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Research Article

Impact of HIV-1 subtype and Korean Red Ginseng on AIDS progression: comparison of subtype B and subtype D

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ABSTRACT

Background: To date, no study has described disease progression in Asian patients infected with HIV-1 subtype D.

Methods: To determine whether the disease progression differs in patients infected with subtypes D and B prior to starting combination antiretroviral therapy, the annual decline (AD) in $CD4^+$ T cell counts over 96 ± 59 months was retrospectively analyzed in 163 patients and compared in subtypes D and B based on the *nef* gene.

Results: $CD4^+$ T cell AD was significantly higher in the six subtype D–infected patients than in the 157 subtype B–infected patients irrespective of Korean Red Ginseng (KRG) treatment ($p < 0.001$). Of these, two subtype D–infected patients and 116 subtype B–infected patients had taken KRG. AD was significantly lower in patient in the KRG-treated group than in those in the KRG-naïve group irrespective of subtype ($p < 0.05$). To control for the effect of KRG, patients not treated with KRG were analyzed, with AD found to be significantly greater in subtype D–infected patients than in subtype B–infected patients ($p < 0.01$). KRG treatment had a greater effect on AD in subtype D–infected patients than in subtype B –infected patients (4.5-fold vs. 1.6-fold). Mortality rates were significantly higher in both the 45 KRG-naïve ($p < 0.001$) and all 163 ($p < 0.01$) patients infected with subtype D than subtype B.

Conclusion: Subtype D infection is associated with a >2-fold higher risk of death and a 2.9-fold greater rate of progression than subtype B, regardless of KRG treatment.

1. Introduction

The rate of AIDS progression has been reported to depend on HIV-1 subtype [1,2], with disease progressing twice as rapidly in patients infected with HIV-1 subtype D than subtype A, despite similar virus concentrations [3,4]. However, other studies report that disease progression rates do not differ significantly with HIV-1 subtype, probably because these findings were obtained in studies with short (<5 years) follow-up periods [5,6] or analysis of non-D subtypes [6]. For example, the rates of decline of $CD4^+$ T cell counts were found to be 4- and 2-fold greater among individuals infected with subtype D than subtypes B [7] and A [8], respectively. Moreover, Ugandan and Zimbabwean women infected with subtype C exhibited 2.5-fold slower rates of $CD4^+$ T cell count decline and higher frequencies of long-term nonprogression than those infected with subtype A or D [9]. Studies of the mechanism underlying the faster disease progression in patients infected with subtype D show that subtype D isolates tend to have a higher frequency of syncytium formation, CXCR4 (X4) coreceptor usage, and rapidly replicating virus than isolates of other subtypes [10]. In addition, the faster progression observed in subtype D may be due to its earlier switch to X4 virus [11]. Moreover, treatment failure and drug resistance rates are more frequent in Ugandans infected with HIV-1 subtype D than subtype A [12].

Worldwide, the majority of HIV infections (70%) have been attributed to subtypes C (48%), A, and B, whereas the prevalence of subtype D has decreased from 3.2% to 2% [13]. In Asia, CRF01_AE (35%), B (33.5%), C (18%), and CRF07_BC (6.2%) are the predominant subtypes (87%), whereas only about 0.1–0.2% of patients are infected with subtype D [13–15]. In Asia and Australia, the overall

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prevalence rates of subtype D infection were approximately 0.1% based on sequences as of February 2017 and 0.4% based on patients [16], respectively, although HIV sequences deposited in the Los Alamos sequence database are not representative of the relative distributions in their countries of origin (https://www.hiv.lanl.gov). The paucity of information has led to a lack of reporting on disease progression in subtype D–infected patients in Asia and Australia.

Mandatory testing of overseas sailors (OSs) in Korea for antibodies to HIV-1 from 1988 to 1993 identified several who were infected with HIV-1 or HIV-2, including six who were infected with HIV-1 subtype D and the remainder with subtype B [17].

The *nef* gene is a major determinant of virulence in primate lentiviruses. Mutations in *nef* attenuate the replication capacity and pathogenicity of simian immunodeficiency viruses [18–23]. In particular, the evolutionary loss of a specific *nef* function may contribute to the high virulence of HIV-1 in humans [24]. The importance of the *nef* gene as a virulence factor led to our use of this gene to determine HIV-1 subtype in 380 infected patients and to compare disease progression in patients infected with subtypes B and D.

In a nationwide study, we found that disease progression was significantly more rapid in patients infected with subtype D than subtype B and that the disease progression rate in both groups was affected by treatment with Korean Red Ginseng (KRG).

