

Clinical and Genotypic Findings in HIV-Infected Patients With the K65R Mutation Failing First-Line Antiretroviral Therapy in Nigeria

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Introduction: The HIV-1 epidemic in African countries is largely due to non-B HIV-1 subtypes. Patterns and frequency of antiretroviral drug resistance mutations observed in these countries may differ from those in the developed world, where HIV-1 subtype B predominates.

Methods: HIV-1 subtype and drug resistance mutations were assayed among Nigerian patients with treatment failure on first-line therapy (plasma HIV RNA >1000 copies/mL). Sequence analysis of the reverse transcriptase and protease gene revealed drug resistance mutations and HIV-1 viral subtype. Specific patterns of mutations and clinical characteristics are described in patients with the K65R mutation.

Results: Since 2005, 338 patients were evaluated. The most prevalent subtypes were CRF02_AG [152 of 338 (44.9%)] and G [128 of 338 (37.9%)]. Three hundred seven of 338 (90.8%) patients had previously received stavudine and/or zidovudine + lamivudine + efavirenz or nevirapine; 41 of 338 (12.1%) had received tenofovir (TDF). The most common nucleoside reverse transcriptase inhibitor mutations observed were M184V (301, 89.1%) and K70R (91, 26.9%). The K65R mutation was present in 37 of 338 patients (10.9%). The Q151M ($P < 0.05$), K219R, and T69del ($P < 0.01$) mutations were more common in patients with K65R who had not received TDF.

Conclusions: The K65R mutation is increasingly recognized and is a challenging finding among patients with non-B HIV subtypes, whether or not they have been exposed to TDF.

Key Words: drug mutations, K65R, non-B HIV-1 subtypes

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BACKGROUND

The development of drug resistance among patients on antiretroviral therapy (ART) remains an important challenge in the management of HIV disease. Patterns of drug resistance mutations in response to antiretroviral drugs and their impact on ART management have been well characterized in patients with HIV-1 subtype B in the developed world,¹ but less frequently in patients from resource-limited settings who bear a disproportionate burden of the AIDS epidemic and are infected by a diverse pool of non-B HIV-1 subtypes. The President's Emergency Plan For AIDS Relief (PEPFAR) was established in 2004 in Nigeria and genotypic analysis of patients with ART failure has been conducted since September 2005, initially supported by the National Institutes for Health. We describe preliminary results from these genotype analyses and provide a more detailed description of mutational and clinical characteristics in a smaller cohort of patients in which the K65R mutation was observed. The K65R mutation is selected by tenofovir (TDF), didanosine, abacavir, and possibly stavudine (d4T) usage and causes a variable loss of susceptibility to TDF, didanosine, and abacavir depending on the presence of thymidine analogue mutations (TAM).^{2–4} It has been described frequently in both populations with HIV-1 subtype B^{5–8} and non-B subtype infection.^{2,9–11} More recently, the K65R mutation has also been reported in non-subtype B patients receiving non-TDF-containing first-line antiretroviral regimens.^{9,12} This finding has significant implications for second-line and third-line ART regimen choices in resource-limited settings, where TDF is often reserved for use due to its relatively high cost and resultant sustainability considerations. Responses to these TDF-containing second-line or third-line regimens among patients in whom K65R developed while on first line therapy may thus be significantly impacted.

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METHODS

Study Site

The AIDS Prevention Initiative in Nigeria Plus (APIN Plus) Harvard PEPFAR program has been providing highly active antiretroviral therapy to eligible HIV-infected patients in Nigeria since September 2004. ART is provided free of charge, and funding is provided for additional program activities including baseline assessment, clinical, immunological, and virological monitoring, and prophylaxis and treatment of opportunistic infections. APIN Plus supports and collaborates with a total of 29 sites, including 11 tertiary care hospitals in 10 different Nigerian states. Patients enrolled in the program are treated according to Nigerian national ART guidelines and international standards. Standard first-line ART regimens include d4T or zidovudine (AZT), lamivudine (3TC), plus efavirenz (EFV) or nevirapine (NVP). More recently, Truvada (TDF + emtricitabine) has been recommended as a first-line alternative in Nigeria, particularly, for patients coinfecting with hepatitis B. Since program enrollment began, 46,975 patients have been initiated on or provided with ART. The Government of Nigeria's ART program began in 2002 with the provision of generic ART at many of the APIN Plus clinical centers; 6920 patients who were already on ART provided through the Nigerian Government program or privately were enrolled and continued on therapy.

