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Efficient monitoring of HIV-1 vertically-infected children in Kenya on first-line antiretroviral therapy

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Abbreviations: antiretroviral therapy (ART), antiretroviral drug (ARV), viral load (VL), reverse-transcriptase (RT), nucleoside reverse-transcriptase inhibitors (NRTI), non-nucleoside reverse-transcriptase inhibitor (NNRTI).

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1 **ABSTRACT**

2 **Background:** Worldwide access to antiretroviral therapy (ART) in low- and middle-income
3 countries has significantly increased. Although this presents better treatment options for HIV-
4 infected individuals, the challenge of monitoring ART in these settings still remains.

5 **Objective:** To investigate efficient and cost-effective criteria for assessing ART failure
6 among HIV-1-infected children on first-line ART in resource-limited settings.

7 **Study design:** Retrospective analysis of 75 HIV-1 vertically-infected Kenyan children with a
8 follow-up period of 24 months after initiating ART. Plasma viral load, peripheral CD4⁺T-cell
9 counts and HIV-1 drug-resistance mutations were monitored biannually.

10 **Results:** Plasma viral load (VL) was suppressed to undetectable level or more than 1.5 log₁₀
11 from baseline levels in 53 (70.7%) children within 24 months. VL in the remaining 22
12 (29.3%) children was not suppressed significantly. Of the 22 children, 21 were infected with
13 HIV-1 strains that developed drug-resistance mutations; 9 within 12 months and 12 between
14 12-24 months. Among the 53 who were successfully treated, VL was suppressed in 33 within
15 12 months and in 20 between 12-24 months. There was no significant difference in VL at
16 baseline and the change of CD4⁺T-cell counts after initiating ART between those treated
17 successfully and the failure groups.

18 **Conclusion:** After initiating ART, children may require longer times to achieve complete
19 viral suppression. Plasma viral load testing 24 months after initiating ART could be used to
20 differentiate ART failures among HIV-1 vertically-infected children in resource-limited
21 settings. Additionally, drug resistance testing, if affordable, would be helpful in identifying
22 those failing therapy and in choosing second-line regimens.

23

24 **Key Words:** HIV-1, Children, Antiretroviral therapy, Kenya.

25

26 **BACKGROUND**

27 Increased availability of antiretroviral therapy (ART) has mitigated HIV-1/AIDS prognoses,
28 especially in resource-poor settings.¹⁻² However, many factors such as bad adherence,
29 treatment interruption, and importantly, the emergence of antiretroviral drug (ARV)-
30 resistance mutations could lead to ART failure.³⁻¹³ According to the world health organization
31 (WHO) treatment guidelines, ART failure is defined as: 1) clinical failure, indicated by new
32 or recurrent WHO stage 3 and 4 conditions after 24 weeks of ART; 2) immunologic failure,
33 indicated by CD4⁺T-cell count of less than 200 cells/mm³ or percentage CD4⁺T-cell count of
34 less than 10 for children between two and five years of age and CD4⁺T-cell count of less than
35 100 cells/mm³ for children at the age of five years or older, and 3) virologic failure, indicated
36 by a persistent plasma viral load (VL) above 5,000 copies/ml after at least 24 weeks of
37 ART.¹⁴ Once a patient is found to be failing ART, change of regimen should be considered,
38 but choice of second line regimens is limited in resource-poor settings.¹⁵⁻¹⁷ Therefore,
39 monitoring of response to ART is important to determine treatment success. Although plasma
40 VL is the main index for determining treatment failure in developed countries, it is rare in
41 resource-poor settings because of high cost. This leaves the clinical and immunologic
42 markers as the only means of assessing treatment failure.¹⁸⁻²¹

43 In children, ART effectively reduces morbidity and hospital admissions, and increases
44 long-term survival.²² However, monitoring ART is more challenging than in adults. Children
45 in sub-Saharan Africa seem to be at higher risk for treatment failure, with reported prevalence
46 of 31.6%, 19.7%, 26%, 23-50% in Tanzania, KwaZulu-Natal (South Africa), Uganda and
47 Cote d'Ivoire, respectively.²³⁻²⁷ In addition, clinical and immunologic markers have been
48 reported to be poor predictors of virologic suppression.^{16, 28-30} There is need to investigate
49 efficient and cost-effective criteria for assessing treatment failure among HIV-1-infected
50 children in resource-limited settings.

