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Distribution of HIV Viral Load According Age Group and Sex in Treated HIV Iraqi Patients

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Abstract

Background: Human Immunodeficiency Virus (HIV) infects humans and can lead to acquired immunodeficiency syndrome (AIDS), a condition where the immune system becomes weak, making the body vulnerable to serious infections and cancers. The infection often starts without symptoms, but the virus becomes detectable in the blood over time. Starting treatment and regularly monitoring viral load is essential for maintaining health and preventing transmission, as viral load testing is the most reliable method to assess the efficacy of antiretroviral therapy (ART). **Aim:** The aim of this study was to evaluate the viral load of HIV-infected patients undergoing antiviral therapy in Iraq to observe treatment effectiveness, and to identify the genotype of the HIV-1 strain using Next-Generation Sequencing (NGS). **Methods:** Viral load was measured for 112 HIV-infected patients using real-time PCR. Additionally, NGS analysis was performed on an Iraqi strain to determine its specific subgroup and subtype. **Results:** Demographic analysis showed that 92 patients (82.1%) were males and 20 patients (17.9%) were females, with the largest age group being between 31 and 50 years, accounting for 77 patients (68.8%). Regarding treatment response, a high viral load was maintained in 98 patients (87.5%), while the remaining 14 patients (12.5%) responded successfully to treatment and achieved an undetectable viral load. Furthermore, the duration of treatment revealed a positive correlation with viral load levels, and NGS results officially confirmed the infection of HIV-1, subgroup M, subtype B in Iraq. **Conclusion:** A high proportion of HIV-1 infected patients in Iraq showed elevated viral loads, indicating either recent infection, poor treatment adherence, or drug resistance. Additionally, the HIV-1 subgroup M, subtype B genotype is confirmed in Iraq, and the outcomes appear to be age-dependent rather than gender-dependent.

Keywords: Human Immunodeficiency Virus (HIV-1), Next-Generation Sequencing (NGS), Antiretroviral therapy (ART).
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Introduction

HIV/AIDS is without doubt the worst epidemic to hit humankind since the Black Death. As of 2004 an estimated 40 million people were living with the disease, and about 20 million had died (1). Despite rapid scientific advances there is still no cure and the drugs are expensive and toxic (2). In the developing world, The HIV/AIDS epidemic has raised unique challenges. Total health care expenditure is low in comparison to developed countries. The overwhelming effect of infectious diseases, malnutrition, and inadequate education, singularly and in combination seriously limit the capacity of such countries to deal with HIV/AIDS with effective long-term strategies (3). The overall prevalence of HIV infection varies from 5-20% depending on the age group, stage of the epidemic, and relative efficiency of prevention strategies (4). Iraq can be characterized as a low-prevalence HIV epidemic, with a low number of officially reported HIV cases. The Iraqi Parliamentary Health and Environment Committee has reported 2,638 HIV/AIDS cases, including 470 deaths in recent years. The committee, suggests the actual number may be higher due to the social stigma associated with the disease, deterring many from seeking hospital treatment (5).

One of the most useful indicators test for predicting HIV progression is Viral load, determining when anti-HIV treatment is necessary, and assessing how well it is working, along with CD4 cell count (6). The presence of viral RNA is a sign that a virus is actively replicating, or multiplying. Viral loads below 10,000 copies/ml are regarded as low,

while those 100,000 copies/ml and higher are considered high. Higher viral loads have been repeatedly linked to faster HIV disease progression and a higher risk of death, according to research(7) (8) .

Material and Methods

Study subjects: A total of 112 patients (92 were males and 20 were females) (20 (17.90%) aged ≤ 30 years, 78, 69.6% ranging from to 31-50 years and 14, 17.9% ranging from 51≥ years) infected with HIV-1 and treated with antiviral drugs were included in this study, they were referred for the diagnosis and treatment to Ibn Zuhur Hospital and AL- Karama Hospital in Baghdad during the period from 1-6-2023 to 1-5-2024. Anti-HIV antibodies were detected in their sera by ELISA technique and confirmed by western blot test and real-time PCR analyses to expose the viral genetic material. The patients received antiviral (Trinistem) treatment for different durations. This investigation with the diagnosis was done by the advisor medical staff at the previous hospital. Data were collected from each patient using a special form, and all participants provided informed consent before enrollment in the study, which was approved by the Biology Department, College of Science, University of Baghdad Ethics Committee.

