Table 3. Characteristics, plasma AZT and intracellular AZT-MP, -DP and -TP concentrations in adult HIV-infected patients

Patients	Age (yrs)/sex	Weight (kg)	AZT dose (mg)	concurrent ARV	Latency (hrs)*	Cell number ($x10^6$)	plasma AZT (nM)	(fmol/10^6 cells)			CD4 cell count	HIV RNA (copies/ml)	Hb	MCV	Remarks
								AZT-MP	AZT-DP	AZT-TP					
1	49/M	61	400	3TC, NVP	1	5.3	2240	5.88	23.2	46.8	1099	UD	16.1	113	
2	40/F	64	400	3TC, LPV/r	12	5.3	21.6	<LLOQ	2.87	3.41	784	UD	13.1	114	
3	34/F	60	400	3TC, LPV/r	2	1.7	219	10.7	16.5	28.5	243	2700	10.2	90.4	Pregnant woman
4	40/M	74	400	3TC, NVP	12	5.5	18.3	2.02	21.7	15.9	635	UD	16	107	
5	38/M	75	400	3TC, LPV/r	4	2	158	4.07	20.0	14.7	971	UD	15.2	105	
6	56/M	64	400	3TC, LPV/r	4	7.2	64.7	1.71	30.5	22.3	811	UD	13.8	111	Samples collected at different days
					6	2.1	16.8	<LLOQ	<LLOQ	5.29	723	UD	13.6	111	
7	36/F	47	400	3TC, LPV/r	13	4.6	2.12	<LLOQ	<LLOQ	<LLOQ	371	UD	12.4	103	Samples collected at different days
					10	3.4	34.1	4.21	3.78	9.56	296	UD	12.8	105	
8	38/F	59	400	3TC, LPV/r	12	5.4	12.3	<LLOQ	<LLOQ	<LLOQ	371	UD	11.1	116	Pregnant woman. Samples collected on same day
					4	5.8	22.4	1.06	1.89	1.98					
9	42/M	78	600	3TC, EFV	12	10	5.62	1.47	2.22	4.21	612	UD	15.3	112	Samples collected on same day
					4	10	163	<LLOQ	2.19	3.28					

* Latency, time between previous AZT dosing and blood sampling; ARV, antiretrovirals; 3TC, lamivudine; NVP, nevirapine; LPV/r, lopinavir/ritonavir; EFV, efavirenz; UD, Undetectable; Hb, hemoglobin; MCV, mean corpuscular volume; AZT, zidovudine; MP, monophosphate; DP, diphosphate; TP, triphosphate; LLOQ, lower limit of quantification

Conclusion

We described here a highly sensitive method for analysis of intracellular AZT phosphates by improving PBMC extraction procedures, alkalization of LC buffer, alkaline-stable HPLC column and the use of low concentration of tetrabutylammonium hydroxide (TBAH) as ion pair. We were able to determine the presence of extremely low concentrations of intracellular AZT metabolites in AZT-treated adult patients. This improved method can be applied to measure other NRTI metabolites because their chemical characteristics are closely similar to those of AZT phosphates. Our method requires small volume of blood samples for determination of intracellular concentrations of AZT metabolites, which can be highly advantageous in children and infants.

References

[1] United States Department of Health and Human Services. Panel on antiretroviral guidelines for adults and adolescents – a working group of the Office of AIDS Research Advisory Council (OARAC). Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. November 13, 2014. Available at http://aidsinfo.nih.gov/guidelines

[2] S. Blanche, M. Tardieu, P. Rustin, A. Slama, B. Barret, G. Firtion, N. Ciraru-Vigneron, C. Lacroix, C. Rouzioux, L. Mandelbrot, I. Desguerre, R. Agnes, M. J. Mayaux, J. F. Delfraissy. Persistent mitochondrial dysfunction and perinatal exposure to antiretroviral nucleoside analogues. *Lancet* **1999**, *354*, 1084–1089.

[3] B. Barret, M. Tardieu, P. Rustin, C. Lacroix, B. Chabrol, I. Desguerre, C. Dollfus, M. J. Mayaux, S. Blanche for the French Perinatal Cohort Study Group. Persistent mitochondrial dysfunction in HIV-1-exposed but uninfected infants: clinical screening in a large prospective cohort. *AIDS* **2003**, *17*, 1769–1785.

[4] L. Durand-Gasselin, A. Pruvost, A. Dehee, G. Vaudre, M. D. Tabone, J. Grassi, G. Leverger, A. Garbarg-Chenon, H. Benech, C. Dolfus. High levels of zidovudine (AZT) and its intracellular phosphate metabolites in AZT- and AZT-lamivudine-treated newborns of human immunodeficiency virus-infected mothers. *Antimicrob. Agents Chemother.* **2008**, *62*, 2555–2563.

[5] A. Pruvost, F. Becher, P. Bardouille, C. Guerrero, C. Creminon, J. F. Delfraissy, C. Goujard, J. Grassi, H. Benech. Direct determination of phosphorylated intracellular anabolites of stavudine (d4T) by liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 1401–1408.

[6] F. Becher, A. Provost, C. Goujard, C. Cuerreiro, J. F. Delfraissy, J. Grassi, H. Benech. Improved method for the simultaneous determination of d4T, 3TC and ddI intracellular phosphorylated anabolites in human peripheral-blood mononuclear cells using high-performance liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 555–565.

[7] A. Pruvost, F. Theodoro, L. Agrofoglio, E. Negredo, H. Benech. Specificity enhancement with LC-positive ESI-MS/MS for the measurement of nucleotides: application to the quantitative determination of carbovir triphosphate, lamivudine triphosphate and tenofovir diphosphate in human peripheral blood mononuclear cells. *J. Mass Spectrom.* **2008**, *43*, 224–233.

[8] P. M. Flynn, J. Rodman, J. C. Lindsey, B. Robbins, E. Capparelli, K. M. Knapp, J. F. Rodriquez, J. McNamara, L. Serchuck, B. Heckman, J. Martinez, and the PACTG1012 Team. Intracellular pharmacokinetics of once versus twice daily zidovudine and lamivudine in adolescents. *Antimicrob. Agents Chemother.* **2007**, *51*, 3516–3522.

[9] E. Font, O. Rosario, J. Santana, H. Garcia, J. P. Sommadossi, J. F. Rodriguez. Determination of zidovudine triphosphate intracellular concentrations in peripheral blood mononuclear cells from human immunodeficiency virus-infected individuals by tandem mass spectrometry. *Antimicrob. Agents Chemother.* **1999**, *43*, 2964–2968.

[10] J. F. Rodriguez, J. L. Rodriguez, J. Santana, H. Garcia, O. Rosario. Simultaneous quantitation of intracellular zidovudine and lamivudine triphosphates in human immunodeficiency virus-infected individuals. *Antimicrob. Agents Chemother.* **2000**, *44*, 3097–3100.

[11] J. D. Moore, F. Valette, A. Darque, X. J. Zhou, J. P. Sommadossi. Simultaneous quantitation of the 5'-triphosphate metabolites of zidovudine, lamivudine, and stavudine in peripheral mononuclear blood cells of HIV infected patients by high-performance liquid chromatography tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 1134–1143.

[12] T. King, I. Bushman, J. Kiser, P. L. Anderson, M. Ray, T. Delahunty, C. V. Fletcher. Liquid chromatography-tandem mass spectrometric determination of tenofovir-diphosphate in human peripheral blood mononuclear cells. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2006**, *843*, 147–156.

[13] B. L. Robbins, P. A. Poston, E. F. Neal, C. Slaughter, J. H. Rodman. Simultaneous measurement of intracellular triphosphate metabolites of zidovudine, lamivudine and abacavir (carbovir) in human peripheral blood mononuclear cells by combined anion exchange solid phase extraction and LC-MS/MS. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2007**, *850*, 310–317.

[14] S. Compain, L. Durand-Gasselin, J. Grassi, H. Benech. Improved method to quantify intracellular zidovudine mono- and triphosphate in peripheral blood mononuclear cells by liquid chromatography-tandem mass spectrometry. *J. Mass Spectrom.* **2007**, *42*, 389–404.

[15] R. Tanaka, H. Hanabusa, E. Kinai, N. Hasegawa, M. Negishi, S. Kato. Intracellular efavirenz levels in peripheral blood mononuclear cells from human immunodeficiency virus-infected individuals. *J. Mass Spectrom.* **2008**, *52*, 782–785.

[16] T. Shirasaka, R. Yarchoan, M. C. O'Brien, R. N. Husson, B. D. Anderson, E. Kojima, T. Shimada, S. Broder, H. Mitsuya. Changes in drug sensitivity of human immunodeficiency virus type 1 during therapy with azidothymidine, dideoxycytidine, and dideoxyinosine: An *in vitro* comparative study. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 562–566.