51 In Kenya, we have longitudinally followed up HIV-1 vertically-infected children,
52 monitored CD4⁺T-cell counts quarterly and plasma VL biannually since 1999 and 2000,
53 respectively, and reported the emergence of ARV-resistance mutations among the infecting
54 HIV-1 strains together with evolutionary changes in the HIV-1 envelope gene.^{31,32}

55

56 **OBJECTIVE**

57 To examine the response to ART, the proportion of treatment failure, and the profile
58 of HIV-1 drug resistance-associated mutations among HIV-1 vertically-infected Kenyan
59 children in order to determine appropriate marker(s) and frequency of monitoring children on
60 ART in a resource-limited setting.

61

62 **STUDY DESIGN**

63 This was a retrospective study involving HIV-1 vertically-infected children from
64 Nyumbani children's home in Nairobi, Kenya. The study subjects have been described
65 previously.³¹ Consent was obtained from the caretaker board of the orphanage, and the study
66 proposal approved by the ethical committees of Kenya Government and Kanazawa
67 University, Japan. Seventy-five children were included in this study. Their baseline
68 characteristics are shown in Table 1. None of them had prior ART exposure. No death
69 occurred during the follow-up.

70 The ART initiation dates varied from March 1999 to October 2007. First-line ART
71 consisted of two nucleoside reverse-transcriptase inhibitors (NRTI): mostly zidovudine and
72 didanosine or lamivudine, and one non-nucleoside reverse-transcriptase inhibitor (NNRTI):
73 nevirapine or efavirenz, as described in the WHO guidelines.¹⁴ In the current study, "ART
74 success" was defined as plasma VL suppression to undetectable level or 1.5 log₁₀ decrease

75 from baseline at 24 months after ART initiation, and “ART failure” was defined as two
76 consecutive plasma VL above 5,000 copies/ml by the 24th month.

77 Peripheral CD4⁺T-cell counts were determined using the FACSCOUNT (Becton-
78 Dickinson, Beiersdorf, Germany) quarterly and plasma HIV-1 RNA quantified biannually
79 using the Amplicor HIV-1 Monitor kit version 1.5 (Roche Diagnostics, Alameda, CA) with
80 detection limit of 400 copies/ml according to the manufacturer’s instructions. Absolute
81 CD4⁺T-cell count was used instead of percentage CD4⁺T-cell count because it was the only
82 available option during study period.

83 HIV-1 RNA was extracted from plasma using SMITEST EX-R and D (Genome
84 Science Laboratories, Fukushima, Japan) according to the manufacturer’s instructions. The
85 HIV-1 reverse-transcriptase (RT) gene was amplified and population sequencing was done as
86 previously described.³¹⁻³⁴ The HIV-1 RT nucleotide sequences obtained from each child
87 biannually during the first 24 months of ART were analyzed for previously-reported drug-
88 resistance mutations using the Stanford university HIV database
89 (<http://hivdb.stanford.edu/pages/algs/HIVdb.html/>). The REGA HIV-1 subtyping tool
90 (<http://hivdb.stanford.edu/>) was used to determine the HIV-1 subtype. Pairwise comparisons
91 were done in demographic, virologic, and immunologic parameters between treatment
92 success and treatment failure groups using the student’s t-test.

93

94 **RESULTS**

95 Plasma VL changes before and after ART initiation is shown in Figure 1. Of the 75
96 children studied, 53 (70.7%) suppressed plasma VL to undetectable level or more than 1.5
97 log₁₀ from baseline after 24 months of ART [Treatment success group]. Of the 53 treatment
98 success children, 33 suppressed VL to undetectable level within 12 months [rapid responders]
99 and 16 between 12-24 months (nine children within 18 months and seven within 24 months),

100 and four had detectable VL but suppressed their VL more than 1.5 log₁₀ from baseline at 24
101 months without any known HIV-1 RTI-resistance mutations [slow responders]. There was no
102 significant difference in plasma VL at baseline between treatment success and failure groups
103 (Table 1).