Sample collection: 5 ml of blood sample was collected in EDTA blood collection tubes from the patients. The plasma was separated by centrifugation at 3000 rpm/min. And frozen at −20°C until use for RNA extraction.

Anti-HIV Ab detection

HIV ELISA kit (Biocompare/USA) was used for HIV antibodies detection.

HIV confirm test

The second assay used to confirm a positive HIV ELISA result is the Western Blot. THE Biotechnology education company/USA was used.

Extraction of HIV Viral RNA

HIV viral RNA was extracted and purification from 200 µL of plasma using a specialized viral nucleic acid extraction kit III (Geneaid, Taiwan) according to manufacturer instructions.

Viral load detection

Real Best HIV PCR" reagent kit (Vector Best/ Russia) for the detection and quantification of HIV- RNA in plasma using reverse transcription of pathogen's RNA with subsequent cDNA amplification by real-time polymerase chain reaction (RT-PCR) with fluorescence detection. 50 µl of positive control, calibrations, internal control and plasma were added to all the tubes according to manufacturer instructions and placed in the thermal cycler for qPCR. the reaction conditions were as follows; enzyme activation at 45˚C for 30 min for 1 cycle, Initial denaturation at 94˚C for 1 min for 1 cycle, after those 50 cycles of PCR were performed. Each cycle consisted of denaturation at 94˚C for 10 Sec., and annealing elongation at 60˚C for 20 Sec. The viral load quantification after a period in which the therapy is considered effective. The undetected value is recommended as an effective form of treatment (46).

Next generation sequencing (NGS) method of HIV genome

Next generation sequencing was used for the first time in Iraq to analyzed HIV-1 isolates from Iraqi HIV positive patients as a confirmatory method in this study as follows; RNA was extracted from 4 HIV positive samples (with high viral load) and converted to cDNA, then sent for Macrogen company/ Kore for sequence.

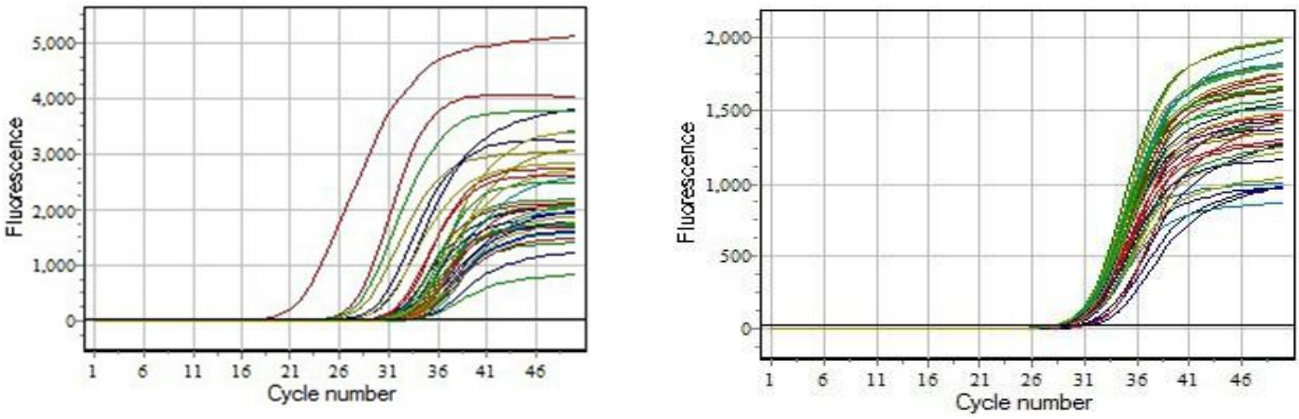
Statistical analysis

All statistical analyses were performed using statistical package for social science (SPSS) program 25 for windows (SPSS INC., Chicago IL, USA). Data were expressed as mean ± SD. Comparisons between two groups were performed using T test for categorical data. P value of <0.05 were considered to indicate statistical significance.