[17] J. Chen, C. Flexner, R. G. Liberman, P. L. Skipper, N. Louissaint, S. R. Tannenbaum, C. Hendrix, E. Fuchs. Biphasic elimination of tenofovir diphosphate and nonlinear pharmacokinetics of zidovudine triphosphate in a microdosing study. *J. Acquir. Immune Defic. Syndr.* **2012**, *61*, 593–599.

[18] A. Lavie, I. Schlichting, I. R. Vetter, M. Konrad, J. Reinstein, R. S. Goody. The bottleneck in AZT activation. *Nat. Med.* **1997**, *3*, 922–924.

[19] H. Nakata, M. Amano, Y. Koh, E. Kodama, G. Yang, C. M. Bailey, S. Kohgo, H. Hayakawa, M. Matsuoka, K. S. Anderson, Y. C. Cheng, H. Mitsuya. Activity against human immunodeficiency virus type 1, intracellular metabolism, and effects on human DNA polymerases of 4'-ethynyl-2-fluoro-2'-deoxyadenosine. *J. Mass Spectrom.* **2007**, *51*, 2701–2708.

[20] W. Y. Gao, R. Agbaria, J. S. Driscoll, H. Mitsuya. Divergent anti-human immunodeficiency virus activity and anabolic phosphorylation of 2',3'-dideoxynucleoside analogs in resting and activated human cell. *J. Biol. Chem.* **1994**, *269*, 12633–12638.

[21] S. Jorajuria, N. Dereuddre-Bosquet, F. Becher, S. Martin, F. Porcheray, A. Garrigues, A. Mabondzo, H. Benech, J. Grassi, S. Orlowski, D. Dormont, P. Clayette. ATP binding cassette multidrug transporters limit the anti-HIV activity of zidovudine and indinavir in infected human macrophages. *Antivir. Ther.* **2004**, *9*, 519–528.

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RESEARCH ARTICLE

Routine Eye Screening by an Ophthalmologist Is Clinically Useful for HIV-1-Infected Patients with CD4 Count Less than 200 /μL

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Abstract

Objective

To investigate whether routine eye screening by an ophthalmologist in patients with HIV-1 infection is clinically useful.

Methods

A single-center, retrospective study in Tokyo, Japan. HIV-1-infected patients aged over 17 years who visited our clinic for the first time between January 2004 and December 2013 and underwent full ophthalmologic examination were enrolled. At our clinic, ophthalmologic examination, including dilated retinal examination by indirect ophthalmoscopy was routinely conducted by ophthalmologists on the first visit. The prevalence of ophthalmologic diseases and associated factors including the existence of ocular symptoms were analyzed.

Results

Of the 1,515 study patients, cytomegalovirus retinitis (CMV-R) was diagnosed in 24 (2%) patients, HIV retinopathy (HIV-R) in 127 (8%), cataract in 31 (2%), ocular syphilis in 4 (0.3%), and uveitis with unknown cause in 8 (0.5%). Other ocular diseases were diagnosed in 14 patients. The CD4 count was <200 /μL in all CMV-R cases and 87% of HIV-R. The prevalence of any ocular diseases, CMV-R, and HIV-R in patients with CD4 <200 /μL were 22%, 3%, and 15%, respectively, whereas for those with CD4 ≥200 /μL were 5%, 0%, and 2%, respectively. No ocular symptoms were reported by 71% of CMV-R cases and 82% of patients with any ocular diseases.

Conclusions

Routine ophthalmologic screening is recommended for HIV-1-infected patients with CD4 <200 /μL in resource-rich settings based on the high prevalence of ocular diseases within this CD4 count category and because most patients with ocular diseases, including those with CMV-R, were free of ocular symptoms.

Introduction

Antiretroviral therapy (ART) has dramatically improved the prognosis of patients with HIV-1 infection [1]. However, many patients are still diagnosed of advanced HIV-1 infection, with concurrent opportunistic infections [2,3]. HIV-1-infected patients are prone to develop ocular opportunistic infections, such as cytomegalovirus (CMV) retinitis, cryptococcosis, toxoplasmosis, and tuberculosis [4–6]. HIV retinopathy can also occur in patients with HIV-1 infection [7]. Of these ocular opportunistic infections, CMV retinitis occurs among patients with severely advanced HIV-1 infection, and can result in total blindness and increased mortality even in the ART era [8].

To date, there is no consensus on the importance of routine screening of HIV-1-infected patients for ophthalmologic diseases with dilated retinal examination using indirect ophthalmoscopy. The revised 2013 American CDC guidelines for opportunistic infections did not state recommendations for routine ophthalmologic screening for such patients and only described the expert opinion that "some specialists recommend yearly funduscopic examinations performed by an ophthalmologist for patients with CD4 counts <50 /μl" [9]. The aims of the present study were; 1) to determine the prevalence of ocular diseases according to CD4 count and 2) to determine factors associated with CMV retinitis and other ocular diseases, with a special focus on the presence of ocular symptoms, in order to assess the clinical utility of routine ophthalmologic screening by an ophthalmologist for patients with HIV-1 infection in a resource-rich setting.

Methods

Study design, setting, and participants

We conducted a single-center retrospective study to investigate the usefulness of routine ophthalmologic screening for patients with HIV-1 infection at AIDS Clinical Center, National Center for Global Health and Medicine (NCGM), Tokyo. AIDS Clinical Center is one of the largest referral centers for HIV-1 infection in Japan with more than 3,800 registered patients [10], and considering that the total reported number of patients with HIV-1 infection is 24,454 by the end of 2013, this clinic treats approximately 15% of the HIV-1 infected patients in Japan [11]. At our clinic, ophthalmologic examination, including dilated retinal examination using indirect ophthalmoscopy, is routinely conducted during the first visit to the clinic by an ophthalmologist, and if it is not possible during the first visit, patients are referred to an ophthalmologists during the second/third visit [12]. The following criteria were applied for enrollment of patients in the study. Inclusion criteria: 1) HIV-1-infected patients aged over 17 years who visited our clinic for the first time between January 2004 and December 2013 and underwent full ophthalmologic examination within one year from the first visit. Exclusion criteria: 1) patients who were already diagnosed with ocular diseases at the time of referral to our clinic, because the aim of this study was to evaluate the usefulness of routine ophthalmologic screening. Furthermore, it

is often difficult to confirm retinal photography which are required for the diagnosis of CMV retinitis according to the standard ACTG criteria [12,13].

The study protocol was approved by the Human Research Ethics Committee of National Center for Global Health and Medicine (G-001623-01). Informed consent was waived because this study solely used the data gained from clinical practice. Patient information was anonymized and de-identified prior to analysis. The study was conducted according to the principles expressed in the Declaration of Helsinki.

Definitions of ophthalmologic diseases and measurements

The results of the first ophthalmologic examination conducted in each patient were extracted from the medical records, together with any or no ocular symptoms. CMV retinitis which fulfilled the standard ACTG criteria of "confirmed CMV retinitis" was included as CMV retinitis cases which required a diagnosis by an experienced ophthalmologist and documentation of CMV retinitis by retinal photography [13]. The diagnosis of HIV retinopathy was based on the findings of microaneurysms, telangiectasia, retinal hemorrhages, and cotton wool spots (CWS) [7]. The diagnosis of ocular syphilis was based on 1) ophthalmological examination documenting findings specific to ocular syphilis and 2) evidence of syphilis infection defined by a positive serum quantitative rapid plasma reagin (RPR) (titer $\geq$8) and positive *Treponema pallidum* hemagglutination assay [14,15]. When ruling out other ocular diseases was difficult, the response to syphilis treatment was also taken into account. At our hospital, if the diagnosis of ocular diseases could not be confirmed, ophthalmological examination was repeated within one to four weeks, and the diagnosis was confirmed by at least two ophthalmologists. Old lesions considered inactive were excluded. All ocular diseases were reviewed and confirmed by an experienced ophthalmologist (SY) and a HIV expert (TN). The basic characteristics [age, sex, ethnicity, the day (if not available, the month) of diagnosis of HIV-1 infection, history of AIDS, route of HIV-1 transmission, and treatment status of HIV-1 infection (either treatment-naïve or experienced)], CD4 count, HIV-1 viral load, quantitative RPR were also collected. For the latter three variables, the data closest to and preceding the day of the first ophthalmologic examination were used. Quantitative RPR was routinely measured on the day of the first visit and on discretion of treating physician at our clinic. The variable CMV end-organ diseases other than retinitis was also collected; their diagnosis of each disease was based on the standardized ACTG criteria and confirmed within 4 weeks of ophthalmological examination [12,13]. The variables systemic steroid use, anti-CMV treatment, and chemotherapy were also recorded. They were defined as steroid, anti-CMV treatment, and chemotherapy, which were administered either orally or intravenously within one month preceding the ophthalmological examination [12].