104 The remaining 22 children (29.3%) did not suppress their VL more than 1.5 log₁₀
105 from baseline after 24 months of ART [Treatment failure group], though four of them
106 suppressed plasma VL to undetectable level but their VL rebounded to above 5,000 copies/ml
107 by the end of 24 months. In the 22 children, 21 subsequently developed HIV-1 RTI-
108 resistance mutations; cumulatively, seven children by six months, nine by 12 months, 13 by
109 18 months, and 21 by 24 months. One female child failed therapy, but no known HIV-1 RTI-
110 resistance mutation was detected in this child after 24 months of ART (Table 2).

111 Changes of CD4⁺T-cell counts before and after ART initiation are shown in Figure 2.
112 CD4⁺T-cell counts increased in the first 12 months after ART initiation and kept the level
113 thereafter irrespective of children's ART outcome. Although CD4⁺T-cell counts showed
114 higher tendency in slow responder group than in rapid responder group, which was
115 significant at baseline and after 24 months of ART (p<0.02 and p<0.05, respectively), there
116 was no significant difference in CD4⁺T-cell counts at any time point between the treatment
117 success and failure groups (data not shown).

118 When the mean age at start of ART was compared between the treatment success and
119 failure groups (8.3 years and 7.3 years, respectively), the difference was not statistically
120 significant despite some trend showing younger age as being associated with treatment
121 failure. In addition, mean age at start of ART was higher in rapid responders (9.1 years) than
122 in slow responders (7.1 years), though the difference was not statistically significant (Table
123 1).

124 HIV-1 genotypic analysis based on RT sequences showed that all the children were
125 infected with non-B subtype HIV-1. There was no significant difference in the distribution of
126 HIV-1 subtypes between treatment success and failure groups (Table 1). Treatment failure
127 was higher in the nevirapine group (16/52, 30.8%) than in the efavirenz group (4/20, 20.0%),
128 though the difference was not significant.

129 The profiles of RTI-resistance mutations are summarized in Table 2. Of the 22
130 children who failed treatment 21 developed mutations. None of the treatment success
131 children had such mutations. Seven children developed drug-resistance mutations within 6
132 months after initiating ART, two between 6 and 12 months, four between 12 and 18 months,
133 and eight between 18 and 24 months. Of the 21 children, 20 had NRTI-resistance mutations,
134 and 16 had NNRTI-resistance mutations. The common NRTI-resistance mutations were
135 M184V, which appeared in 11 children, and thymidine analogue-associated mutations
136 (TAMs): TAM 1 (M41L, L210W, and T215Y/F) and TAM 2 (D67N, K70R, and K219Q/E),
137 which appeared in 13 and 13 children, respectively. Two of them had both TAM 1 and TAM
138 2 profiles detected together. M184V appeared as the first primary NRTI-resistance mutation
139 in six of the 20 children, with TAMs in five, TAMs without M184V in eight children, and
140 T69N in one child. The most common NNRTI mutation was K103N, which appeared in 13 of
141 the 21 children.

142 The sequences obtained in this study were deposited at the Genbank under accession
143 numbers: HQ586062-HQ586272.

144

145 **DISCUSSION**

146 In the current study, we followed up 75 HIV-1 vertically-infected children who were
147 on first-line ART for at least 24 months. After 24 months of ART, the proportion of treatment
148 success was 70.7% (53 children). During the two-year follow-up, the cumulative treatment

149 success rate was 29%, 44%, 56% and 71% at 6, 12, 18 and 24 months, respectively. It is of
150 note that 20 “slow responders” were identified among the 53 treatment success children; their
151 VL was suppressed to undetectable level or more than 1.5 log₁₀ decrease from baseline
152 between 12 and 24 months. These results suggest that HIV-1-infected children may need
153 longer periods of ART to control virus replication comparing with adults.

154 Twenty-two children (29.3%) failed therapy at 24 months after ART initiation. They
155 maintained high VL: less than 1.5 log₁₀ decrease from baseline, or rebounded after an initial
156 good response during follow-up. The treatment failure rate in this study is within the range of
157 previous reports from sub-Saharan Africa: 19.7% - 50%.²³⁻²⁷ Thus, ART failure appears to be
158 more frequent in children than in adults, possibly due to the difference in the prevalence and
159 profile of drug-resistance mutations in children compared with adults.³⁵⁻³⁸ In this study, at the
160 time ART was initiated, ARV dosing was based on body weight and body surface area of
161 children. Though children grow constantly hence it may be advisable to change dosing
162 regularly,^{36,37} this was not possible in our settings. Furthermore, in this setting, all the
163 children were carefully monitored by the staff of the children’s home and were therefore
164 ART compliant. However, by mid-2000, ARVs were acquired through charity and
165 prescription depended on drug availability. This might have affected ART compliance hence
166 the higher failure rate.