Study Population

All HIV-infected adult patients on ART who were enrolled at one of the APIN Plus PEPFAR-funded ART clinics and had genotype testing performed were considered for this analysis. Genotype testing has been performed as a part of patient clinical follow-up in the program since September 2005, supported by the United States National Institutes of Allergy and Infectious Diseases/National Institutes for Health. Patients who were eligible for genotype testing included those with evidence of virological failure (detectable viral load >1000 copies/mL after 6 months on first-line ART), to which they were adherent (defined as adhering to scheduled drug pickups 3 months before failure). Criteria for inclusion in this analysis were eligibility for resistance testing and the presence of one or more reverse transcriptase resistance mutations. If more than 1 genotype was performed, the first result fitting these inclusion criteria was used in the analysis.

Patients were recruited for participation and enrolled in the APIN Plus ART treatment program after written informed consent, which was subject to ethical review by the Institutional Review Boards of the University of Ibadan/University College Hospital, National Institute for Medical Research, Lagos, Jos University Teaching Hospital, University of Maiduguri Teaching Hospital, and the Harvard School of Public Health.

Study Design and Data Collection

We describe HIV-1 subtype and drug resistance mutation characteristics in a cohort of eligible patients and further characterize nucleoside reverse transcriptase inhibitor (NRTI) mutations in the subset with the K65R mutation. We also compared genotypic mutation patterns among patients

with and without the K65R mutation and among patients with the K65R mutation on standard first-line ART, which did not contain TDF to those on TDF-containing first-line ART. Finally, we describe immunologic and virologic outcomes among patients with the K65R mutation, who were switched to second-line ART. Genotypic analysis was performed retrospectively on samples collected from the patient closest to the time virological failure was detected. For genotypic analysis, plasma samples from EDTA-separated blood were aliquotted and shipped in liquid nitrogen to the Harvard School of Public Health where testing was performed using Abbott's ViroSeq HIV-1 Genotyping System 2.0 assay. RNA was isolated from plasma, reverse transcribed and amplified, and sequenced on an ABI 3100 capillary system. The sequence chromatograms were then edited using Abbott's ViroSeq software. Clinical data on each patient with the K65R mutation was recorded from standardized electronic forms which capture demographic, clinical, laboratory, and therapeutic information at baseline and at each visit. Patients enrolled into the APIN Plus program who are on ART have scheduled visits every 3 months or as medically indicated until they are clinically stable and the viral load is undetectable. Thereafter, visits occur every 6 months. CD4⁺ cell count and viral loads, in addition to laboratory tests for toxicity monitoring, are routinely collected at each visit. Data collected for the purposes of this analysis included date of antiretroviral failure, date of first genotypic analysis, ART regimen and duration before each genotypic analysis, and ART regimen and date of ART regimen switch if switch to second-line ART occurred. CD4⁺ cell count (cells/mm³) and viral load (copies/mL) were also collected at the time of antiretroviral failure, initial genotypic testing, and at 6 months after ART switch.

Statistical Analysis

Summary statistics were performed in Stata v.9 (StataCorp, College Station, TX). Comparative analyses were performed using Fisher exact test.

RESULTS

Genotype testing and subtype determination was performed on 338 patients with virologic failure. HIV-1 subtypes included: CRF02_AG [152 of 338 (44.9%)], G [128 of 338 (37.9%)], CRF06 [15 of 338 (4.4%)], A [12 of 338 (3.6%)], and other subtypes or recombinants [31 of 338 (9.2%)]. Three hundred seven of 338 patients (90.8%) had received ARV regimens containing d4T usually in combination with 3TC and NVP or EFV before genotypic testing. Eighteen patients (5.3%) had previous exposure to abacavir or didanosine, and 41 patients (12.1%) had received a prior regimen containing TDF. The most common NRTI mutations observed were M184V (301, 89.1%), K70R (91, 26.9%), D67N (75, 22.2%), T215Y (61, 18.0%), T215F (51, 15.1%), M41L (46, 13.6%), K219Q (45, 13.3%), S68G (43, 12.7%), and K65R (37, 10.9%). The most common nonnucleoside reverse transcriptase inhibitor mutations included: Y181C (168, 49.7%), K103N (123, 36.4%), G190A (89, 26.3%), A98G (66, 19.5%), K101E (59, 17.5%), V108I (52, 15.4%), and V90I (41, 12.1%).

