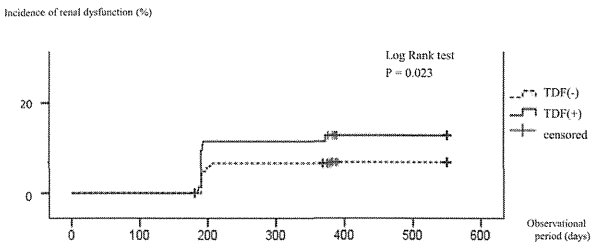

Fig. 1. Kaplan–Meier curve showing the time to renal dysfunction in patients of TDF-switched group and non-TDF-containing groups. Compared to patients of the non-TDF group, those of the TDF-switched group were significantly more likely to develop renal dysfunction ($p = 0.023$, Log-rank test).

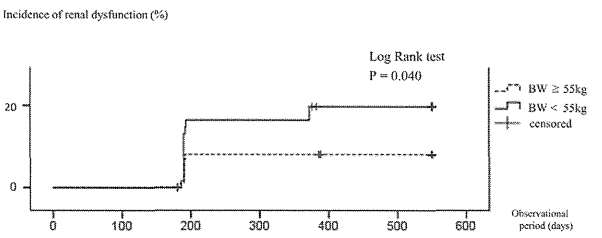

Fig. 2. Kaplan–Meier curve showing the time to renal dysfunction in patients of TDF-switched group classified according to body weight. Compared to patients with body weight ≥55 kg, those weighing <55 kg were significantly likely to develop renal dysfunction ($p = 0.040$, Log-rank test).

We reported previously that low body weight and TDF use were factors significantly associated with chronic kidney disease in a cross-sectional study of this cohort in Hanoi [12]. The present study confirmed that TDF exposure and low body weight bear a causative relationship to renal dysfunction. We also reported low body weight (<59 kg) as a risk factor for renal dysfunction in Japanese patients treated with TDF [10], whereas high body weight of >67 kg was not the risk, similar to the body weight of the patients reported by Cooper et al. [8]. In light of the fact that the average body weight of the patients in this cohort was 55 kg, which is around 30 kg lighter than that of average American males (88 kg) (URL:http://www.cdc.gov/nchs/data/nhsr/nhsr010.pdf), the impact of these risk factors on renal function remain unknown in patients with low body weight in the long-run, thus, observational studies will need to be continued for a longer term.

In addition to low body weight, the presence of glucosuria at baseline was identified as a risk factor for renal dysfunction. This result is consistent with the most recent WHO guidelines which suggest urinary glucose as one of the cost-effective screening test for serious TDF-induced kidney injury (URL: http://apps.who.int/iris/bitstream/10665/85321/1/9789241505727_eng.pdf). Since the number of patients with glucosuria was small in this study (about 1% of total population), and glucosuria was not followed until the end of the observation period, further evaluation of this factor is necessary.

Other risk factors for renal dysfunction described in previous studies, such as cotrimoxazole, LPV/r, hepatitis C virus co-infection and diabetes mellitus [13–16] were not identified as risk factors in this study. This discrepancy could be explained by the fact that patients who could be affected by these factors were already excluded according to the study design, which excluded patients with renal dysfunction. With regard to the use of LPV/r, which is known as a risk for renal dysfunction [14,17], especially in cases of co-use with TDF, a number of patients with LPV/r were excluded from the study since most of the patients with LPV/r were co-treated with TDF at baseline. Thus, the impact of co-use of LPV/r and TDF on renal function could be underestimated in this study. Given that LPV/r is used as a salvage regimen and often administered with TDF in Vietnam, long-term monitoring of renal function is required in patients treated with both LPV/r and TDF.

The present study has several limitations. First, data on hypertension, which is a risk factor for renal dysfunction, were not available in this study. Although the average age of patients in this study was around 36 years and the prevalence of hypertension may not high, measurement of blood pressure could lead to better management of renal dysfunction and hypertension should be evaluated for potential risk. Regarding diabetes mellitus as well, the degree of diabetes mellitus was not checked in detail. However, severe patients such as insulin dependence were not in this study, thus, the lack of data could be limited. Second, the observation period of 18 months is relatively short to evaluate long-term adverse event for renal function as mentioned above. Some studies advocated stabilization of decline in eGFR later after the first 6 months of TDF exposure [18] and reversibility of eGFR decline after cessation of TDF therapy [19], while several studies argued incomplete reversibility of eGFR decline following TDF exposure [20–22]. In this study, most of the patients who developed the decline in Crcl continued the same ART regimen because of their moderate and/or stabilized renal dysfunction. However, the observational period of the present study is relatively short compared to other studies, thus, whether or not the stabilization and reversibility will be observed in this cohort of averagely small body weight should be evaluated in the longer period.