167 Of the 22 children who failed treatment, 21 developed drug-resistance mutations.
168 Cumulatively, they were detected in 7, 9, 13 and 21 children at 6, 12, 18 and 24 months of
169 ART, respectively. In seven children, TAMs emerged as first mutations without M184V,
170 which is known to be associated with the use of lamivudine. Two of them had both TAM 1
171 and TAM 2 profiles detected together. Despite being on a lamivudine-containing regimen, six
172 children developed NRTI-resistance mutations devoid of M184V (Table 2). These findings
173 may indicate a pathway different from what has been previously suggested.³¹ Nonetheless,

174 this is consistent with previous observations on the response to ART in children comparing
175 with adults.^{38,39} Besides, one child failed therapy, but did not harbor HIV-1 strains with
176 known RTI-resistance mutations. This may be due to unknown factors, such as low
177 compliance, or other unknown drug-resistance mutations located outside of the RT region
178 that was examined. The possible cause of treatment failure in this child is under investigation.
179 The most observed NNRTI-resistance mutation was K103N, which is easily transmitted and
180 persistent, and has become the most common mutation in patients failing first-line
181 regimens.⁴⁰

182 The WHO recommends early initiation of ART for all HIV-1 vertically-infected
183 children.⁴¹ To get a better clinical outcome, plasma VL and drug-resistance testing should be
184 done before starting ART and as many times as possible during therapy to allow timely and
185 optimized therapeutic change. However, most infected children reside in resource-limited
186 countries where such monitoring is nonexistent.⁴¹ So far, clinical and immunological markers
187 have been used for this purpose.⁴² However, there was no significant difference in the change
188 of CD4⁺T-cell counts before and after ART initiation between the treatment success and
189 failure groups. In addition, there was no difference in the plasma VL at baseline between the
190 two groups. From these findings, it would be advisable that plasma viral load testing at 24
191 months of ART be done to differentiate failing ART regimens. Additionally, drug-resistance
192 testing, if affordable, at 24 months after initiating ART would also be helpful in choosing
193 second-line regimens.

194

195 **ACKNOWLEDGEMENTS AND CONFLICT OF INTEREST**

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FIGURE LEGENDS

Figure 1: Changes in plasma viral load after initiating antiretroviral therapy among HIV-1–infected children.




Treatment success (TS12, ): undetectable viral load within 12 months after initiating antiretroviral therapy; Treatment success (TS24, ): viral load suppressed to less than 400 copies/ml or more than 1.5 log from baseline between 12–24 months; Treatment failure (TF, ): failed therapy within 24 months. 0 = baseline.

Figure 2: Changes in peripheral CD4⁺ T-cell counts after initiating antiretroviral therapy among HIV-1–infected children.




Treatment success (TS12, ): undetectable viral load within 12 months after initiating antiretroviral therapy; Treatment success (TS24, ): viral load suppressed to less than 400 copies/ml or more than 1.5 log decline from baseline between 12–24 months; Treatment failure (TF, ): failed therapy within 24 months. 0 = baseline.