2. Materials and methods

2.1. Study patients

Sequences of the *nef* gene were determined in 381 patients [17,25]. Of these, six were infected with subtype D and 157 with subtype B; of the latter, 36 were infected with Western subtype B and 121 with the Korean subclade of subtype B (KSB) [25] (Table 1). The six patients infected with subtype D included five OSs and one soldier (93-01) who had not gone abroad (Table 2). The phylogenetic tree indicated that these six sequences may have originated from three different sources (Supplementary Figs. 1 and 2). Additional information on these patients, including age, sex, mode of transmission, and year of diagnosis, is shown in Table 2. This study was approved by the Institutional Review Board of Asan Medical Center.

Table 1
Demographic and HIV-1–associated characteristics of patients

Characteristic	Subtype		
	B (n = 157)	D (n = 6)	p-value
Sex, no. (%)			
Female	16 (10)	0	>0.05
Male	141 (90)	6 (100)	>0.05
Overseas sailors and spouses	10 (6)	5 (83)	<0.0001
Age, y, mean ± SD (median)	29 ± 10 (28.5)	29 ± 7 (30.5)	>0.05
Year of diagnosis of HIV-1 infection			
1986–1987	6	0	
1988–1989	17	1	
1990–1991	44	2	
1992–1993	65	2	
1994–2004	25	1	
No. (%) of long-term slow progressors	34 (22%)	0	>0.05
Follow-up months based on $CD4^+$ T cells	97 ± 59	79 ± 50	>0.05
Amount of KRG administered to all patients, grams	3,221 ± 4,814	2,453 ± 5,093	>0.05
Amount of KRG administered to KRG-treated patients (116 vs. 2), grams	4,359 ± 5,141	7,360 ± 7,580	>0.05
Amount of KRG per year, grams	539 ± 453	643 ± 600	>0.05
$CD4^+$ T cell count at diagnosis/μL (mean ± SD) (range)	496 ± 254 (131–1,484)	557 ± 362 (7–926)	>0.05
HIV-1 load, copies/mL, mean ± SD (n = 109 vs. 5)	23,911 ± 55,871	47,156 ± 59,206	> 0.05
β2-microglobulin (mg/L), mean ± SD	2.44 ± 0.94	2.50 ± 0.59	>0.05
Study endpoint reached, no. (%)			
Death	56 (36)	5 (83)	<0.05
Survival from diagnosis to death or last $CD4^+$ T cell count before cART, months, mean ± SD	105 ± 58	82 ± 51	>0.05
AD in CD4+ T cells >200/μL	44 ± 58	251 ± 132	<0.0001
AD in CD4+ T cells <200/μL	77 ± 72	105 ± 133	>0.05

AD, annual decline; KRG, Korean Red Ginseng; SD, standard deviation; cART, combinational antiretroviral therapy.

Table 2
Characteristics of the seven patients infected with HIV-1 subtype D.

Patient code	Sex/age at diagnosis	Transmission mode	Visited country	Time of foreign visit	Presumed primary infection	Diagnosis of HIV-1 infection	Plasma RNA copy (/mL)	SD (months)	AD
88-05	M/25	Unknown	Kenya, JP	Feb 86-Jul 1987	Feb 1987	Feb 1988	7,831–3,262	128	77
90-15	M/29	Heterosexual	Kenya	After Nov 1988	Aug 1989	Apr 1990	122,712	59	198
90-16	M/35	Bisexual	Africa	1986-Jul 1988	Jan 1988	Apr 1990	4,459[1)]	28	234
92-65	M/31	Heterosexual	Kenya	1992	July 1992	Oct 1992	>100,000	110	95
93-01	M/21	Homosexual	None	1988[2)]	Dec 1988	Dec 1992	781–2,561	140	−12
94-29	M/37	Heterosexual	JP, other	After 1989	Jan 1990	May 1994	ND	24	229

JP, Japan; NA, not applicable; AD, annual decline in $CD4^+$ T cell count; SD, survival duration from diagnosis to death (0/μL) or last CD4+ T cell count before starting combination antiretroviral therapy; ND, not determined.
[1)] On the 5th day of zidovudine treatment.
[2)] First sexual contact when he was high school student in 1988.