Statistical analysis

All analyses were performed and all results were presented at the subject-level, rather than eye level. Baseline characteristics were described for the entire study patients, those with CMV retinitis, HIV retinopathy, cataract, and ocular syphilis. A univariate logistic regression model was constructed to estimate the association of each variable with CMV retinitis, HIV retinopathy, and cataract, respectively, and variables with $p < 0.05$ in univariate analysis were incorporated into the multivariate model. For CMV retinitis, the variables anti-CMV treatment and history of AIDS were not added to the multivariate model because of multicollinearity with CMV diseases other than retinitis and CD4 count, respectively, and because of the small number of cases with CMV retinitis. For cataract, the variable systemic steroid use was added to the multivariate model because systemic steroid use is an established risk factor for cataract [16,17]. Sex

was not added to any of the models because the study included only a small number of female patients. 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

1,515 (66%) patients were analyzed as the study patients (Fig 1). They were mostly Asian men who had sex with men and were treatment-naïve for HIV-1 infection (Table 1). The median CD4 count and HIV-1 load were 210 /μL [interquartile range (IQR) 66–353 /μL)] and 4.76 $\log_{10}$copies/mL (IQR 4.04–5.28 $\log_{10}$copies/mL), respectively. Median time from diagnosis of HIV-1 infection to ophthalmological examination was 1 month (IQR 0.3–2.6 months). Of the study patients, 204 (13%) presented with ocular diseases; ocular diseases included CMV retinitis in 24 (1.6%), HIV retinopathy in 127 (8.4%), cataract in 31 (2%), ocular syphilis in 4 (0.3%), uveitis with unknown cause in 8 (0.5%), and other diseases listed in Fig 1 in 14. Two patients

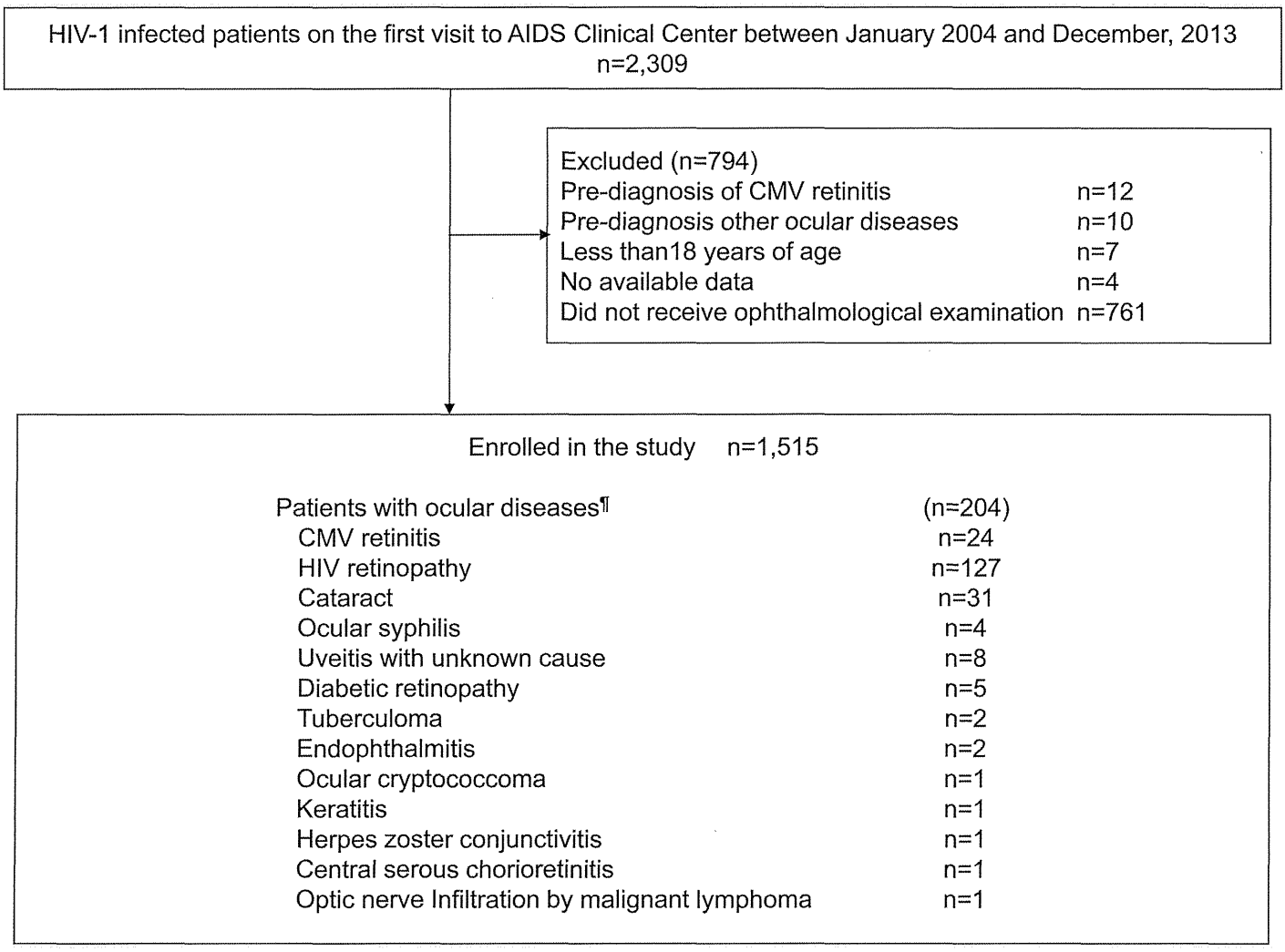

Fig 1. Patient enrollment process. ¶Two patients had both HIV retinopathy and cataract, one had both cataract and diabetic retinopathy, and one had both HIV retinopathy and diabetic retinopathy. CMV: cytomegalovirus.

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Table 1. Baseline characteristics of study patients and those with various ocular diseases.

	Study patients n = 1515	Any ocular diseases n = 204	CMV retinitis n = 24	HIV retinopathy n = 127	Cataract n = 31	Ocular syphilis n = 4
Ocular symptoms, n (%)[¶]	50 (3)	27 (13)	7 (29)	10 (8)	10 (32)	2 (50)
Sex (male), n (%)	1428 (94)	199 (98)	24 (100)	123 (97)	31 (100)	4 (100)
Age[†]	36 (30–44)	42 (36–54)	42 (34–53)	40 (36–51)	63 (44–66)	37 (34–44)
Asian, n (%)	1456 (96)	200 (98)	22 (92)	124 (99)	30 (97)	4 (100)
CD4 count (/μl)[†]	210 (66–353)	67 (21–168)	27 (15–71)	51 (18–91)	224 (89–323)	181 (163–499)
HIV RNA load (log_{10}/ml)[†]	4.76 (4.04–5.28)	5.22 (4.65–5.71)	5.28 (4.50–5.81)	5.36 (4.93–5.85)	4.59 (3.90–5.20)	4.35 (3.23–5.43)
Treatment-naive, n (%)	1320 (87)	185 (91)	21 (88)	118 (93)	26 (84)	4 (100)
CMV diseases other than retinitis, n (%)	17 (1)	8 (4)	2 (8)	6 (5)	0	0
Anti-CMV treatment, n (%)	36 (2)	11 (5)	3 (13)	7 (6)	0	0
Rapid plasma reagin titer ≥8, n (%)	210 (14)	27 (13)	4 (17)	14 (11)	3 (10)	4 (100)
Chemotherapy, n (%)	14 (1)	1 (0.1)	1 (4)	0	0	0
Systemic steroid use, n (%)	157 (10)	43 (21)	5 (21)	34 (27)	4 (13)	0
History of AIDS, n (%)	477 (32)	125 (61)	24 (100)	83 (65)	10 (32)	2 (50)
Route of transmission						
Homosexual contact, n (%)	1221 (81)	161 (79)	18 (75)	105 (83)	24 (77)	3 (75)
Heterosexual contact, n (%)	229 (15)	32 (16)	5 (21)	17 (14)	4 (13)	1 (25)
Contaminated blood product, n (%)	11 (1)	2 (1)	0	1 (1)	1 (3)	0
Injection drug, n (%)	24 (2)	3 (1.5)	0	1 (1)	1 (3)	0
Unknown, n (%)	30 (2)	6 (3)	1 (4)	3 (2)	1 (3)	0
Months between diagnosis of HIV-1 infection and ophthalmologic examination[†]*	1 (0.3–2.6)	NA	NA	NA	NA	NA

[†]Median (interquartile range). CMV: cytomegalovirus, NA: not applicable.

Two patients had both HIV retinopathy and cataract.

[¶]Ocular symptoms were not assessed in five patients because of altered mental status

*Data for the day of diagnosis of HIV-1 infection are missing for 39 patients.

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had both HIV retinopathy and cataract, one had both cataract and diabetic retinopathy, and one had both HIV retinopathy and diabetic retinopathy.