Results

In the present study, the plasma samples were collected from 245 susceptible Iraqi HIV infected patients. 173 (70.6%) were positive for HIV Ab by ELISA assay. However, 112 (64.7%) Iraqi HIV infected patients out of a total 137 patients were confirmed by western blot. 92 (82.1%) were males and 20 (17.9 %) were females out of a total 112 patients. HIV infected patient ages were divided into three age groups; first one (10-30) years which represented by 21 (18.8%) patients, the second age group (31-50) years included the largest number of patients 77 (68.8%) and the third age group (51-70) years included 14 (12.4 %) patients. The primary routes of HIV/AIDS transmission were sexual contact 80 (71.42%) patients, homosexual routes (12.3%) and (16.4%) other routs (surgery and transmission from mothers to their infants). most of the samples had a high viral load 98 (87.5 %) with the exception of a small number of those who responded to treatment, and their viral load was undetectable 14 (12.5%), as shown in Figure 1. Positive viral load patients were divided based on the viral copies/ ml into 4 groups. The first group A included 57 (58.2%) patients which their viral load ranged from 1000-5000 copies/ml, while the second B group included 14(14.3%) patients which their viral load ranged from 5001-9000 copies/ml, third group C included 12(12.2%) patients which their viral load ranged from 9001-13000 copies/ml, and the fourth group D included 15(13.3%) patients which their viral load ranged from 13001-17000 copies/ml, as shown in Figure 2.

Figure-1: The viral load of infected people was evaluated using real-time PCR, ROX CT peaks or HIV-1 viral load (A), FAM CT peaks for internal control (B)



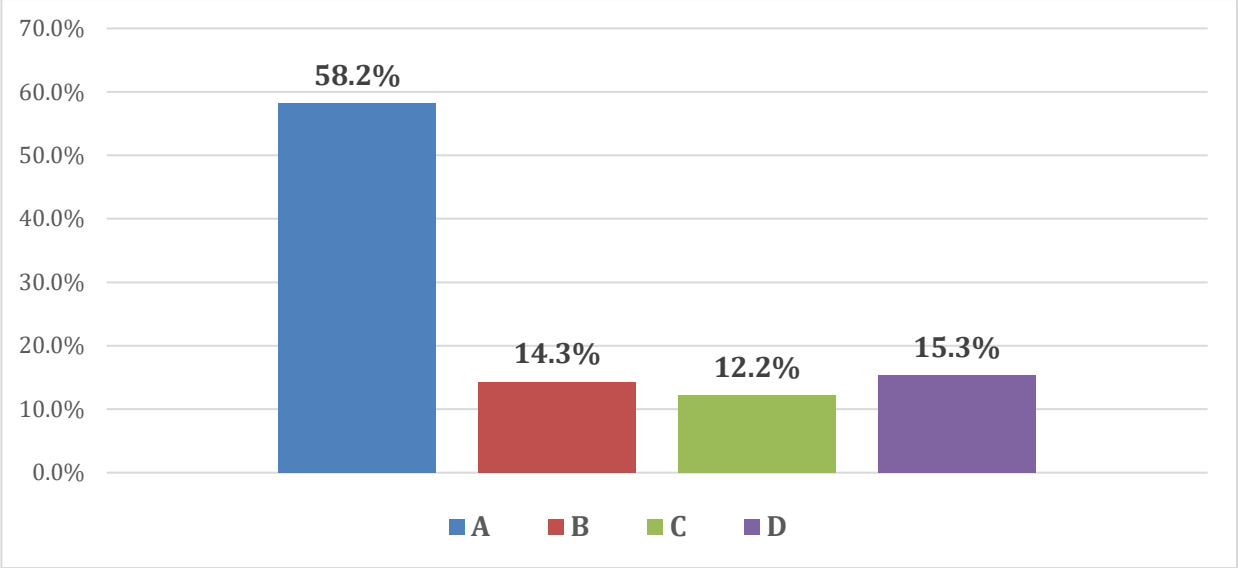


Figure 2: Distribution of positive viral load patients according to concentration of viral copies /ml.

In the present study, there is no statistically effect (Pearson Chi-Square = 3.650, P-Value = 0.455) of sex on the viral load, However, Age groups showed statistically significant (Pearson Chi-Square = 10.078 P-Value = 0.040) effect on viral load; the highest 78 (69.6%) infection rate (viral load) in HIV patients with 31-50 years age group, while the lowest infection rate 16 (14.28%) was within the 51-70 years age group, and 18 (16.07%) within 10-30 years the age group, as shown in figure 3and 4