2.2. Treatment with KRG and data collection

Data on $CD4^+$ T cell count were from the National Registry, which was established by the division of AIDS, Center for Immunology and Pathology, Korea National Institute of Health (KNIH). Study of KRG treatment in HIV-1 infected patients was initiated at the KNIH in 1991 [26–27] and lasted until February 1996. In parallel, research on KRG from HIV-1 infections began in University of Ulsan College of Medicine in May 1993 and lasted until 2018. Male and female were instructed to take orally six and three capsules of KRG three times daily, respectively [26–30]. One capsule contained 300 mg powder without any additives. There were several interruptions prior to July 2000 including an interruption in KRG intake for 4–5 months after the first 6-month pilot study initiated in November 1991. Therefore, amount and duration of KRG treatment are varied. KRG-treated patients were defined as those who had taken at least 150 g KRG per year. In subtype B–infected and subtype D–infected patients, yearly intake of KRG in the 116 and two KRG-treated patients was 539 ± 453 g and 643 ± 600 g, respectively (Table 1).

2.3. $CD4^+$ and $CD8^+$ T cell measurements

Peripheral blood mononuclear cells were obtained from patients every 6 months. The peripheral blood mononuclear cell preparations were incubated with phycoerythrin- and fluorescein isothiocyanate-conjugated antibodies against CD4 and CD8 antigens, respectively (Simultest reagents; Becton Dickinson, San Jose, CA, USA), and $CD4^+$ and $CD8^+$ T cells were counted by flow cytometry (FACScan; Becton Dickinson) [26–30].

2.4. RNA copy measurement

HIV-1 RNA copy numbers (/mL) in serum (before 1997) and plasma (beginning in 1997) were measured using AMPLICOR HIV-1 monitor kits (Roche Diagnostics Systems, Branchburg, NJ, USA). Because RNA copy numbers are 0.28 logs lower in serum than in plasma [31], copy numbers in sera were converted to plasma-equivalent numbers by the addition of 0.28 logs.

2.5. Statistical analysis

All data were expressed as mean ± standard deviation. Statistical significance was estimated by Student's two-tailed *t* test using MedCalc Software (Ostend, Belgium). Kaplan–Meier survival analyses were used to explore the relationship between subtypes (MedCalc Software). Statistical significance was defined as $p < 0.05$.

2.6. Sequence data

The GenBank accession numbers for the sequences in this study are AF063920, AF462775-7, AF584794-9, AY121473, AY221703, AY260808, AY899400-01, DQ054367, KU588843-5, KU588847-50, KY557555-8, KY557830-5, KY558144-7, KY558156-70, KY683964-72, KY683993-5, MF176214-19, and MH396195-36.

3. Results

3.1. Patient characteristics

HIV-1 disease progression was analyzed in all patients infected with subtypes D and B, as determined by *nef* sequences, and with at least two measurements prior to starting combinational antiretroviral therapy (cART). $CD4^+$ T cell counts were followed up for 96 ± 59 months. The mean time from diagnosis to last $CD4^+$ T cell count before cART or death was 104 ± 58 months. The demographic characteristics of the 163 patients infected with HIV-1 subtypes B and D are shown in Table 1. The proportion of OSs and their spouses was significantly higher in patients infected with subtype D than subtype B (83% vs. 6%, $p < 0.0001$). Sex and age at diagnosis did not differ in these two groups (Table 1).

3.2. Viral load level

Plasma RNA copy numbers were measured in 95 subtype B–infected patients and five subtype D–infected patients. Although initial viral loads were 1.8-fold higher in subtype D–infected patients (47,156 ± 59,206 copies/mL) than in the subtype B–infected patients (23,911 ± 55,871 copies/mL) (Table 1), the RNA copy number at the last time point was higher in patients infected with subtype B (123,241 ± 187,987 copies/mL) than in those infected with subtype D (53,246 ± 55,214 copies/mL) at the same level of $CD4^+$ T cells (303 ± 208/μL vs. 305 ± 383/μL). This finding showed that there was no significant difference in plasma viral load between patients infected with subtypes B and D. The two subtype D–infected patients with the highest viral loads, patients 90-15 and 92-65, rapidly progressed to AIDS (Table 2, Supplementary Fig. 3). By contrast, three subtype D–infected patients (88-05, 90-16, and 93-01) had lower viral loads (mean: 2,007 ± 2,123 copies/mL) before the introduction of KRG or zidovudine (Table 2). Following diagnosis, two subtype D–infected patients with low virus concentrations (88-05 and 93-01) survived for more than 10 years in the absence of cART (Supplementary Fig. 3). Both patients were CCR5 virus carriers. In particular, patient 93-01 had a virus concentration as low as 781/mL beginning in April 1993, and this low viral load was maintained for over 10 years after taking KRG with a normal range of high-sensitivity C-reactive protein concentrations (0.3, 0.2, and 0.3 mg/L in December 1992, October 1993, and August 2004, respectively). Despite his first sexual contact in 1988 and a low viral load, his earlier decline in $CD4^+$ T cell counts to 200/μL was very rapid (Supplementary Fig. 3).