The K65R mutation was detected in 37 of 338 patients (10.9%) (Table 1). The most prevalent HIV-1 subtype among patients with K65R was CRF02_AG (21/37; 56.8%). Twenty-four of 37 patients (64.9%) with K65R were on TDF-containing first-line ART. The remaining 13 patients (35.1%) with K65R had only received a combination of d4T or AZT, 3TC, and NVP and had no documented prior exposure to TDF. Eight of these patients were previously enrolled in the Nigerian government ART program and had been receiving the Government of Nigeria first-line regimen of d4T, 3TC, and NVP before PEPFAR enrollment. Among patients with the K65R mutation, the median duration of ART therapy before the first genotypic test was 13.4 months (range 6.4–45.2 months), the median time between detection of failure and first genotypic test was 5.72 months (range 0–26.2), and the median viral load and CD4⁺ cell count at the time of genotypic testing was 70,469.5 copies/mL (range 2318–1,037,171) and 84 cells per cubic millimeter (range 4–469), respectively.

A variety of NRTI mutations were observed in association with the K65R mutation (Table 2), with some notable differences between patients with and without TDF in their first-line ART regimen. Among the 13 patients on non-TDF ART, the following NRTI mutations were observed in association with the K65R mutation: Q151M, F77L, F116Y, V75I, M184V, K219R, T69del, and S68G. Among 21 patients primarily on TDF-containing ART, the following mutations were observed: M184V, M184I, S68G, A62V and Y115F, and K219E. Mutations that occurred significantly more commonly in patients on non-TDF ART than those on TDF-containing ART included Q151M complex mutations ($P < 0.05$) and the combination of K219R and T69del mutations, which always appeared together [6 of 13 (46.2%) vs. 0 of 21 (0%); $P < 0.01$]. The S68G mutation was also seen more frequently in patients on non-TDF ART, but this finding was not significant [9 of 13 (69.2%) vs. 9 of 21 (42.9%); $P > 0.05$]. In contrast, the M184V [15 of 21 (71.4%) vs. 3 of 13 (23.1%); $P = 0.01$] and Y115F mutations [7 of 21 (33.3%) vs. 0 of 13 (0%); $P = 0.03$] were significantly more common among patients on TDF-containing ART compared with patients on non-TDF ART. TAMs were very infrequent among both groups of patients with the K65R mutation, and there were no significant differences between them ($P > 0.05$).

A comparison of drug resistance mutations between patients with ($n = 37$) and without ($n = 301$) the K65R mutation was also performed. The following mutations were observed in significantly more patients with K65R: S68G, A62V, Y115F, Q151M complex, T69del, and K219R [all $p \leq 0.01$]. In contrast, the M184V mutation [280 of 301 (93.0%) vs. 21 of 37 (56.8%), $P < 0.01$] and TAMs, M41L [45 of 301 (15.0%) vs. 1 of 37 (2.7%), $P = 0.04$] and K219Q [44 of 301 (14.6%) vs. 1 of 37 (2.7%), $P = 0.04$], were significantly more prevalent among patients without K65R.

Twenty-five patients were switched to second-line ART after the diagnosis of antiretroviral failure to first-line ART (Table 1). The median time to switch from ART failure detection was 10.5 months (range 1.8–31.3). Nineteen of the switch regimens included TDF. The median viral load at 6 months of follow-up after ART switch among 15 of these switch patients decreased to 200 (range 200–7918) copies per

milliliter. The median CD4 at 6 months among these patients increased to 221 (range 90–651) cells per cubic millimeter. There was 1 reported death in our K65R cohort with no further information. Missed drug pickups (≥ 1 missed monthly drug pickup during time on ART before initial genotypic test) were observed in all the patients. In addition, interruptions to therapy as a result of stockouts could not be ruled out among 6 of 8 patients who initiated first-line ART before enrollment in our program; there have been no stockouts, however, during the APIN Plus PEPFAR program.