TABLE1: The characteristics of vertically-infected Kenyan children on ART

| Variable | All (n=75) | Treatment Success (TS) | | | Treatment failure (n=22) |
|--|------------------------|------------------------|-------------------------|-------------------------|-----------------------------|
| | | All TS (n= 53) | TS12† (n= 33) | TS24‡ (n= 20) | |
| Gender | | | | | |
| Male, n (%) | 41 (54.7) | 28 (52.8) | 19 (57.6) | 9 (45.0) | 13 (59.1) |
| Female, n (%) | 34 (45.3) | 25 (47.2) | 14 (42.4) | 11 (55.0) | 9 (40.9) |
| Age at ART start (years) | | | | | |
| Mean (SD) | 7.8 (3.9) | 8.3 (4.1) | 9.1 (4.3) | 7.1 (3.3) | 7.3 (3.1) |
| Baseline viral load (log₁₀copies/ml) | | | | | |
| Mean (SD) | 5.1 (0.7) ^a | 5.0 (0.8) | 5.0 (0.8) ^c | 4.9 (0.7) ^d | 5.2 (0.5) ^d |
| Baseline CD4⁺T-cell counts (/mm³) | | | | | |
| Mean (SD) | 431 (310) ^b | 445 (270) | 376 (224) ^{e*} | 584 (309) ^{f*} | 469 (376) ^g |
| HIV-1 subtype, n (%) | | | | | |
| A1 | 59 (78.7) | 44 (83.0) | 27 (81.8) | 17 (85.0) | 15 (68.2) |
| C | 3 (4.0) | 2 (3.8) | 2 (6.1) | 0 (0) | 1 (4.55) |
| D | 12 (16.0) | 7 (13.2) | 4 (12.1) | 3 (15.0) | 5 (22.7) |
| CRF02_AG | 1 (1.3) | 0 (0) | 0 (0) | 0 (0) | 1 (4.55) |
| Drug Combinations, n (%) | | | | | |
| AZT/3TC/NVP | 50 (66.7) | 36 (67.9) | 22 (66.7) | 14 (70.0) | 14 (63.6) |
| AZT/ 3TC/ EFV | 16 (21.3) | 14 (26.4) | 9 (27.3) | 5 (25.0) | 2 (9.1) |
| AZT/3TC/ABC | 3 (4.0) | 1 (1.9) | 0 (0) | 1 (5.0) | 2 (9.1) |
| AZT/ddI/EFV | 3 (4.0) | 1 (1.9) | 1 (3.0) | 0 (0) | 2 (9.1) |
| AZT/ddI/NVP | 2 (2.7) | 0 (0) | 0 (0) | 0 (0) | 2 (9.1) |
| d4T/3TC/EFV | 1 (1.3) | 1 (1.9) | 1 (3.0) | 0 (0) | 0 (0) |

ART: antiretroviral therapy; AZT: zidovudine; 3TC: lamivudine; ddI: didanosine; ABC: abacavir; NVP: nevirapine; EFV: efavirenz; SD: standard deviation. † undetectable viral load within 12 months;; ‡ viral load to <400 copies/ml or >1.5log decline from baseline between 12-24 months; ^a n=57; ^b n=59; ^c n=27; ^d n=12; ^e n=26; ^f n=13; ^g n=20; * p value = 0.02

TABLE 2: Characteristics of children who failed therapy and time to emergence of first major RTI drug resistance-associated mutations

| ID | GENDER | AGE AT ART START | REGIMEN | 6 MONTHS | 12 MONTHS | 18 MONTHS | 24 MONTHS |
|-----|--------|------------------|-------------|--------------------------------|--|--|--|
| 38 | M | 8.6 | AZT/3TC/NVP | D67N | D67N/K70R/L210W/K219E | D67N/K70R/L210W/K219E | D67N/K70R/L210W/K219E |
| 62 | M | 5.4 | AZT/ddI/NVP | D67N/K70R/G190A | D67N/K70R/G190A / T215F/K219E | | D67N/K70R/G190A /T215F/ K219E /Y181C |
| 85 | F | 4.7 | AZT/3TC/NVP | D67N/M184V/K103N | D67N/M184V/K103N | | |
| 103 | M | 3.2 | AZT/3TC/NVP | M184V/K103N | M184V /L74V/K103N | L74V/M184V/K103N | L74V/M184V/K103N |
| 98 | F | 5.3 | AZT/3TC/NVP | M184V/K103N | M184V/K103N | M184V/K103N | M184V/K103N |
| 89 | F | 1.4 | AZT/3TC/NVP | M184V/L210W/T215F/ K101E/G190A | M184V/L210W/T215F/K101E/ G190A/D67N/K70R | M184V/L210W/T215F/K101E/ G190A/D67N/K70R | M184V/L210W/T215F/K101E/ G190A/D67N/K70R |
| 36 | M | 7.4 | AZT/3TC/ABC | M184V/T215F/K103N | M184V/T215F/K103N /G190A | M184V/T215F/K103N /G190A | M184V/T215F/K103N /G190A |
| 48 | M | 11.4 | AZT/3TC/NVP | NA | M184V/K103N | M184V/K103N | M184V/K103N |
| 74 | F | 6.8 | AZT/3TC/NVP | NONE | NONE | NONE | T69N /K103N |
| 73 | F | 6.7 | AZT/3TC/NVP | NONE | NONE | | K103N |
| 87 | M | 3.4 | AZT/3TC/NVP | NONE | NONE | | M41L |
| 80 | M | 5.5 | AZT/3TC/NVP | NONE | NONE | | M41L//A62V/T69P/K103N |
| 41 | M | 10.9 | AZT/3TC/NVP | NONE | | M184V/K103N | NA |
| 10 | M | 12.8 | AZT/ddI/NVP | NONE | | M41L | E44D/V118I |
| 122 | M | 11 | AZT/3TC/NVP | | M184V/K103N | M184V/K103N | M184V/K103N |
| 91 | F | 3.5 | AZT/3TC/NVP | | | M184V/G190A | M184V/G190A |
| 19 | M | 10 | AZT/ddI/EFV | | | M184V/T215F/K103N/G190A | M184V/T215F/K103N/G190A / M41L/D67N |
| 42 | F | 6.9 | AZT/ddI/EFV | | | | D67N/K70R |
| 33 | F | 7.6 | AZT/3TC/EFV | | | | K219Q/K101Q |
| 11 | M | 9.9 | AZT/3TC/EFV | | | | M41L//T215Y/K103N |
| 65 | M | 7.6 | AZT/3TC/NVP | | | | M41L/K103N/M184V |
| 7 | F | 11 | AZT/3TC/ABC | | | | NONE |