3.3. Definition of AD, AD >200, and AD <200

Since 1988, Korean OSs had undergone compulsory testing for anti-HIV-1 antibody [17]. If each OS returns to Korea once every 2 or 3 years, the time of primary infection can be estimated relatively precisely in conjunction with previous negative results for anti-HIV-1 antibody. Setting a baseline $CD4^+$ T cell count at 1,000/μL, the annual decline (AD) from this level to about 200/μL was calculated (AD >200). Subsequently, the AD from a $CD4^+$ T cell of about 200/μL to the last $CD4^+$ T cell count or death (0/μL) was also calculated (AD <200). The overall AD was defined as the rate from the first to the last measurement of $CD4^+$ T cell count or to death (0/μL).

3.4. Comparison of AD in patients infected with subtypes B and D

Patients infected with subtype D showed a rapid decline in $CD4^+$ T cell counts and progressed to AIDS within 10 years, although their exact times of initial infection were not known (Supplementary Fig. 3). Specifically, patients 90-15, 90-16, and 94-29 progressed to AIDS within 4–6 years. Their mean AD ($n = 6$) was 140 ± 97 cells/μL over 79 ± 50 months (Table 2 and Fig. 1). By comparison, the 157 patients infected with subtype B had a mean AD of 49 ± 58 cells/μL over 97 ± 59 months (Fig. 1). Taken together, the AD of subtype D–infected patients was 2.9-fold faster than that of subtype B–infected patients ($p < 0.001$; Fig. 1).

Of the six patients with subtype D, two were treated with KRG (643 ± 600 g/y) and four were not, with AD being mild significantly (4.5-fold) lower in the former ($p = 0.07$) (Fig. 2). Of the 157 patients

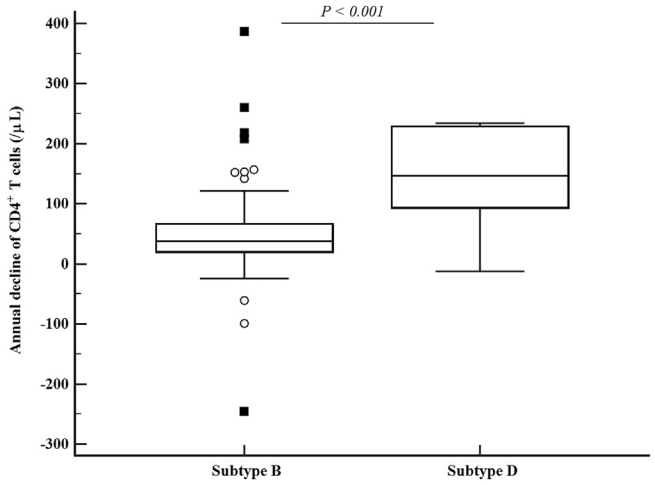

Fig. 1. Annual decline (AD) in CD4$^+$ T cell counts in patients infected with HIV-1 subtypes B and D. The intervals between the first and last CD4$^+$ T cell measurements before combinational antiretroviral therapy (cART) or death in patients with subtypes D and B were 79 ± 50 months and 97 ± 59 months, respectively. In Table 2, the AD of subtype D–infected patients was 140 ± 97/μL, which was significantly higher than that of subtype B–infected patients (49 ± 58). Thus, the AD of subtype D–infected patients was 2.9-fold faster than that of subtype B–infected patients.

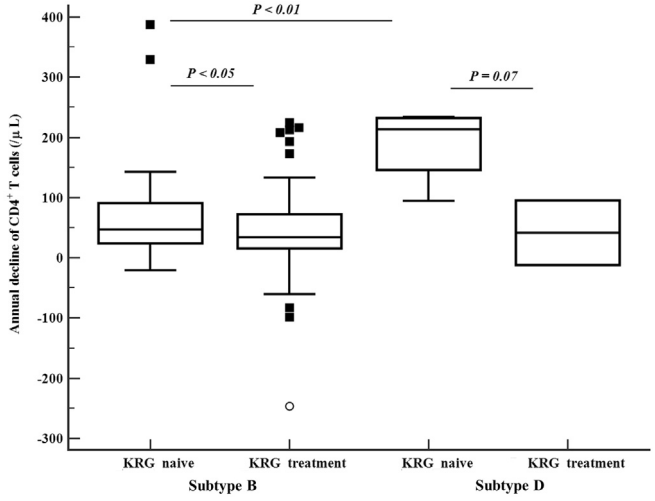

Fig. 2. Korean Red Ginseng (KRG) significantly affects the annual decline (AD) in CD4$^+$ T cell counts in patients with HIV-1 subtypes B and D. AD was significantly higher in KRG-naïve patients with subtype D than in those with subtype B and was significantly lower in KRG-treated patients than in KRG-naïve patients with subtype B. These findings suggest that the effect of KRG treatment might be greater in patients infected with subtype D than subtype B (4.5-fold vs. 1.6-fold).