Third, the timing of switch to TDF and total duration of ART were not unified in the present study, since the study was an observational cohort in which patients were already on ART at enrollment. The reasons for switch to TDF were mainly related to adverse events caused by d4T and AZT or treatment for HBV infection, thus the timing of switch to TDF was not strictly controlled. However, more than 70% and 90% of the patients were switched to TDF within 3 and 6 months from baseline, respectively, thus influence of this limitation on the result of this study could be restricted.

Despite concern on nephrotoxicity, TDF remains an important drug with enough anti-HIV potency and less mitochondrial toxicity among NRTIs. In order to use it safely in the long term, serum creatinine should be monitored in patients with aforementioned risk factors even in resource-limited situations. Further longitudinal studies are required to determine the impact of TDF, low body weight and glucosuria on renal function in Vietnamese and other Asian people with low body weight.

Conflict of interest

S.O. has received honoraria and research grants from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Pfizer, Co., and Roche Diagnostics K.K.; received honoraria from Astellas Pharmaceutical K.K., Bristol-Myers K.K., Daiichisankyo, Co., Dainippon Sumitomo Pharma, Co., GlaxoSmithKline, K.K., Taisho Toyama Pharmaceutical, Co., Torii Pharmaceutical, Co., and ViiV Healthcare. H.G. has received honoraria from MSD K.K., Abbott Japan, Co., Janssen Pharmaceutical K.K., Torii Pharmaceutical, Co., and ViiV Healthcare, Co. All other authors declare no conflict of interest.

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References

[1] Wyatt CM, Winston JA, Malvestutto CD, Fishbein DA, Barash I, Cohen AJ, et al. Chronic kidney disease in HIV infection: an urban epidemic. AIDS 2007;21:2101–3.
[2] Mocroft A, Kirk O, Gatell J, Reiss P, Gargalianos P, Zilmer K, et al. Chronic renal failure among HIV-1-infected patients. AIDS 2007;21:1119–27.
[3] Deti EK, Thiebaut R, Bonnet F, Lawson-Ayayi S, Dupon M, Neau D, et al. Prevalence and factors associated with renal impairment in HIV-infected patients, ANRS C03 Aquitaine Cohort, France. HIV Med 2010;11:308–17.
[4] Menezes AM, Torelly Jr J, Real L, Bay M, Poeta J, Sprinz E. Prevalence and risk factors associated to chronic kidney disease in HIV-infected patients on HAART and undetectable viral load in Brazil. PLoS One 2011;6:e26042.
[5] Wyatt CM, Arons RR, Klotman PE, Klotman ME. Acute renal failure in hospitalized patients with HIV: risk factors and impact on in-hospital mortality. AIDS 2006;20:561–5.
[6] Franceschini N, Napravnik S, Eron Jr JJ, Szczech LA, Finn WF. Incidence and etiology of acute renal failure among ambulatory HIV-infected patients. Kidney Int 2005;67:1526–31.
[7] Schwartz EJ, Szczech LA, Ross MJ, Klotman ME, Winston JA, Klotman PE. Highly active antiretroviral therapy and the epidemic of HIV+ end-stage renal disease. J Am Soc of Nephrol JASN 2005;16:2412–20.
[8] Cooper RD, Wiebe N, Smith N, Keiser P, Naicker S, Tonelli M. Systematic review and meta-analysis: renal safety of tenofovir disoproxil fumarate in HIV-infected patients. Clin Infect Dis 2010;51:496–505.
[9] Van Rompay KK, Durand-Gasselin L, Brignolo LL, Ray AS, Abel K, Cihlar T, et al. Chronic administration of tenofovir to rhesus macaques from infancy through adulthood and pregnancy: summary of pharmacokinetics and biological and virological effects. Antimicrob Agents Chemother 2008;52:3144–60.
[10] Nishijima T, Komatsu H, Gatanaga H, Aoki T, Watanabe K, Kinai E, et al. Impact of small body weight on tenofovir-associated renal dysfunction in HIV-infected patients: a retrospective cohort study of Japanese patients. PLoS One 2011;6:e22661.