ART: antiretroviral therapy; RTI: reverse transcriptase inhibitor; AZT: zidovudine; 3TC: lamivudine; ddI: didanosine; ABC: abacavir; NVP: nevirapine; EFV: efavirenz. F: Female; M: Male; NA: Not amplified; Blank: No sample available for testing; NONE: No major mutation detected.

Figure 1.

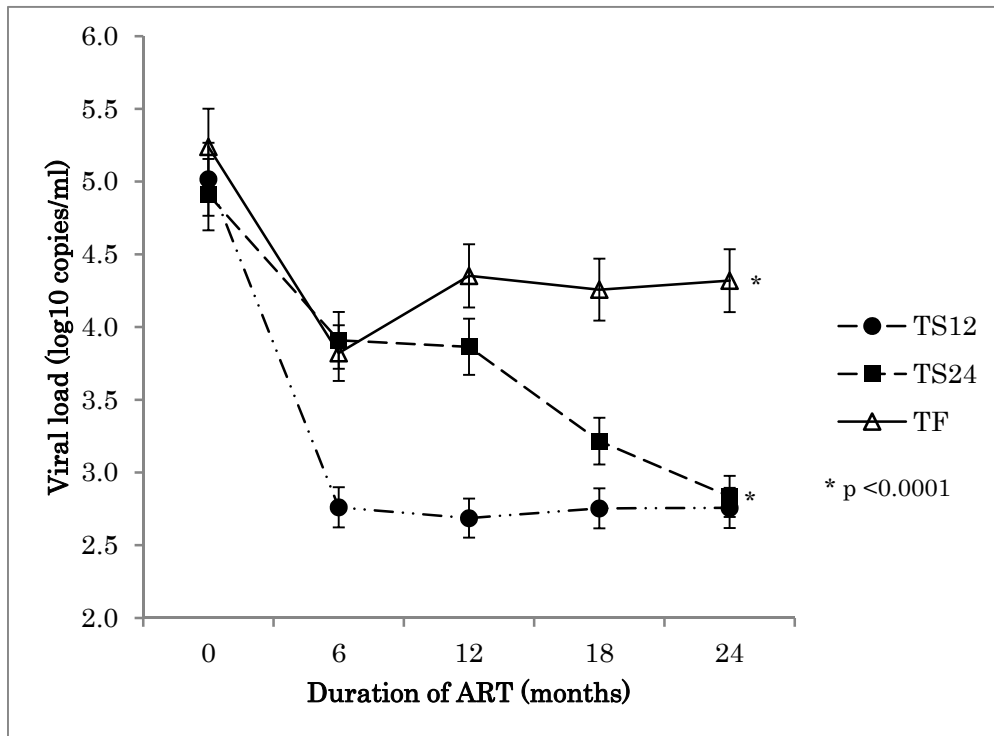


Figure 2