infected with subtype B, 116 were treated with KRG (539 ± 453 g/y) and 41 were not (Table 1). Mean AD was significantly lower in those treated (44 ± 61 cells/μL for 107 ± 59 months) than in those not (70 ± 82 cells/μL over 80 ± 44 months) treated with KRG ($p < 0.05$; Fig. 2). A comparison in patients not treated with KRG showed that AD was 2.8-fold higher in patients infected with subtype D than subtype B ($p < 0.01$; Fig. 2). Moreover, these findings suggest that the effect of KRG treatment might be greater in patients infected with subtype D than subtype B (4.5-fold vs. 1.6-fold).

3.5. Comparison of AD by CD4$^+$ T cell counts in patients infected with subtypes B and D

A CD4$^+$ T cell count below 200/μL is defined as AIDS. Therefore, the rate of progression may differ in patients experiencing declines in CD4$^+$ T cell counts from 1,000 to 200/μL and from 200 to 0/μL. Independent of KRG treatment, AD during the decline from 1,000 to 200/μL was significantly higher (4.9-fold) in patients infected with subtype D (251 ± 132 cells/μL) than in those infected with subtype B (52 ± 68 cells/μL) ($p < 0.0001$), whereas the difference was not significant (1.4-fold) during the decline from <200 to 0/μL (Fig. 3). This may due to the difference in the proportion of KRG-treated patients among subtype D–infected patients between both periods.

3.6. Effect of KRG treatment on AD

Of the six patients infected with subtype D, two had taken KRG, patient 92-65 when CD4+ T cell count was >200/μL and patient 93-01 when CD4$^+$ T cell count was <200/μL. Patient 93-01 maintained CD4$^+$ T cell counts for 13 years without cART (Supplementary Fig. 3). Of the 157 patients infected with subtype B, 116 had taken a mean 4,359 ± 5,141 g of KRG (median, 2,400 g; range, 30–25,602 g), beginning in November 1991 (Table 1).

In the KRG-naive patients, AD was significantly greater in patients infected with subtype D than subtype B during the decline in CD4$^+$ T cell counts from 1,000 to 200/μL (3.9-fold, $p < 0.0001$; Fig. 4A). In the same way, AD was greater in patients infected with subtype D than in those infected with subtype B during the decline in CD4$^+$ T cell counts from <200 to 0/μL (1.8-fold, $p > 0.05$; Fig. 4B).

Regarding the KRG effect, during the decline in CD4$^+$ T cell counts from 1,000 to 200/μL, AD was significantly lower in subtype B–infected patients treated with KRG than in those not treated with KRG (KRG-naïve patients) ($p < 0.05$; Fig. 4A). During the decline in CD4$^+$ T cell counts from 200 to 0/μL, AD was lower in the one KRG-treated than in the four KRG-naïve patients infected with subtype D (Fig. 4B).

3.7. Survival analysis

The survival durations (SDs) from diagnosis to death or last CD4$^+$ T cell count in patients infected with subtypes D and B were 82 ± 51 months (range, 24–140 months) and 105 ± 58 months (range, 4–339 months), respectively (Table 1). Kaplan–Meier survival analysis showed that mortality rates were significantly higher

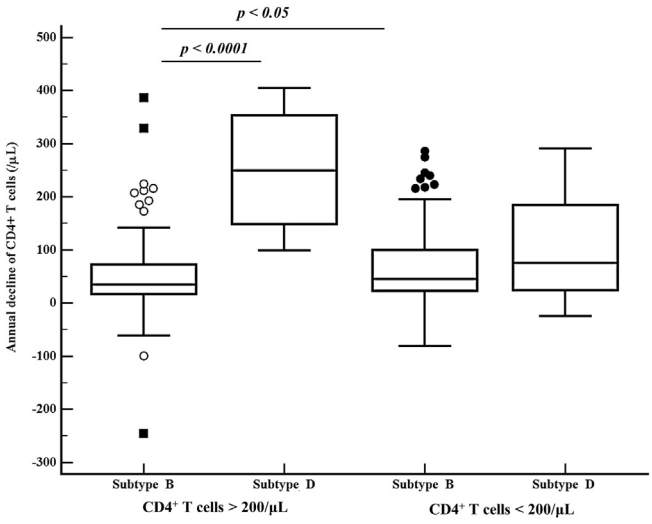

Fig. 3. Effect of CD4$^+$ T cell counts and HIV-1 subtypes on the annual decline (AD) in CD4$^+$ T cell counts in patients with HIV-1 subtypes B and D. AD was significantly higher in subtype D–infected patients than in subtype B–infected patients when CD4$^+$ T cell counts were >200/μL.