Table 2 shows the prevalence of ocular diseases according to CD4 cell count. Ocular diseases were identified in 81 (26%) of 308 patients with CD4 <50 /μL, 130 (27%) of 490 with CD4 <100 /μL, 162 (22%) of 731 with CD4 <200 /μL, and 28 (5.4%) of 784 with CD4 count ≥200 /μL. CMV retinitis was diagnosed in 24 (1.6%) of the 1,515 study patients, 14 (5%) of 308 patients with CD4 <50 /μL, 20 (4%) of 490 with CD4 <100 /μL, 24 (3%) of 731 with CD4 <200 /μL, and none among patients with CD4 count ≥200 /μL. For patients with CD4 count <200 /μL, three uveitis with unknown cause, two endophthalmitis, two diabetic retinopathy, two tuberculoma, one ocular cryptococcoma, and one keratitis were diagnosed other than those listed in Table 2.

At the time of ophthalmological examination, 50 patients (3%) of the total 1,515 patients complained of ocular symptoms (Table 1). Among 204 patients with any ocular diseases, only 27 (13%) had ocular symptoms. Similarly, only 7 (29%) out of 24 patients with CMV retinitis, 10 (8%) out of 127 with HIV retinopathy, 10 (32%) of 31 with cataract, and 2 (50%) of 4 with ocular syphilis, respectively, had ocular symptoms.

The median CD4 count of 24 cases with CMV retinitis was 27 /μL (IQR 15–71 /μL, range 7–158 /μL) (Table 1). Two (8%) patients also had CMV diseases other than retinitis, and three

Table 2. Prevalence of ocular diseases according to CD4 cell count.

	All patients n = 1515	CD4 <50 /μl n = 308	CD4 <100 /μl n = 490	CD4 <200 /μl n = 731	CD4 ≥200 n = 784
Any ocular diseases	204 (14)	81 (26)	130 (27)	162 (22)	42 (5.4)
CMV retinitis	24 (1.6)	14 (4.5)	20 (4.1)	24 (3.3)	0
HIV retinopathy	127 (8.4)	62 (20)	97 (20)	111 (15)	16 (2)
Cataract	31 (2)	2 (0.6)	9 (1.8)	15 (2.1)	16 (2)
Ocular syphilis	4 (0.3)	0	0	3 (0.4)	1 (0.1)

Data are numbers (percentages).
CMV: cytomegalovirus

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(13%) had received anti-CMV treatment. The results of multivariate analysis showed that the presence of ocular symptom versus no symptoms was significantly associated with CMV retinitis (OR 13, 95% CI 4.59–36.0, p<0.001) (Table 3). Lower CD4 count (per 100 /μL decrement, OR 3.9, 95% CI 1.85–8.30, p<0.001) was also significantly associated with CMV retinitis, whereas presence of CMV diseases other than retinitis showed a trend toward association that was not statistically significant (OR 3.9, 95% CI 0.78–19.1, p = 0.097).

111 (87%) of 127 patients with HIV retinopathy had CD4 count less than 200 /μL (median 51 /μL, IQR 18–91 /μL, range 1–403 /μL). 34 (27%) patients were using systemic steroid, and 83 (65%) patients were with history of AIDS. In multivariate model, older age (per 10 year increment, OR 1.4, 95% CI 1.15–1.61, p<0.001), lower CD4 count (per 100 /μL decrement, OR 1.7, 95% CI 1.36–2.13, p<0.001), and higher HIV-1 load (per 1 $\log_{10}$copies/mL, OR 1.6, 95% CI 1.20–2.06, p = 0.001) were significantly associated with HIV retinopathy, whereas presence of ocular symptoms and history of AIDS showed a trend toward association that was not statistically significant (Table 4).

31 patients with cataract were relatively old (median age 63, IQR 44–66), and had a median CD4 count of 224 /μL (IQR 89–323 /μL). In multivariate analysis, presence of ocular symptoms and older age were significantly associated with cataract (ocular symptoms, OR 13, 95% CI 4.86–33.4, p<0.001) (age per 10 year increment, OR 3.1, 95% CI 2.26–4.34, p<0.001), whereas

Table 3. Uni- and multi-variate analyses to estimate the associations of various factors with cytomegalovirus retinitis.

	Crude model			Adjusted model		
	OR	95%CI	P value	Adjusted OR	95%CI	P value
Ocular symptoms versus no ocular symptoms	15	5.75–37.6	<0.001	13	4.59–36.0	<0.001
Age per 10 year increment	1.5	1.09–2.07	0.012	1.2	0.86–1.77	0.26
CD4 count per 100 /μl decrement	4.3	2.07–8.92	<0.001	3.9	1.85–8.30	<0.001
HIV-1 viral load per 1 $\log_{10}$copies/ml increment	1.5	0.96–2.28	0.079			
CMV diseases other than retinitis	8.9	1.93–41.5	0.005	3.9	0.78–19.1	0.097
Anti-CMV treatment	6.3	1.79–22.2	0.004			
History of AIDS	6.7	2.66–17.1	<0.001			
Antiretroviral therapy	1.0	0.29–3.27	0.96			
Chemotherapy	4.9	0.62–39.4	0.13			
Systemic steroid use	2.3	0.85–6.30	0.099			

Variables with p <0.05 in the univariate analysis were incorporated into the multivariate model. Anti-CMV treatment and history of AIDS were not added to the multivariate model because of multicollinearity with CMV diseases other than retinitis and CD4 count, respectively. History of AIDS was not added to the multivariate model because CMV retinitis is one of the AIDS-defining illnesses.

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Table 4. Uni- and multi-variate analyses to estimate the associations of various factors with HIV retinopathy.

	Crude model			Adjusted model		
	OR	95%CI	P value	Adjusted OR	95%CI	P value
Ocular symptoms versus no ocular symptoms	3.0	1.44–6.06	0.003	2.2	0.99–4.76	0.052
Age per 10 year increment	1.5	1.31–1.78	<0.001	1.4	1.15–1.61	<0.001
CD4 count per 100 /μl decrement	2.3	1.92–2.84	<0.001	1.7	1.36–2.13	<0.001
HIV-1 viral load per 1 $\log_{10}$copies/ml increment	2.5	1.95–3.17	<0.001	1.6	1.20–2.06	0.001
History of AIDS	4.8	3.24–6.99	<0.001	1.6	0.97–2.57	0.064
Antiretroviral therapy	0.5	0.25–0.99	0.046	0.8	0.37–1.83	0.63
Systemic steroid use	3.8	2.44–5.81	<0.001	1.2	0.71–1.96	0.52

Variables with p <0.05 in univariate analysis were incorporated into the multivariate model.

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systemic steroid use was not associated (OR 1.0, 95% CI 0.28–3.42, p = 0.97). In 4 patients with ocular syphilis, the titer of RPR was 256, 64, 64, and 16, and higher RPR value was associated with ocular syphilis (univariate analysis, per 2^n RPR titer increment, OR 1.6, 95% CI 1.18–2.15, p = 0.002, where RPR titer <8 was treated as RPR = 2).

Discussion

In this single center cohort where an ophthalmologist routinely screens ocular diseases with dilated retinal examination using indirect ophthalmoscopy for HIV-1-infected patients who first visited the clinic [12], we aimed to determine whether routine ophthalmologic screening is clinically useful. The results of the present study showed the presence of ocular diseases and CMV retinitis in 26% and 5% of patients with CD4 <50 /μL, and in 22% and 3% of patients with CD4 <200 /μL, respectively, but only in 5.4% and 0% of patients with patients with CD4 count ≥200 /μL. Of the 24 patients with CMV retinitis, 14 patients had CD4 count <50 /μL, whereas 6 patients had CD4 50–99 /μL and 4 patients had CD4 100–199 /μL. Importantly, only 27 (13%) of 204 patients with any ocular diseases and 7 (29%) of 24 patients with CMV retinitis had ocular symptoms. The importance of early detection of ocular diseases, especially CMV retinitis is well established [18]. Based on the high prevalence of ocular diseases among patients with CD4 <200 /μL and high percentage of asymptomatic patients among those with ocular diseases, we recommend ophthalmological screening with dilated retinal examination using indirect ophthalmoscopy in HIV-1-infected patients with CD4 count of <200 /μL in resource-rich settings.

To our knowledge, this is the first study that implemented systemic screening by ophthalmologists for all HIV-1-infected patients during the first visit to the HIV referral clinic regardless of CD4 count. The study design allowed comparison of the prevalence of ocular diseases according to CD4 count. The result showed that the prevalence of ocular diseases among HIV-1-infected patients with CD4 ≥200 /μL was 5.4% (CMV retinitis 0%), far lower than 22% (CMV retinitis 3%) among patients with CD4 <200 /μL. Other studies that investigated the incidence and prevalence of ocular diseases among HIV-1-infected patients, such as the Longitudinal Study of the Ocular Complications of AIDS (LSOCA) [19], only enrolled AIDS patients with very low median nadir CD4 count of 30 /μL. Furthermore, in this study, most study patients underwent ophthalmological examination soon after the diagnosis of HIV-1 infection (median 1 month, IQR 0.3–2.6 months). Thus, the results of ophthalmological examination in this study would be similar to the results at the time of diagnosis of HIV-1 infection.