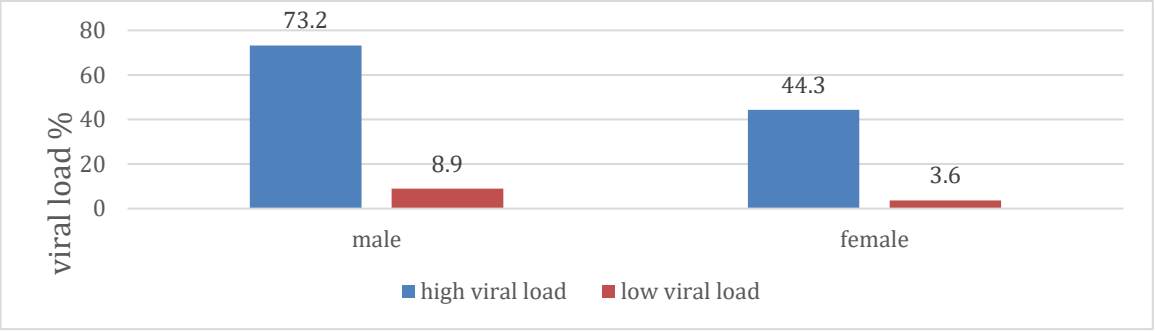


Figure 3: Distribution of viral load according to sex.

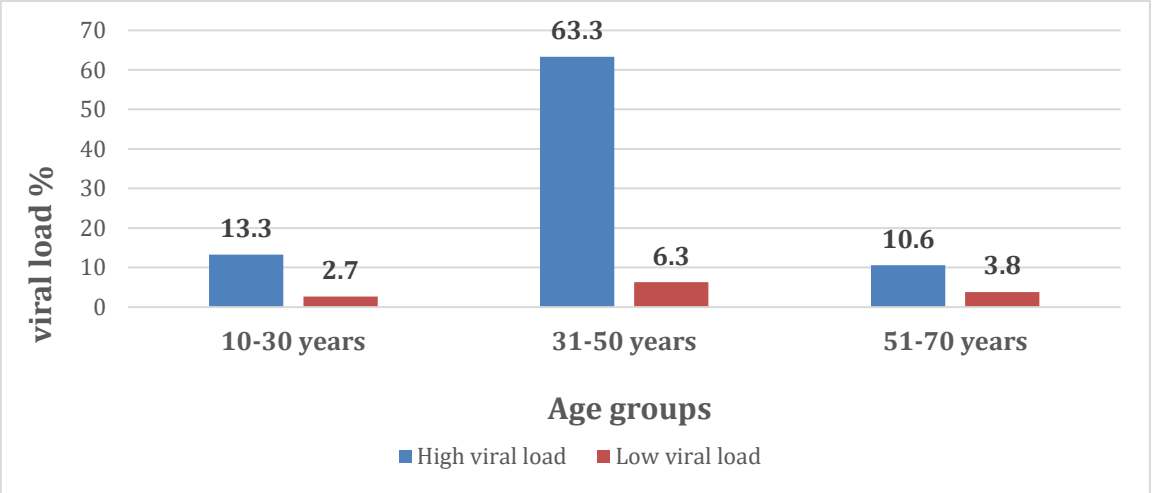
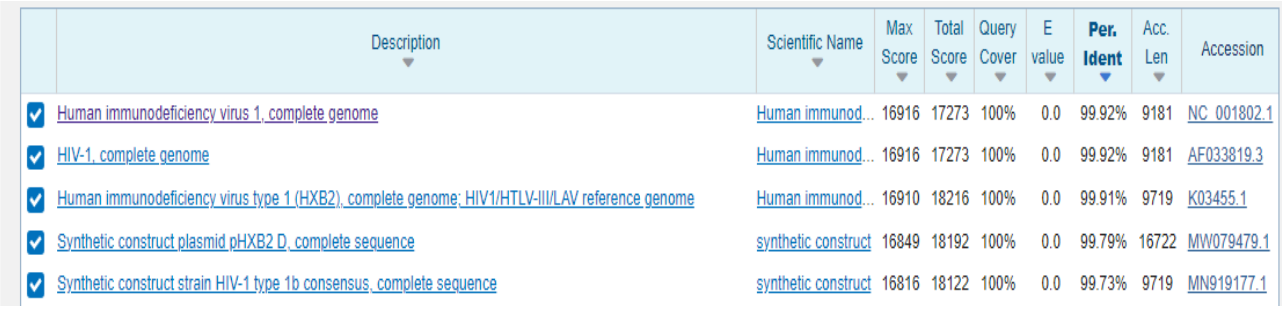


Figure 4: Distribution of viral load according to age group.