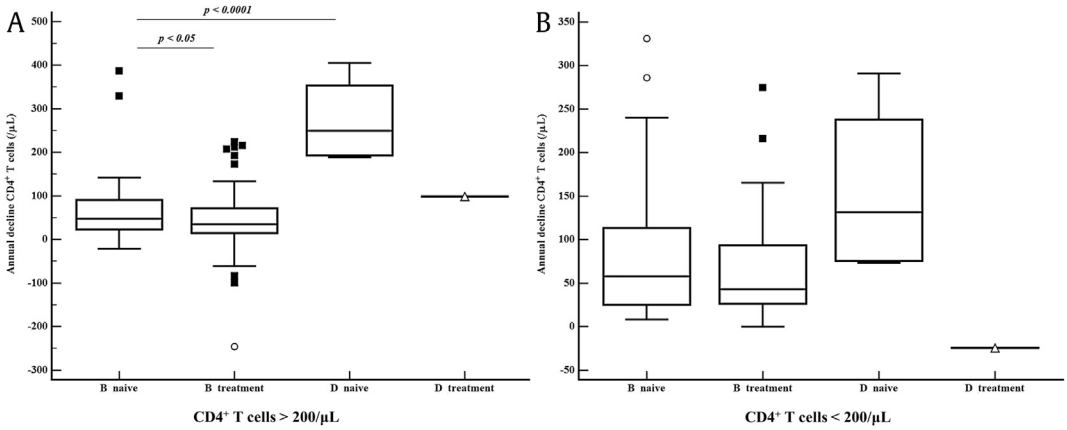

Fig. 4. Effect of Korean Red Ginseng (KRG) treatment and $CD4^+$ T cell counts on the annual decline (AD) in $CD4^+$ T cell counts. (A) $CD4^+$ T cell counts were > 200/μL. (B) $CD4^+$ T cell counts were <200/μL. KRG treatment reduced AD significantly in subtype B–infected patients when $CD4^+$ T cell counts were>200/μL ($p < 0.05$). Excluding patients treated with KRG, AD was 3.9-fold ($p < 0.0001$) and 1.7-fold more rapid in patients with subtype D than subtype B when $CD4^+$ T cell counts were >200/μL and <200/μL, respectively.

in subtype D–infected patients than subtype B–infected patients, both for the 45 who were KRG-naïve ($p < 0.001$; Fig. 5) and for all patients ($p < 0.01$).

Among patients infected with subtype B, SD was significantly longer in those treated with KRG (112 ± 59 months) than in the KRG-naïve group (88 ± 48 months) ($p < 0.05$) (Fig. 6). Among patients infected with subtype D, SD was 2.1-fold longer in those who were treated with KRG than in the KRG-naïve group (125 ± 21 months vs. 60 ± 48 months; Fig. 6). Taken together, the prolongation of SD due to KRG intake was 2.7-fold longer in subtype D–infected patients (65 months) than in subtype B–infected patients (24 months), although infection with subtype D was associated with a >2-fold higher risk of death than infection with subtype B ($p < 0.05$; Table 1).

3.8. CXCR4 versus CCR5 tropism

We determined *env* tropism using the program Geno2pheno [coreceptor] 2.5 (http://coreceptor.bioinf.mpi-inf.mpg.de/). Patient 93-01 had dual tropism (CCR5 > CXCR4), patients 90-15 and 92-65 had CXCR4 tropism, and patients 88-05 and 90-16 had CCR5 tropism with a false positive rate of 10%. However, the 11/25 rule

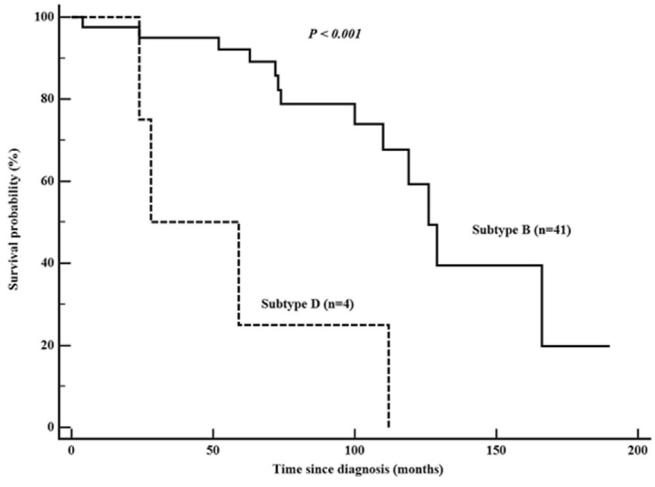

Fig. 5. Kaplan–Meier survival curves for 45 Korean Red Ginseng (KRG)-naïve patients with HIV-1 subtypes B and D from the time of diagnosis of HIV-1 infection to death or start of combination antiretroviral treatment.