Little evidence is available for the clinical usefulness of routine ophthalmological screening in HIV-1-infected patients. Although both the 2013 American CDC guidelines for the treatment of opportunistic infections and 2013 Primary Care Guidelines by Infectious Diseases Society of America recommend referral of patients with CD4 count <50 /μL to an ophthalmologist for examination, the recommendation is mostly based on expert opinion [9,20]. It is well-known that CMV retinitis typically occurs in patients with CD4 count <50 /μL [21,22]. However, CMV retinitis can also occur in patients with CD4 >50 /μL [23], as in the present study the CD4 count was 50–199 /μL in 10 (42%) out of 24 CMV retinitis cases. Considering that 71% of CMV cases had no ocular symptoms, we recommend that routine screening by ophthalmologists of patients with CD4 <200 /μL is rational in resource-rich settings.

In the assessment of associated factors for each ocular disease, lower CD4 count and higher HIV-1 load were associated with HIV retinopathy, in agreement with the results of previous studies [24,25]. On the other hand, to our knowledge, this is the first study to report a significant association between old age and HIV retinopathy. HIV retinopathy is a well-known ocular disease characterized by micro aneurysms, telangiectasia, retinal hemorrhages, and CWS [7]. Pathogenesis and clinical significance of HIV retinopathy are not fully understood, and number of probable etiologies or associated factors, including viral immune complex, direct HIV infection of endothelial cells, and hyperviscosity due to red cell aggregation, have been suggested [7,26,27]. Clinically, most patients with HIV retinopathy do not have visual complaints [7] and CWS can disappear within a few weeks [28]. However, abrupt visual loss can occur in patients with HIV retinopathy [29], and HIV-induced CWS can cause permanent retinal destruction [26]. Our finding that old age is associated with HIV retinopathy might suggest the importance of active ophthalmologic screening for the elderly with HIV-1 infection.

The association between cataract and HIV-1 infection has been poorly documented. However, one Danish nationwide population-based cohort study reported almost twice higher risk of cataract surgery in patients with HIV-1 infection than in the general population [30]. One possible explanation for the susceptibility of HIV-1-infected patients to cataract is the occurrence of immune recovery uveitis/vitritis after the introduction of ART, with subsequent development of secondary ocular manifestations, including cataract [30–32]. The prevalence of cataract in this study was 2%, but it is difficult to directly compare this number with other studies, because older age is an established risk factor for cataract [33] and such comparison requires age matching. Although the Danish study identified that CD4 count ≤200 /μl and introduction of ART as risk factors for cataract [30], these findings were not reproduced in the present study.

Several limitations need to be acknowledged. First, due to the nature of a single-center cohort study, selection bias of study patients could not be ruled out. However, due to the very low prevalence of HIV-1 infection in Japan, our clinic treats approximately 15% of the HIV-1 infected patients in Japan [10,11]. Second, the majority of patients diagnosed with ocular diseases in the present study had HIV retinopathy, for which the clinical significance and sequelae are still not fully elucidated as described above. However, HIV-induced CWS can serve as a portal for CMV entry to the retina [34], and HIV retinopathy is an established risk factor for subsequent CMV retinitis [5,34,35]. Some experts even recommend ophthalmological examination every three months for patients with HIV retinopathy [7,27]. Third, 34% of the HIV-1-infected patients on the first visit to our clinic during the study period, including 12 patients with pre-diagnosed CMV retinitis, were excluded from the study (Fig 1), mostly because they did not receive ophthalmologic examination. If the characteristics of these patients differ substantially from the included cohort, this could influence the interpretation of the results.

In conclusion, the present study reported the results of systematic screening of HIV-1-infected patients by ophthalmologists with dilated retinal examination using indirect ophthalmoscopy

among HIV-1-infected patients shortly after the diagnosis of HIV-1 infection. The prevalence of any ocular diseases and CMV retinitis were 22% and 3% in patients with CD4 <200 /μL, respectively, but were only 5.4% and 0% in patients with CD4 count ≥200 /μL. Furthermore, only 13% of patients with any ocular diseases and 29% of CMV cases complained of ocular symptoms. We recommend routine ophthalmologic screening by ophthalmologists for HIV-1-infected patients with CD4 count of <200 /μL in resource-rich settings.

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Author Contributions

Conceived and designed the experiments: TN SY. Analyzed the data: TN SY. Contributed reagents/materials/analysis tools: YK KT. Wrote the paper: TN SY NK SO HG.

References

1. Helleberg M, May MT, Ingle SM, Dabis F, Reiss P, Fatkenheuer G, et al. (2015) Smoking and life expectancy among HIV-infected individuals on antiretroviral therapy in Europe and North America. Aids 29: 221–229. doi: 10.1097/QAD.0000000000000540 PMID: 25426809
2. Lahuerta M, Wu Y, Hoffman S, Elul B, Kulkarni SG, Remien RH, et al. (2014) Advanced HIV disease at entry into HIV care and initiation of antiretroviral therapy during 2006–2011: findings from four sub-saharan African countries. Clin Infect Dis 58: 432–441. doi: 10.1093/cid/cit724 PMID: 24198226
3. Hall HI, Halverson J, Wilson DP, Suligoi B, Diez M, Le Vu S, et al. (2013) Late diagnosis and entry to care after diagnosis of human immunodeficiency virus infection: a country comparison. PLoS One 8: e77763. doi: 10.1371/journal.pone.0077763 PMID: 24223724
4. Kestelyn PG, Cunningham ET Jr. (2001) HIV/AIDS and blindness. Bull World Health Organ 79: 208–213. PMID: 11285664
5. Holland GN (2008) AIDS and ophthalmology: the first quarter century. Am J Ophthalmol 145: 397–408. doi: 10.1016/j.ajo.2007.12.001 PMID: 18282490
6. Kaleem MA, Ramsahai S, Del Fierro K, Rasul S, Onumah C, Lerebours V, et al. (2012) Ocular findings in human immunodeficiency virus patients in Washington, DC. Int Ophthalmol 32: 145–151. doi: 10.1007/s10792-012-9543-3 PMID: 22450559
7. Stewart MW (2012) Human immunodeficiency virus and its effects on the visual system. Infect Dis Rep 4: e25. doi: 10.4081/idr.2012.e25 PMID: 24470932
8. Jabs DA, Holbrook JT, Van Natta ML, Clark R, Jacobson MA, Kempen JH, et al. (2005) Risk factors for mortality in patients with AIDS in the era of highly active antiretroviral therapy. Ophthalmology 112: 771–779. PMID: 15878056
9. 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: http://aidsinfo.nih.gov/contentfiles/lvguidelines/adult_oi.pdf.
10. Nishijima T, Komatsu H, Gatanaga H, Aoki T, Watanabe K, Kinai E, et al. (2011) Impact of small body weight on tenofovir-associated renal dysfunction in HIV-infected patients: a retrospective cohort study of Japanese patients. PLoS One 6: e22661. doi: 10.1371/journal.pone.0022661 PMID: 21799928
11. AIDS Surveillance Committee. The Japanese Ministry of Health, Labour, and Welfare. Available: http://api-net.jfap.or.jp/status/2013/13nenpo/nenpo_menu.htm. in Japanese.
12. Mizushima D, Nishijima T, Yashiro S, Teruya K, Kikuchi Y, Katai N, et al. (2015) Diagnostic Utility of Quantitative Plasma Cytomegalovirus DNA PCR for Cytomegalovirus End-Organ Diseases in Patients With HIV-1 Infection. J Acquir Immune Defic Syndr 68: 140–146. doi: 10.1097/QAI.0000000000000410 PMID: 25590268
13. Wohl DA, Kendall MA, Andersen J, Crumpacker C, Spector SA, Feinberg J, et al. (2009) Low rate of CMV end-organ disease in HIV-infected patients despite low CD4+ cell counts and CMV viremia:

results of ACTG protocol A5030. HIV Clin Trials 10: 143–152. doi: 10.1310/hct1003-143 PMID: 19632953