DISCUSSION

We report results of genotype testing in patients with non-B HIV subtypes who failed first-line ART. In general, drug resistance mutations most frequently observed were similar to those described in patients infected with HIV subtype B¹³; however, a notable finding was the significant proportion of patients in this cohort with the K65R mutation, a number of whom had no previous exposure to TDF or other antiretroviral drugs commonly known to select for K65R. The development of K65R among patients on non-TDF ART is uncommon, especially among patients on first-line combinations commonly used in resource-limited settings (d4T or AZT plus lamivudine and NVP or EFV). To our knowledge, only 2 other studies have reported K65R developing in patients on these antiretroviral combinations in resource-limited settings. Patients in these studies were predominantly infected with HIV-1 subtypes, CRF01_AE (Thailand) and C (Malawi). In the study by Sungkanuparph et al,¹² the K65R mutation was observed in 8 of 122 failing patients (7%) on a first-line regimen of fixed-dose d4T, 3TC, and NVP, which was provided by the Thailand national government. Factors associated with the presence of K65R included viral load at virologic failure and duration of ART. In a second study by Ferradini et al,⁹ of a cohort of Malawian patients, 10 of 52 patients (5%) failing first-line d4T or AZT plus lamivudine and NVP or EFV developed the K65R mutation.

The route of K65R acquisition among our patients on non-TDF ART is unknown. Transmission of the K65R mutation remains rare, especially in resource-limited settings.^{14,15} Selection of K65R by d4T, which was part of initial ART in all but one of the patients on non-TDF-containing regimens, has been described previously in patients with HIV-1 subtype B. In a study by Garcia-Lerma et al,¹⁶ which investigated the *in vitro* pathways of acquisition of resistance to d4T in a panel of viruses, the K65R mutation was selected for by d4T in 7 of 9 viruses under investigation. Clinical studies to support this finding include a study by Margot et al,¹⁷ which compared the development of resistance among treatment-naïve patients receiving TDF or d4T with 3TC and EFV. The K65R mutation was noted to develop in 2 of 301 patients (0.7%) in the d4T-containing arm. In another study by Valer et al,⁷ 2 of 53 patients (3.8%) on d4T and 3TC developed K65R. The K65R has also been observed in 2 studies conducted in Botswana and South Africa of patients infected with HIV subtype C on d4T-containing regimens. It should be noted that patients in these studies were also receiving didanosine.^{18,19}