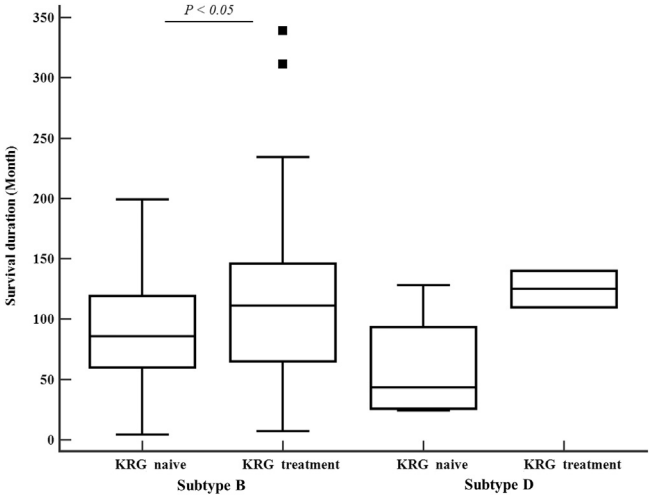

Fig. 6. Effects of HIV-1 subtype and treatment with Korean Red Ginseng (KRG) on mean survival duration (SD). SD was significantly longer in KRG-treated (B treatment) than in KRG-naïve patients with subtype B (B naive) and tended to be longer in KRG-treated patients (D treatment) than in KRG-naïve patients with subtype D (D naïve), with KRG treatment enhancing SD more in patients with subtype D than with subtype B.

[32] would predict the sequence from four patients (90-15, 90-16, 92-65, and 93-01) to be from X4 virus. Interestingly, the two patients who survived for more than 10 years (patients 88-05 and 93-01) were CCR5 carriers (Supplementary Fig. 3).

4. Discussion

This study of HIV-1–infected Korean men followed since 1988 showed that infection with subtype D was associated with a more rapid decline in $CD4^+$ T cell counts and a 2.8-fold higher mortality rate, regardless of $CD4^+$ T cell counts and KRG treatment than infection with subtype B. This study is unique, in that all of the patients with subtype D were men, and HIV-1 subtyping was based on the *nef* gene although the *env* gene was also determined in five patients. In addition, subtype D–infected patients in this study were followed up for a long period of time. Moreover, SD in subtype B–infected patients who were not treated with KRG (88 ± 48 months) was significantly longer than in our previous study of KRG-naïve patients in whom the HIV-1 subtype was not determined (59 ± 40 months) ($p < 0.001$) [28]. To our knowledge, this report is

the first to compare disease progression in Asian patients infected with HIV-1 subtypes B and D. Our results are similar to those of Esterbrook et al [7], who reported that subtype D–infected patients had a 4-fold higher rate of $CD4^+$ T cell decline than subtype B–infected patients in England. The faster rate of CD4 decline in subtype D–infected patients remained significant after adjustment for ethnicity, gender, and baseline $CD4^+$ T cell count [7]. In the present study, the AD of subtype D–infected patients was 2.9-fold faster than that of subtype B–infected patients, which was slightly slower than that among English patients described earlier. This is probably due to the fact that the efficacy of KRG in subtype D–infected patients was higher (4.5-fold) than that in subtype B–infected patients (1.6-fold), as shown in Fig. 2.

Among Asian patients infected with non-B subtypes, CRF01_AE is the dominant subtype, with subtype D being very rare [13–15]. To date, no report has compared Asian patients infected with subtypes D and B, although one study compared disease progression rates in Asian patients infected with CRF01_AE and subtype B [33]. $CD4^+$ T cell decline is generally significantly more rapid (4-fold) in patients infected with subtype D than with other subtypes, a decline reported to result from subtype differences in the levels of replicative fitness and virulence in the order D > A > C ($p < 0.001$) [34]. That is, patients infected with subtype D would be expected to have a high plasma viral load accompanying the rapid decline in $CD4^+$ T cell counts. The replication capacity is significantly higher for subtype B than subtype C [35]. However, only one report to date has compared the progression rates of subtypes D and B, although several have compared their replication capacity [36].