14. Tucker JD, Li JZ, Robbins GK, Davis BT, Lobo AM, Kunkel J, et al. (2011) Ocular syphilis among HIV-infected patients: a systematic analysis of the literature. Sex Transm Infect 87: 4–8. doi: 10.1136/sti.2010.043042 PMID: 20798396
15. Kiss S, Damico FM, Young LH (2005) Ocular manifestations and treatment of syphilis. Semin Ophthalmol 20: 161–167. PMID: 16282150
16. Curtis JR, Westfall AO, Allison J, Bijlsma JW, Freeman A, George V, et al. (2006) Population-based assessment of adverse events associated with long-term glucocorticoid use. Arthritis Rheum 55: 420–426. PMID: 16739208
17. Da Silva JA, Jacobs JW, Kirwan JR, Boers M, Saag KG, Ines LB, et al. (2006) Safety of low dose glucocorticoid treatment in rheumatoid arthritis: published evidence and prospective trial data. Ann Rheum Dis 65: 285–293. PMID: 16107513
18. Heiden D, Ford N, Wilson D, Rodriguez WR, Margolis T, Janssens B, et al. (2007) Cytomegalovirus retinitis: the neglected disease of the AIDS pandemic. PLoS Med 4: e334. PMID: 18052600
19. Jabs DA, Van Natta ML, Holbrook JT, Kempen JH, Meinert CL, Davis MD (2007) Longitudinal study of the ocular complications of AIDS: 1. Ocular diagnoses at enrollment. Ophthalmology 114: 780–786. PMID: 17258320
20. Aberg JA, Gallant JE, Ghanem KG, Emmanuel P, Zingman BS, Horberg MA (2013) Primary Care Guidelines for the Management of Persons Infected With HIV: 2013 Update by the HIV Medicine Association of the Infectious Diseases Society of America. Clinical Infectious Diseases 58: e1–e34. doi: 10.1093/cid/cit665 PMID: 24235263
21. Jabs DA (1995) Ocular manifestations of HIV infection. Trans Am Ophthalmol Soc 93: 623–683. PMID: 8719695
22. Gallant JE, Moore RD, Richman DD, Keruly J, Chaisson RE (1992) Incidence and natural history of cytomegalovirus disease in patients with advanced human immunodeficiency virus disease treated with zidovudine. The Zidovudine Epidemiology Study Group. J Infect Dis 166: 1223–1227. PMID: 1358986
23. Jacobson MA, Zegans M, Pavan PR, O'Donnell JJ, Sattler F, Rao N, et al. (1997) Cytomegalovirus retinitis after initiation of highly active antiretroviral therapy. Lancet 349: 1443–1445. PMID: 9164318
24. Furrer H, Barloggio A, Egger M, Garweg JG (2003) Retinal microangiopathy in human immunodeficiency virus infection is related to higher human immunodeficiency virus-1 load in plasma. Ophthalmology 110: 432–436. PMID: 12578793
25. Iwasaki Y, Yamamoto N, Kawaguchi T, Ozaki N, Tomita M, Ajisawa A, et al. (2013) Human immunodeficiency virus-related retinal microangiopathy and systemic cytomegalovirus disease association. Jpn J Ophthalmol 57: 372–378. doi: 10.1007/s10384-013-0247-7 PMID: 23700203
26. Gomez ML, Mojana F, Bartsch DU, Freeman WR (2009) Imaging of long-term retinal damage after resolved cotton wool spots. Ophthalmology 116: 2407–2414. doi: 10.1016/j.ophtha.2009.05.012 PMID: 19815278
27. Vrabec TR (2004) Posterior segment manifestations of HIV/AIDS. Surv Ophthalmol 49: 131–157. PMID: 14998690
28. Iragui VJ, Kalmijn J, Plummer DJ, Sample PA, Trick GL, Freeman WR (1996) Pattern electroretinograms and visual evoked potentials in HIV infection: evidence of asymptomatic retinal and postretinal impairment in the absence of infectious retinopathy. Neurology 47: 1452–1456. PMID: 8960726
29. Cunningham ET Jr., Levinson RD, Jampol LM, Engstrom RE Jr., Lewis H, Holland GN (2001) Ischemic maculopathy in patients with acquired immunodeficiency syndrome. Am J Ophthalmol 132: 727–733. PMID: 11704034
30. Rasmussen LD, Kessel L, Molander LD, Pedersen C, Gerstoft J, Kronborg G, et al. (2011) Risk of cataract surgery in HIV-infected individuals: a Danish Nationwide Population-based cohort study. Clin Infect Dis 53: 1156–1163. doi: 10.1093/cid/cir675 PMID: 21998282
31. Thorne JE, Jabs DA, Kempen JH, Holbrook JT, Nichols C, Meinert CL (2006) Causes of visual acuity loss among patients with AIDS and cytomegalovirus retinitis in the era of highly active antiretroviral therapy. Ophthalmology 113: 1441–1445. PMID: 16781775
32. Goldberg DE, Smithen LM, Angelilli A, Freeman WR (2005) HIV-associated retinopathy in the HAART era. Retina 25: 633–649; quiz 682–633. PMID: 16077362
33. Lindblad BE, Hakansson N, Philipson B, Wolk A (2008) Metabolic syndrome components in relation to risk of cataract extraction: a prospective cohort study of women. Ophthalmology 115: 1687–1692. doi: 10.1016/j.ophtha.2008.04.004 PMID: 18538408

34. Hodge WG, Boivin JF, Shapiro SH, Lalonde RG, Shah KC, Murphy BD, et al. (2004) Clinical risk factors for cytomegalovirus retinitis in patients with AIDS. Ophthalmology 111: 1326–1333. PMID: 15234132

35. Sugar EA, Jabs DA, Ahuja A, Thorne JE, Danis RP, Meinert CL (2012) Incidence of cytomegalovirus retinitis in the era of highly active antiretroviral therapy. Am J Ophthalmol 153: 1016–1024 e1015. doi: 10.1016/j.ajo.2011.11.014 PMID: 22310076

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RESEARCH ARTICLE

Prevalence of Anal Human Papillomavirus Infection and Risk Factors among HIV-positive Patients in Tokyo, Japan

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Abstract

Background

Oncogenic human papillomavirus (HPV) infection, particularly multiple HPV types, is recognized as a necessary cause of anal cancer. However, a limited number of studies have reported the prevalence of anal HPV infection in Asia. We determined the prevalence, genotypes, and risk factors for anal HPV infection in Japanese HIV-positive men who have sex with men (MSM), heterosexual men, and women.

Methods

This cross-sectional study included 421 HIV-positive patients. At enrollment, we collected data on smoking, alcohol, co-morbidities, drugs, CD4 cell counts, HIV RNA levels, highly active anti-retroviral therapy (HAART) duration, sexually transmitted infections (STIs), and serological screening (syphilis, hepatitis B virus, *Chlamydia trachomatis*, *Entamoeba histolytica*). Anal swabs were collected for oncogenic HPV genotyping.

Results

Oncogenic HPV rate was 75.9% in MSM, 20.6% in heterosexual men, and 19.2% in women. HPV 16/18 types were detected in 34.9% of MSM, 17.7% of heterosexual men, and 11.5% of women. Multiple oncogenic HPV (≥2 oncogenic types) rate was 54.6% in MSM, 8.8% in heterosexual men, and 0% in women. In univariate analysis, younger age, male sex, MSM, CD4 <100, HIV viral load >50,000, no administration of HAART, and having ≥2 sexually transmitted infections (STIs) were significantly associated with oncogenic HPV infection, whereas higher smoking index and corticosteroid use were marginally associated with oncogenic HPV infection. In multivariate analysis, younger age (OR, 0.98 [0.96–0.99]), MSM (OR, 5.85 [2.33–14.71]), CD4 <100 (OR, 2.24 [1.00–5.01]), and having ≥2

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STIs (OR, 2.81 [1.72–4.61]) were independently associated with oncogenic HPV infection. These 4 variables were also significant risk factors for multiple oncogenic HPV infection.

Conclusions

Among Japanese HIV-infected patients, approximately two-thirds of MSM, one-fifth of heterosexual men, and one-fifth of women have anal oncogenic HPV infection. Younger *age*, MSM, ≥2 STIs, and immunosuppression confer a *higher risk* of infection with oncogenic HPV and multiple oncogenic types.

Introduction

Human papillomavirus (HPV) infections are the most common sexually transmitted infections (STIs) worldwide[1]. HPV types that infect the anorectal area can be divided into oncogenic types and non-oncogenic types, and approximately 85% of anal cancers worldwide are attributed to oncogenic HPV[2,3]. In addition, infection with multiple HPV types has been associated with HPV persistence and longer duration of infection[4,5]; thus, co-infection with multiple oncogenic HPVs may be an important risk factor for anal cancer. Although anal cancers are rare in the general population[6], HIV-positive men who has sex with men (MSM) have an approximately 1.5 times higher prevalence of anal HPV infection and a 5 times higher risk of anal cancer than HIV-negative MSM[7,7–9], even in this era of highly active antiretroviral therapy (HAART) use[10].

Most data on anal HPV infection have been obtained in Western countries. A limited number of studies have reported the prevalence of anal HPV infection in Asia including Taiwan [7,11], India[12], Thailand[7], and China[7,8,13]. In Japan, 14,706 HIV and 6719 AIDS cases were reported at the end of 2012[14]. Although the prevalence of HIV in the general population remains low at 0.018%, the number of newly reported cases of HIV in MSM more than doubled from 314 in 2001 to 724 in 2012, and it is possible that the number of HIV-positive MSM could reach 10.4% in 2040[14,15]. Therefore, there have been concerns about the increasing trend of anal HPV infection as well as anorectal STIs such as syphilis[16] and invasive amebiasis[17]. However, no data are available on the prevalence of anal HPV infection in Japan. Prior data suggested an increase of anal HPV infection-associated anal intraepithelial neoplasia (AIN) in Asian MSM[18]. However, as it stands, most HIV-infected patients do not undergo anal screening in Japanese hospitals; thus, obtaining information on HPV prevalence and genotypes in Japanese HIV-positive patients is necessary for HPV-related AIN prevention.