TABLE 1. Clinical Characteristics of Patients With the K65R Mutation

Patient	First-line ART Regimen(s) Before ART Failure and First K65R Genotype	Time on ART at First Genotype (m)*	RNA Viral Load at Genotype (copies/mL)	CD4 Count at Genotype (cells/mm ³)	Second-line ART Switch Regimen	Time to Switch† (m)	6-Month RNA Viral Load (copies/mL)	6-month CD4 (cells/mm ³)
No TDF in first-line ART								
1	d4T 3TC NVP	26.9	72,939	142	TDF DDI LPV/R	9.24	562	482
2	d4T 3TC NVP	12.1	18,000	4	AZT TDF LPV/R	2.07	900	212
3	d4T 3TC NVP	39.5	8547	27	TDF LPV/R AZT 3TC	17.86	5783	122
4	AZT 3TC NVP	6.4	869,004	15	TDF LPV/R AZT 3TC	10.63	200	302
One dose d4T 3TC NVP								
5	AZT 3TC NVP	12.4	1,037,171	207	TDF FTC 3TC LPV/R	12.66	—	—
d4T 3TC NVP								
6	d4T 3TC NVP	29.0	68,000	168	AZT TDF LPV/R	18.26	NA	NA
7	d4T 3TC NVP	6.4	150,311	50	LPV/R TDF FTC	5.59	833	651
8	d4T 3TC NVP	8.6	3516	34	—	—	—	—
9	d4T 3TC NVP	9.5	50,628	46	AZT LPV/R TDF FTC	17.37	NA	NA
10	d4T 3TC NVP	26.4	42,322	34	TDF LPV/R AZT 3TC	17.96	1653	297
11	d4T 3TC NVP	10.7	—	460	—	—	—	—
12	d4T 3TC NVP	31.1	23,393	110	AZT TDF LPV/R	1.84	200	90
13	d4T 3TC NVP	45.2	191,512	86	AZT TDF LPV/R	31.25	NA	NA
TDF in first-line ART								
14	TDF FTC NVP	21.8	5440	192	AZT 3TC LPV/R	20.76	—	—
15†	AZT 3TC NVP	18.3	9001	337	—	—	—	—
TDF FTC NVP								
16	TDF FTC NVP	11.8	44,599	121	TDF LPV/R AZT 3TC	8.91	200	184
17	TDF FTC NVP	13.4	82,998	53	—	—	—	—
18	TDF FTC NVP	12.3	145,110	53	AZT 3TC LPV/R	12.89	200	199
One dose d4T 3TC NVP								
19	TDF FTC NVP	12.2	168,303	128	—	—	—	—
20	TDF FTC NVP	24.4	20,802	37	—	—	—	—
21	TDF FTC NVP	24.0	113,462	239	LPV/R AZT 3TC	18.45	NA	NA
22	TDF FTC NVP	20.5	19,905	9	AZT 3TC LPV/R	8.75	200	256
23	TDF FTC NVP	12.2	245,011	11	LPV/R AZT 3TC	6.64	7918	222
24	TDF FTC NVP	12.2	91,463	10	AZT 3TC LPV/R	9.44	200	105
25	TDF FTC NVP	11.4	412,246	71	—	—	—	—
26	TDF FTC NVP	12.0	44,318	30	—	—	—	—
One dose AZT 3TC NVP								
27	TDF 3TC NVP	10.7	282,441	13	—	—	—	—
One dose ABC 3TC NVP								
28	TDF FTC NVP	18.2	98,243	214	AZT LPV/R TDF FTC	14.11	200	459
One dose TDF FTC EFV								
29	TDF FTC NVP	14.9	23,830	18	TDF SQV AZT 3TC	5.76	NA	NA
TDF FTC EFV								
One dose TDF SQV								
AZT 3TC								
30	TDF FTC NVP	21.4	9879	274	—	—	—	—
One dose d4T 3TC NVP								
31	TDF FTC NVP	20.0	57,215	84	TDF LPV/R AZT 3TC	21.64	NA	NA
32	TDF FTC NVP	17.1	179,588	85	TDF LPV/R AZT 3TC	10.39	200	132
TDF FTC EFV								
One dose AZT 3TC EFV								
33	TDF FTC NVP	12.3	532,772	41	TDF LPV/R AZT 3TC	6.97	200	221
34	TDF FTC NVP	11.8	276,990	140	TDF LPV/R AZT 3TC	7.57	NA	NA
35	TDF FTC NVP	11.6	2318	424	—	—	—	—
36‡	d4T 3TC NVP	16.5	175,206	194	LPV/R TDF FTC	17.50	NA	NA
TDF FTC NVP								

(continued on next page)

TABLE 1. (continued) Clinical Characteristics of Patients With the K65R Mutation

Patient	First-line ART Regimen(s) Before ART Failure and First K65R Genotype	Time on ART at First Genotype (m)*	RNA Viral Load at Genotype (copies/mL)	CD4 Count at Genotype (cells/mm ³)	Second-line ART Switch Regimen	Time to Switch† (m)	6-Month RNA Viral Load (copies/mL)	6-month CD4 (cells/mm ³)
37‡	TDF FTC NVP AZT 3TC NVP	15.9	8643	469	—	—	—	—
Median values (range)	—	13.4 (6.4–45.2)	70,469.5 (2318–1,037,171)	84 (4–469)	—	10.5 (1.8–31.3)	200 (200–7918)	221 (90–651)

LPV/RTV, lopinavir/ritonavir; FTC, emtricitabine; SQV, saquinavir.

*Median time between first-ART failure detection and genotype was 3.9 (range 0–26.2) months (no TDF in first ART) and 6.0 (range 0–14.7) months (TDF in first-line ART).

†Time from first ART failure detection and switch to second-line ART.

‡Patients who were excluded from TDF-containing ART vs. non-TDF ART comparative analyses.

Novel NRTI mutation patterns were also detected in our patient cohort with K65R. They included the Q151M complex, the T69 deletion, and K219R and S68G mutations. All of these mutations were significantly more prevalent than among patients without K65R and significantly more prevalent among patients on non-TDF-containing regimens. The Q151M complex, a set of mutations known to confer multi-NRTI resistance, occurred exclusively among patients in this group. Prior studies with HIV-1 subtype B have demonstrated a strong association between K65R and Q151M.^{7,20–22} The combination of K65R and Q151M mutations has also been observed more frequently among patients on non-TDF-containing ART, suggesting that this particular resistance pattern may be linked to multidrug resistance (MDR) more specifically than to TDF.^{23,24} To support these observations, in 23 of 24 patients in our cohort on TDF-containing ART, Q151M was not present.