In the present study, all subtype D patients showed rapid declines in $CD4^+$ T cell counts from diagnosis or primary infection to around 200/μL and slow decline from a $CD4^+$ T cell count of about 200/μL to the last $CD4^+$ T cell count or death. Three subtype D–infected patients (patients 88-05, 90-16, and 93-01) had relatively low viral loads raising questions regarding their very rapid reductions in $CD4^+$ T cell counts to <200 cells/μL. Furthermore, patients 88-05 and 93-01, with low viral loads at baseline, survived for >10 years after diagnosis in the absence of cART. Their low viral load and long-term survival may have resulted from CCR5 tropism. Patient 93-01 had a greater degree of dual tropism than other patients, which explains, at least in part, the finding that subtype D was associated with a faster rate of disease progression than other HIV-1 subtypes [37]. Additionally, patient 93-01 was treated with KRG. Finally, he had the same 15-bp deletion in the *nef* gene (Δ15-bp; amino acids 9–13), which was present from the earliest samples in December 1992 (AY584798), prior to KRG treatment, to the final samples (Supplementary Fig. 2). Similarly, patient 87-05, who had survived 30 years in the absence of antiretroviral treatment, harbored a 9-bp deletion in *nef* (amino acids 9–11), irrespective of KRG treatment [29,30].

We also determined the sequences of *vif* and *env* genes in six subtype D–infected patients, with the exception of patient 94-29 (Supplementary Figs. 4 and 5). In 1988–1992, various non-B subtypes were transmitted to Korea through OSs [17]. As some of these were homosexual, recombinant strains of KSB and non-B may have arisen, as in patient 92-65, who was infected with subtype D based on the *nef* gene. Some of the OSs are likely to be bisexual, suggesting that they may have spread HIV-1 subtype D to homosexuals who were not OSs, such as patient 93-01. In detail, the *pol* and *vif* genes and 5′ two-thirds of the *env* gene (MH425163-64) of patient 92-65 were found to be KSB, with the 3′ terminal one-third of the *env* gene being subtype D, suggesting that the strain in this patient may be a recombinant of KSB and subtype D. In contrast to the rapid reduction in $CD4^+$ T cell counts observed in other patients infected with subtype D, patient 92-65 experienced a gradual reduction over 9 years, suggesting that the biologically important *env* gene was replaced by KSB. The *vif* and *env* genes in the remaining four patients were of subtype D. Further research is needed to determine whether the six patients classified as having subtype D were infected with full-length HIV-1 subtype D.

The present study provides further evidence that subtype D may be more pathogenic than subtype B. Interestingly, recent studies suggest that subtype D may be decreasing in prevalence [38], consistent with the notion that more pathogenic variants may be less fit for transmission [39]. To date, the biological mechanisms that cause such differences between subtypes are unknown. This study, as well as a previous study [3], showed that increased pathogenesis of subtype D is not simply the result of increased replication because the differences in disease progression were independent of plasma HIV-1 load. Subtype D has been reported more likely to utilize the coreceptor CXCR4 than other subtypes [10] and to develop CXCR4 tropism earlier after infection [2,34]. The presence of a more facile coreceptor switch provides an attractive model for the increased virulence of subtype D because the emergence of CXCR4 variants has been linked to disease progression in patients infected with subtype B [39]. Such differences in coreceptor use could also explain differences in transmissibility [3]. More detailed studies of subtype D variants may help elucidate the biological properties that contribute to HIV-1 virulence.

The mechanism of the anti-HIV action of KRG was mentioned in previous studies [25–29]. In brief, significant decreases in serum p24 antigen as well as soluble CD8 antigen were observed [40–42]. The decrease in soluble CD8 antigen results in the potentiation of cytotoxic $CD8^+$ T cell activity [28]. Furthermore, KRG treatment induces significant genetic defects in the proviral DNA of HIV-1 subtype B [28–30]. In addition, it is well known that many components of ginseng attenuate inflammatory cytokine production [43]. We also observed significant decreases in or a normal range of high-sensitivity C-reactive protein in HIV-1 patients treated with KRG, as evidenced in patient 93-01.

This study had several limitations, including the small number of patients infected with HIV-1 subtype D and the unknown primary infection date. In addition, full-length HIV-1 sequences were not determined. Despite these limitations, treatment with KRG affected the progression rate of both subtypes, with greater efficacy in subtype D patients than in subtype B.

In conclusion, the progression rate to AIDS was about 2.9-fold more rapid in patients infected with HIV-1 subtype D than subtype B. Further studies are needed to determine whether more rapid progression is due to other factors such as socioeconomic factors.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jgr.2018.07.006.

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