Compared with HIV-positive MSM, few studies have reported the prevalence of anal HPV infections with different genotypes in HIV-positive heterosexual men or HIV-positive women [11,12]. In addition, only limited data are available on the risk factors for HPV infection in HIV-positive patients[9,19]. It remains poorly understood whether low CD4 counts, high viral load (VL), long duration of HIV disease, HAART use, or concomitant infection with STIs affect the risk of oncogenic HPV infection.

To address these issues, we prospectively collected information on oncogenic HPV infection as well as STIs and HIV-related factors, and we then determined the prevalence, genotypes, and risk factors for anal HPV infection in Japanese HIV-positive MSM, heterosexual men, and women.

Material and Methods

Study Design, Setting, and Participants

This prospective, cross-sectional study was carried out between September 2009 and March 2015 in the Department of Gastroenterology and Hepatology at the National Center for Global Health and Medicine (NCGM), Tokyo, Japan. Eligible patients were HIV-positive patients, at least 18 years old, who were willing to receive gastrointestinal (GI) tract cancer screening, provide anal swabs and blood for tests, and physically able and willing to provide written informed consent. NCGM has one of the largest HIV clinics in Japan with >3,500 registered patients as of May 2013. These patients were the first patients enrolled in the HIV-GI cohort (V-GI protocol) and were systematically screened for anal HPV infection and STIs during the study period. Written informed consent was obtained from all participants. This study was approved by the ethics committee of the National Center for Global Health and Medicine (Nos.1440) and was implemented in accordance with the provisions of the Declaration of Helsinki.

Data collection

Blood samples were collected for syphilis, hepatitis B virus (HBV), *Chlamydia trachomatis* (*C. trachomatis*), and *Entamoeba histolytica (E. histolytica)*. Positive infection was defined as current or prior infection. Syphilis infection was defined as positive *Treponema pallidum* latex agglutination (TPHA) test and rapid plasma reagin (RPR) titer ≥8[20]. HBV infection was defined as positive hepatitis B surface antigen (HbsAg) or positive anti-HBs antibody; if HbsAg and anti-HBs antibody were negative, the patient was considered to be negative for HBV infection. In Japan, because universal vaccination against HBV has not been introduced and intervention to prevent mother-to-child transmission has been very successful[21], most adult cases with chronic HBV infection are considered to be sexually transmitted[22]. *C. trachomatis* infection was determined by anti-*C. trachomatis* IgG and IgA antibodies using an Enzyme-Linked ImmunoSorbent Assay (*C. trachomatis* IgA/IgG antibody, LSIM; LSI Medience, Tokyo, Japan). *C. trachomatis* infection was defined as positive IgA or IgG results; negative infection was defined as both negative IgA and IgG results. Amebic infection was assessed by anti-*E. histolytica* antibody (Ameba-Spot IF; bioMe´rieux, Marcy l'Etoile, France), as described previously[23]. Serum antibody titers <100 were considered negative, while titers of 100, 200, 400, 800, 1600, and 3200 were considered positive. The structured interview/questionnaire was completed on the day of HPV tests[24]. Patients were asked about i) their lifestyle habits (smoking history and alcohol consumption), ii) systemic steroid use for >2 weeks, iii) HIV-related factors including CD4 cell count, HIV-1 VL, duration (years) of HAART, and route of transmission (MSM/heterosexual infection, injection drug use, transfusion for hemophilia, and unknown) through face-to-face interviews by well-trained researchers in a private room[24]. Because the maximum period of supply for prescriptions is limited to 3 months in the Japanese health care system, patients need to make visits at least every 3 months for prescriptions as well as monitoring of CD4 cell count and HIV-1 VL.

HPV detection and genotyping

A dedicated brush (DNAPAP cervical sampler, Qiagen, Gaithersburg, MD) was used for sampling of the anorectal area. The anal brush was inserted 3–5 cm into the anal verge and the dentate line and it was then used to scrape the anal walls by repeatedly rotating it clockwise and counterclockwise. Nucleic acids were extracted from 500-μL analsamples in the Sure Path solution with a commercial kit (QIAGEN DNA mini kit; Qiagen, Hilden, Germany)[25]. HPV DNA was genotyped by polymerase chain reaction (PCR)-Invader assay, as described

previously[25]. This method could detect 14 oncogenic HPV genotypes (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 67, and 68)[25]. Study participants who were positive for any HPV type were considered to have a current HPV infection. A "multiple type" variable was created such that results at each sampling site were categorized as having 0, 1, or ≥2 oncogenic types. A participant was considered to have infection with multiple oncogenic types if ≥2 oncogenic types were detected at any of the sampled sites.

Statistical analysis

Baseline characteristics were compared between patients with and without anal oncogenic HPV infection using the Mann–Whitney U test or χ^2 test (or Fisher's exact test) for continuous or categorical variables, respectively. We used logistic regression analysis to compute the odds ratios (ORs) and 95% confidence intervals (CIs) as an estimate of anal oncogenic HPV infection associated with clinical factors. For multivariate analysis, we used a multiple logistic regression model that included all factors with $p < 0.05$ on univariate analysis (those included age <40 years, sex, MSM, CD4 <100, HIV VL >50,000, administration of HAART, and more than 2 STIs).

We also elucidated the association between infection with multiple oncogenic HPV types and clinical factors in uni- and multivariate logistic regression analyses. Sex was not included in the logistic regression model because all patients infected with multiple oncogenic HPV types were males.

The Cochran–Armitage test was used to determine trends in the proportion of oncogenic HPV infection according to CD4 count (<200, 200–399, 400–599, and ≥600), HIV VL (undetectable, 50–50,000, and >50,000), and duration of HAART (no administration, <5 years, 5–9 years, and ≥10 years).

Statistical significance was defined as two-sided p values <0.05. All statistical analyses were performed using Stata version 13 software (StataCorp LP, College Station, TX).

Results

Patient characteristics

During the study period, 421 HIV-infected patients were recruited. Patient characteristics are shown in Table 1. There were 395 (93.8%) men and 26 (6.2%) women, and their median age was 44 years. The majority of HIV infection was through anal intercourse (85.8%). Among HIV-positive patients, 18.3% had CD4 <100 cells/μL, 62.5% had an undetectable HIV VL, and 75.1% were receiving HAART with a median duration of 8 years. The rates of syphilis, HBV, *C. trachomatis*, and *E. histolytica* infection were 41.3%, 54.4%, 50.1%, and 26.1%, respectively. The proportion of subjects with ≥2 STIs was 58.2%.

Prevalence of oncogenic HPV infection

The prevalence of anal HPV infection and the distributions of genotypes are shown in Table 2. The oncogenic HPV infection rate among the HIV-infected patients was 69.4%; specifically, it was 75.9% in MSM, 20.6% in heterosexual men, and 19.2% in women. The oncogenic HPV genotypes that were detected most frequently were as follows: HPV-58 (30.2%), HPV-16 (28.8%), HPV-52 (22.2%), and HPV-33 (18.8%) in MSM; HPV-16 (14.7%), HPV-31 (5.9%), HPV-33 (5.9%), and HPV-52 (5.9%) in heterosexual men; HPV-18 (7.7%), HPV-16 (3.9%), HPV-31 (3.9%), and HPV-33 (3.9%) in women. HPV-16/18 types were detected in 34.9% of MSM, 17.7% of heterosexual men, and 11.5% of women. The mean numbers of oncogenic HPV infection were 2.4 in MSM, 0.4 in heterosexual men, and 0.2 in women. Infection with

Table 1. Characteristics of 421 HIV-infected patients.

Variables	n (%)	Median (IQR)
Age (years)		44 (39, 55)
Male sex	395 (93.8)	
Alcohol consumption		
None	208 (49.4)	
Light (1–50 g/week)	100 (23.8)	
Moderate (>360 g/week)	113 (26.8)	
Smoking index†		
Never smoker	164 (39.0)	
1–300	127 (30.2)	
>300	130 (30.9)	
Comorbidities and drug use		
Hypertension	54 (12.8)	
Diabetes mellitus	27 (6.4)	
Dyslipidemia	28 (6.7)	
Chronic kidney disease	9 (2.1)	
Chronic liver disease	94 (22.4)	
Corticosteroid use	25 (5.9)	
HIV-related factors		
Route of HIV infection		
MSM	361 (85.8%)	
Heterosexual	39 (9.3)	
Injection drug use	2 (0.5)	
Hemophilia	17 (4.0)	
Unknown	2 (0.5)	
CD4 cell counts (cells/μL)		369 (179, 582)
CD4 <100 (cells/μL)	77 (18.3)	
HIV VL (copies/mL)		51,500 (770, 320,000)
VL ≤50 (normal range)	263 (62.5)	
50< VL ≤50,000	79 (18.8)	
VL >50,000	79 (18.8)	
Administration of HAART	316 (75.1)	
Duration of HAART (years)*		8.0 (5.1, 11.8)
Duration ≤5 year	78 (24.7)	
5 years< duration ≤10 years	123 (38.9)	
Duration >10 yrs	115 (36.4)	
Sexual transmitted infections		
Syphilis infection	174 (41.3)	
Hepatitis B virus infection	229 (54.4)	
Chlamydia trachomatis infection	211 (50.1)	
Entamoeba histolytica infection	107 (26.1)	
Number of infections		2 (1, 3)
Number of infections ≥2	245 (58.2)	

Abbreviations: HAART, highly active anti-retroviral therapy; IQR, interquartile range; MSM, men who have sex with men; VL, viral load

*Duration of HAART was analyzed in 316 patients who had undergone HAART.