The T69 deletion was also observed in a significant proportion of our non-TDF-exposed patients (6 of 13; 46.2%). The significance of this unusual combination is unknown. In subtype B, it has been reported to occur in close association with S163I.²⁵ In our patient population, with a G or CRF02_AG background, the T69 deletion appeared in association with K219R. Deletions in the β3–β4 region (codons 62–78) of HIV-1 reverse transcriptase, which confer reduced susceptibility to nucleoside analogs, have been reported previously; however, insertions in this region are more common.²⁶ Deletions have been shown to occur most commonly in association with the Q151M complex.^{25,26} In a recent study, the T69 deletion increased the replicative capacity of HIV-1 variants with a MDR background.²⁷

Twenty-one of 37 patients (56.8%) in our cohort with K65R had the M184V mutation. In most published studies, the M184V has occurred in association with K65R, although other studies have observed that the presence of M184V may actually be protective for the development of K65R.^{13,21} The number of patients in our cohort where this mutation occurred simultaneously with the K65R was notable, even though it was less frequent than in patients without the K65R.

Other common mutations that occurred in our patient cohort included the S68G mutation. A strong correlation between K65R and S68G was observed in a previous study by Trotta et al²¹ and may be one of the steps in the mutational pathway toward Q151M mutation. This may explain why it

was seen more frequently among patients on non-TDF ART; those in whom the Q151M complex mutation also predominated. The mutation, S68G, was also found in high frequency in conjunction with K65R in a study by Boucher et al.²³

An important observation in our analysis was the relative absence of TAMS in most of our patients with the K65R mutation. The prevalence of 2 or more TAMS was significantly higher among patients lacking the K65R mutation compared with those with the K65R mutation. The absence of TAMS with the K65R mutation has been previously reported. In a study by Parikh et al,²⁸ which examined the frequency of K65R in a large genotype database, there was a strong negative association between TAMS and K65R with in vitro confirmation of bidirectional phenotypic antagonism. Recently, the rare association between TAMS and K65R was confirmed in vivo; furthermore, when K65R was present with TAMS, it was only found on the same genome with T215F/Y and ≥2 other TAMS in the presence of Q151M.²⁹

The effect of K65R on responses to second-line regimens is yet to be fully ascertained. An interesting finding among our patients was that all the patients who switched to second-line TDF-containing regimens responded well to therapy at 6 months of follow-up. The successful short-term response to second-line therapy observed among these patients is likely due to the inclusion of AZT in the majority of the second-line regimens to which K65R viruses are exquisitely susceptible and the effect of lopinavir/ritonavir.³⁰ It will be important, however, to determine whether this response is sustained over time.

Finally, a diverse number of HIV-1 subtypes were observed in our patients; however, due to small patient numbers, it is not possible to determine whether there was preferential selection of K65R in one particular subtype or another. Two in vitro studies have demonstrated a preferential selection for K65R among HIV-1 subtype C viruses after exposure to didanosine/d4T¹⁹ and TDF/3TC/didanosine³¹; however, some clinical data do not indicate a higher in vivo selection for K65R.¹¹

There were a number of potential limitations to our analysis. First, our cohort of patients with the K65R mutation was small, which makes it difficult to draw conclusions about the significance of this mutation, its impact on future therapies, and whether patterns of coexisting mutations are significantly