[11] Chaisiri K, Bowonwatanuwong C, Kasettratat N, Kiertiburanakul S. Incidence and risk factors for tenofovir-associated renal function decline among Thai HIV-infected patients with low-body weight. Curr HIV Res 2010;8:504–9.
[12] Mizushima D, Tanuma J, Kanaya F, Nishijima T, Gatanaga H, Lam NT, et al. WHO antiretroviral therapy guidelines 2010 and impact of Tenofovir on chronic kidney disease in Vietnamese HIV-infected patients. PLoS One 2013;8: e79885.
[13] Peters L, Grint D, Lundgren JD, Rockstroh JK, Soriano V, Reiss P, et al. Hepatitis C virus viremia increases the incidence of chronic kidney disease in HIV-infected patients. AIDS 2012;26:1917–26.
[14] Ryom L, Mocroft A, Kirk O, Worm SW, Kamara DA, Reiss P, et al. Association between antiretroviral exposure and renal impairment among HIV-positive persons with normal baseline renal function: the D:A:D study. J Infect Dis 2013;207:1359–69.
[15] Kalayjian RC, Lau B, Mechekano RN, Crane HM, Rodriguez B, Salata RA, et al. Risk factors for chronic kidney disease in a large cohort of HIV-1 infected individuals initiating antiretroviral therapy in routine care. AIDS 2012;26:1907–15.
[16] Choi AI, Li Y, Parikh C, Volberding PA, Shlipak MG. Long-term clinical consequences of acute kidney injury in the HIV-infected. Kidney Int 2010;78:478–85.
[17] Mwafongo A, Nkanaunena K, Zheng Y, Hogg E, Samaneka W, Mulenga L, et al. Renal events among women treated with tenofovir/emtricitabine in combination with either lopinavir/ritonavir or nevirapine: analysis from the AIDS clinical trial group A5208 trial. AIDS 2014;28:1135–42.
[18] Gallant JE, Moore RD. Renal function with use of a tenofovir-containing initial antiretroviral regimen. AIDS 2009;23:1971–5.
[19] Bonjoch A, Echeverria P, Perez-Alvarez N, Puig J, Estany C, Clotet B, et al. High rate of reversibility of renal damage in a cohort of HIV-infected patients receiving tenofovir-containing antiretroviral therapy. Antiviral Res 2012;96: 65–9.
[20] Jose S, Hamzah L, Campbell LJ, Hill T, Fisher M, Leen C, et al. Incomplete reversibility of estimated glomerular filtration rate decline following Tenofovir disoproxil fumarate exposure. J Infect Dis 2014;213:363–73.
[21] Wever K, van Agtmael MA, Carr A. Incomplete reversibility of tenofovir-related renal toxicity in HIV-infected men. J Acquir Immune Defic Syndr 2010;55:78–81.
[22] Yoshino M, Yagura H, Kushida H, Yonemoto H, Bando H, Ogawa Y, et al. Assessing recovery of renal function after tenofovir disoproxil fumarate discontinuation. J Infect Chemother 2012;18:169–74.

ORIGINAL

Development and application of a simple LC-MS method for the determination of plasma rilpivirine (TMC-278) concentrations

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Abstract : Rilpivirine (TMC-278) is a second-generation non-nucleoside reverse transcriptase inhibitor that is high potent against both wild-type and drug-resistant HIV-1 strains. Therefore, rilpivirine is expected to treat therapy-experienced patients who failed to use current drugs due to the emergence of drug-resistant HIV mutants. The quantification of rilpivirine in human plasma is important to support clinical studies and determine pharmacokinetic parameters of rilpivirine in HIV-1 infected patients. Consequently, simple and easy system to determine plasma rilpivirine concentrations has been required. In this study, we developed a conventional LC-MS method to quantify plasma rilpivirine. Subsequently the method was validated by estimating the precision and accuracy for inter- and intraday analysis in the concentration range of 18-715 ng/ml. The calibration curve was linear in this range. Average accuracy ranged from 100.0 to 100.6%. Relative standard deviations of both inter- and intraday assays were less than 3.3%. Recovery of rilpivirine was more than 82.0%. These results demonstrate that our LC-MS method provides a conventional, accurate and precise way to determine rilpivirine in human plasma. This method can be used in routine clinical application for HIV-1 infected patients, and permits management of drug interactions and toxicity for rilpivirine. J. Med. Invest. 60 : 35-40, February, 2013

Keywords **:** *rilpivirine, LC-MS, HIV, therapeutic drug monitoring*

INTRODUCTION

The clinical treatment of patients with human immunodeficiency virus (HIV)-1 infection has been advanced by the success of highly active antiretroviral therapy. The latest treatment guidelines recommend regimen including efavirenz, a first-generation non-nucleoside reverse transcriptase inhibitor (NNRTI), as one of the standard first-line regimen (1). However, efavirenz use is limited by low genetic barrier to resistance and central nervous system toxity (2, 3). Therefore, new antiretroviral drugs, which have long-term efficacy and good tolerability, are required to continue effective therapy for the treatment of HIV-1.

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