†The smoking index was evaluated in occasional and daily smokers and defined as the number of cigarettes per day multiplied by the number of smoking years.

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Table 2. Anal oncogenic HPV infection prevalence and genotyping in HIV-infected patients.

	All HIV-infected patients (n = 421)	Men (n = 395)		Women (n = 26)
		MSM (n = 361)	Heterosexual (n = 34)	
Any oncogenic type HPV	286 (67.9)	274 (75.9)	7 (20.6)	5 (19.2)
16	110 (26.1)	104 (28.8)	5 (14.7)	1 (3.9)
18	42 (10.0)	39 (10.8)	1 (2.9)	2 (7.7)
31	58 (13.8)	55 (15.2)	2 (5.9)	1 (3.9)
33	71 (16.9)	68 (18.8)	2 (5.9)	1 (3.9)
35	53 (12.6)	53 (14.7)	0	0
39	45 (10.7)	45 (12.5)	0	0
45	32 (7.6)	32 (8.9)	0	0
51	57 (13.5)	57 (15.8)	0	0
52	82 (19.5)	80 (22.2)	2 (5.9)	0
56	38 (9.0)	38 (10.5)	0	0
58	110 (26.1)	109 (30.2)	1 (2.9)	0
59	76 (18.1)	76 (21.1)	0	0
67	56 (13.3)	55 (15.2)	1 (2.9)	0
68	38 (9.0)	38 (10.5)	0	0
16 or 18	135 (32.1)	126 (34.9)	6 (17.7)	3 (11.5)
Number of oncogenic HPV types, mean±SD	3.0±2.0	2.4±2.2	0.4±1.0	0.2±0.4
Multiple oncogenic HPV types	200 (47.5)	197 (54.6)	3 (8.8)	0

Abbreviations: MSM, men who have sex with men; SD, standard deviation.

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multiple oncogenic HPV types was detected in 54.6% of MSM and 8.8% of heterosexual men, whereas HIV-infected women had no infection with multiple oncogenic HPV types.

Risk factors for oncogenic HPV infection

Risk factors for oncogenic HPV infection are shown in Table 3. In univariate analysis, younger age, male sex, MSM, CD4 <100, HIV VL >50,000, no administration of HAART, and having ≥2 STIs were significantly associated with oncogenic HPV infection, whereas higher smoking index and corticosteroid use were marginally associated with oncogenic HPV infection. In multivariate analysis, younger age, MSM, CD4 <100, and having ≥ 2STIs were independently associated with oncogenic HPV infection.

Risk factors for infection with multiple oncogenic HPV types are shown in Table 4. In univariate analysis, younger age, corticosteroid use, MSM, CD4 <100, HIV VL >50,000, no administration of HAART, and having ≥2 STIs were significantly associated with multiple oncogenic HPV infection. In multivariate analysis, younger age, MSM, CD4 <100, and having ≥2 STIs were independently associated with multiple oncogenic HPV infection.

Oncogenic and multiple oncogenic HPV infection rates tended to increase with the decrease in CD4 ($p<0.001$ and $p<0.001$, respectively; Fig 1A) and with HIV VL increase ($p<0.001$ and $p<0.001$, respectively; Fig 1B), while they tended to decrease with longer HAART duration ($p<0.001$ and $p<0.001$, respectively; Fig 1C).

Discussion

In regard to the prevalence of anal HPV infection, we found that 76% of HIV-infected MSM had an oncogenic HPV type, which was similar to the rates reported in other Asian countries.

Table 3. Risk factors for oncogenic HPV infection of the anorectal area (n = 421).

Variables	HPV (n = 286)/ without HPV (n = 135)	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Age (years)	45.1±11.3/ 49.9±12.1	0.97 (0.95–0.98)	<0.001	0.98 (0.96–0.99)	0.024
Male sex	281 (98.3)/ 114 (84.4)	10.35 (3.81–28.12)	<0.001	1.23 (0.33–4.66)	0.759
Alcohol consumption, none	145 (50.7)/ 63 (46.7)	1 (reference)			
Light (1–50 g/week)	68 (23.8)/ 32 (23.7)	0.92 (0.55–1.54)			
Moderate (>360 g/week)	73 (25.5)/ 40 (29.6)	0.79 (0.49–1.29)	0.645		
Smoking index†, never smoker	105 (36.7)/ 59 (43.7)	1 (reference)			
1–300	96 (33.6)/ 31 (23.0)	1.74 (1.03–2.91)			
>300	85 (29.7)/ 45 (33.3)	1.06 (0.66–1.72)	0.086		
Hypertension	32 (11.2)/ 22 (16.3)	0.65 (0.36–1.16)	0.146		
Diabetes mellitus	15 (5.2)/ 12 (8.9)	0.57 (0.26–1.25)	0.159		
Dyslipidemia	17 (5.9)/ 11 (8.2)	0.71 (0.32–1.57)	0.399		
Chronic kidney disease	6 (2.1)/ 3 (2.2)	0.94 (0.23–3.83)	0.934		
Chronic liver disease	63 (22.0)/ 31 (23.1)	0.94 (0.58–1.53)	0.800		
Corticosteroid use	21 (7.3)/ 4 (3.0)	2.60 (0.87–7.72)	0.086		
Route of HIV infection, MSM	274 (95.8)/ 87 (64.4)	12.60 (6.40–24.79)	<0.001	5.85 (2.33–14.71)	<0.001
CD4 <100 (cells/μL)	66 (23.1)/ 11 (8.2)	3.38 (1.72–6.64)	<0.001	2.24 (1.00–5.01)	0.049
HIV VL >50,000 (copies/mL)	64 (22.4)/ 15 (11.1)	2.31 (1.26–4.22)	0.007	1.21 (0.50–2.96)	0.675
Administration of HAART	205 (71.7)/ 111 (82.2)	0.55 (0.33–0.91)	0.021	1.21 (0.59–2.49)	0.609
Number of STIs ≥2	200 (69.9)/ 45 (33.3)	4.65 (3.00–7.21)	<0.001	2.81 (1.72–4.61)	<0.001

Abbreviations: HAART, highly active anti-retroviral therapy; MSM, men who have sex with men; STIs, sexual transmitted infections; VL, viral load

†The smoking index was evaluated in occasional and daily smokers and defined as the number of cigarettes per day multiplied by the number of smoking years.

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In Shenzhen, China, 71% of HIV-positive MSM had HPV infection[8], while in Beijing, 61% of HIV-positive MSM had oncogenic HPV infection[26]. In Taiwan, 40–64% of HIV-infected men had oncogenic HPV infection[7,11]. In Bangkok, Thailand, 58% of HIV-positive MSM had oncogenic HPV infection[7]. The anal HPV prevalence in our study was lower than the reported 81–92% in Western countries among HIV-positive men[19,27,28].

Few studies have investigated anal HPV infection in HIV-positive heterosexual men and women. In our study, the prevalence of anal oncogenic HPV infection among HIV-positive heterosexual men was 21%. Christophe et al investigated 50 HIV-positive heterosexual male injection drug users with no history of anal intercourse and 44% had oncogenic HPV[29]. In the study of 64 HIV-positive heterosexual men by Chien et al., 38% had anal oncogenic HPV infection[11]. The mechanisms of anal HPV infection in the absence of anal intercourse are not well known, but infection is possibly due to insertion of transiently infected fingers or toys, as well as shedding from other infected genital sites[29], and thus, anal HPV infection may behave as a STI. The reason for the low prevalence of infection among HIV-positive heterosexual men in our study compared with the previous two studies is that 71% of the heterosexual men were hemophilia patients who were infected with HIV through treatment with blood products or blood transfusions[30] and not sexually. In our study, 19% of HIV-positive women had oncogenic HPV infection. In India, 9% of HIV-positive women had anal oncogenic HPV and 17% had oncogenic cervical HPV infection[12]. These findings, and the fact that cervical cancer increases the risk of anorectal cancer in women[31], suggest the potential utility of both cervical and anal cancer screening of HIV-infected women for HPV infection of either the cervical or anal area, or both.