TABLE 2. NRTI Mutations in Patients With the K65R Mutation

Patient	First-line ART Regimen(s) Before ART Failure and First K65R Genotype	Time on ART at First Genotype* (m)	Mutations
No TDF in first-line ART			
1	d4T 3TC NVP	26.9	S68G, K219R, T69del, M41L
2	d4T 3TC NVP	12.1	S68G, V75I, F77L, F116Y, Q151M
3	d4T 3TC NVP	39.5	K219R, T69del
4	AZT 3TC NVP One dose d4T 3TC NVP	6.4	S68G, F77L, F116Y, Q151M T69I, M184V
5	AZT 3TC NVP d4T 3TC NVP	12.4	S68G, F116Y, Q151M M184V
6	d4T 3TC NVP	29.0	S68G, K219R, T69del, V75I, F77L, Q151M
7	d4T 3TC NVP	6.4	K219R, T69del
8	d4T 3TC NVP	8.6	K219R, T69del
9	d4T 3TC NVP	9.5	K219R, T69del, F77L, Q151M V76M
10	d4T 3TC NVP	26.4	S68G, V75I, F77L, F116Y, Q151M
11	d4T 3TC NVP	10.7	S68G, Q151M, M184V
12	d4T 3TC NVP	31.1	S68G, K219E, V76A
13	d4T 3TC NVP	45.2	S68G, F116Y, Q151M, T69I
TDF in first-line ART			
14	TDF FTC NVP	21.8	Y115F, M184V
15	AZT 3TC NVP TDF FTC NVP	18.3	M184V
16	TDF FTC NVP	11.8	S68G, M184V
17	TDF FTC NVP	13.4	M184V
18	TDF FTC NVP One dose d4T 3TC NVP	12.3	M184I
19	TDF FTC NVP	12.2	S68G, K219E, M184V
20	TDF FTC NVP	24.4	Y115F, M184V
21	TDF FTC NVP	24.0	K219Q, M184I
22	TDF FTC NVP	20.5	S68G, Y115F, M184V
23	TDF FTC NVP	12.2	K219E, M184V
24	TDF FTC NVP	12.2	S68G, M184I
25	TDF FTC NVP	11.4	M184V
26	TDF FTC NVP One dose AZT 3TC NVP	12.0	M184V
27	TDF 3TC NVP One dose ABC 3TC NVP	10.7	No additional mutations
28	TDF FTC NVP one dose TDF FTC EFV	18.2	S68G, Y115F, M184V
29	TDF FTC NVP TDF FTC EFV One dose TDF SQV AZT 3TC	14.9	S68G, T69N, M184I
30	TDF FTC NVP One dose d4T 3TC NVP	21.4	A62V, Y115F, M184V

TABLE 2. (continued) NRTI Mutations in Patients With the K65R Mutation

Patient	First-line ART Regimen(s) Before ART Failure and First K65R Genotype	Time on ART at First Genotype* (m)	Mutations
31	TDF FTC NVP	20.0	S68G, A62V, M184V
32	TDF FTC NVP TDF FTC EFV One dose AZT 3TC EFV	17.1	S68G, K219E, M184V
33	TDF FTC NVP	12.3	S68G, A62V, M184V
34	TDF FTC NVP	11.8	A62V, Y115F, M184V
35	TDF FTC NVP	11.6	Y115F, K219E, M184I
36	d4T 3TC NVP TDF FTC NVP	16.5	S68G, F77L, Q151M, V76A, M184V
37	TDF FTC NVP AZT 3TC NVP	15.9	M184V

LPV/RTV, lopinavir/ritonavir; FTC, emtricitabine; SQV, saquinavir.

different compared with those in patients without K65R. Second, we relied on patient recall for details of previous TDF exposure, which may have been inaccurate especially among those who were already on government-provided ART at program enrollment. It is unlikely, however, that any of these patients had received TDF as it was not a recommended antiretroviral at that time, and availability of TDF within Nigeria's private sector was extremely limited. Third, the length of time between the detection of ART failure and when the first genotype was actually performed varied between patients and may have affected the number and type of acquired mutations. It should be noted that in a previous study of resistance mutations associated with similar first-line regimens, higher viral loads at the time of virological failure detection were associated with the K65R mutation.¹² Finally, only 6-month follow-up data were available for patients who switched to second-line ART. A longer duration of follow-up is needed to determine the success of these regimens.

In conclusion, a significantly high rate of K65R was observed among patients infected with predominantly CRF02_AG and G HIV-1 subtypes on first-line ART, in this West African setting. Coexisting NRTI mutations were especially prevalent among patients on non-TDF-containing regimens. The presence of K65R in these situations may limit the success of TDF-containing second-line therapies, particularly in settings where routine genotypic testing is not available and the choice of ART is limited. Because K65R does in fact confer significant resistance to TDF-containing regimens, the rate of development of this drug-resistant mutation may have significant implications for ART international guidelines, where TDF is frequently reserved for second-line use. Further study is therefore needed to assess the exact prevalence of K65R among patients on non-TDF-containing ART in resource-limited settings and their impact on future therapies.

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