The viral load showed significant variation between the HIV infected patients in this study, this vibration related significantly (Pearson Chi-Square = 11.668, P-Value = 0.047) to the treatment (all 112 patients take the same dose of the same medicine in different duration). As well as the medicine uptake revealed positive correlation (r= 0.485) with duration of treatment. the HIV patients divided to 3 groups according to medicine uptake; The first group included 87 (77.7%) patients who uptake the treatment for less than 1- less than 2 years, most of them have high viral load. while the second group included 13 (11.6%) patients who uptake the treatment for a period from 2 –3 years, and the third group included 12 (10.7%) patients who uptake the treatment for a period more than 3 years, most of them with low viral load. The genotype of the sample analyzed by NGS was HIV-1, subgroup M, and subtype B which is the first time recorded in Iraq. This sample matched several samples in the gene bank, as shown in Picture 5.



Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Human immunodeficiency virus 1, complete genome	Human immunod...	16916	17273	100%	0.0	99.92%	9181	NC_001802.1
HIV-1, complete genome	Human immunod...	16916	17273	100%	0.0	99.92%	9181	AF033819.3
Human immunodeficiency virus type 1 (HXB2), complete genome; HIV1/HTLV-III/LAV reference genome	Human immunod...	16910	18216	100%	0.0	99.91%	9719	K03455.1
Synthetic construct plasmid pHXB2 D, complete sequence	synthetic construct	16849	18192	100%	0.0	99.79%	16722	MW079479.1
Synthetic construct strain HIV-1 type 1b consensus, complete sequence	synthetic construct	16816	18122	100%	0.0	99.73%	9719	MN919177.1

Figure 5: sequenced Iraqi strain by NGS was matched with gene bank after the results were analyzed using the electronic program (https://hivdb.stanford.edu/hivdb/by-sequences/report/). The results showed that there was no drug-resistance mutation in this sample.

Desiccation

The HIV infection rate of males in Iraq higher than females in this study may be due to that male have higher rates of risky sexual behaviors than females, such as having a greater number of sexual partners or having unprotected intercourse. On the other hand, the reasons may be due to Iraq's conservative social structure; make females afraid to reach HIV treatment centers. As well as, the presence of a stigma imposed on women infected with HIV may lead them to prefer remaining without treatment rather than have their health status exposed. In Iraq, like other countries, most of the HIV-infected patients from sexually active ages (20-50 years old) as well as the fact that these ages are physically empowered as mixing and traveling which are an important reason for the transmission. In the last decade, there is an increasing number of older HIV infected patients either because of new HIV diagnosis in the older population or because they have acquired HIV infection in young age and in recent time become older due to Highly Active Antiretroviral Therapy (HAART) improved survival.(9) Several previous studies from Iraq reported that the HIV infected cases were most frequent within the age group more than 20 years and less 50 years, with the bulk of cases occurring up to the age of below 40 years (10,11,12,13,14) which were agreed with results of this study as well as, in 2021, a study in Baghdad province reported that HIV infection was more frequent in < 20 to ≥ 50 years age groups (15). In 1986, the first cases of HIV infection recorded in Iraq by the Ministry of Health to hemophiliac patients, and the route of transmission by blood product contamination, and remained the main route of infection to 2003. After that, the transmission route of HIV changed

in Iraq and the sexual transmission subsequently became common in new cases, particularly among males traveling abroad and engaging in unprotected sex. Several studies in Iraq have indicated that sexual transmission is the most common mode of HIV infection (13, 15, 16, 17). The most reliable method for tracking how well HIV antiretroviral therapy (ART) is working is viral load testing. Data from a variety of settings indicate that. while access to ART and viral load monitoring has expanded, a small percentage of HIV-positive individuals receiving ART have detectable viral loads that fall below the threshold for virological failure (i.e., 1000 copies/mL) (18,19,20). Multiple reasons that lead to a high viral load; the most important one is drug-resistant mutations. Ansah (21) found that 23.6% of patients undergoing antiretroviral therapy had a high viral load, while the viral suppression rate was (76.4%). Most resource-constrained environments do not currently allow for viral load monitoring, however low-cost and reliable methods for measuring viral load that will work in these environments in the future are being developed (22,23). This study was consistent with Mosha, (24) who found that after a year of follow-up, there was no statistical significance between of males and females in the level of the viral load. And agreed with Wakooko (25), who revealed that sex was not associated with viral load in HIV positive patients. While Nkambule and Huang, (26) found that males have a higher rate of viral load than females and explained that by the negative impact of the work situation on the possibility of obtaining HIV care. Work status partially explained the relationship between gender (male) and access to HIV care. Lack of time is a possible explanation for this. But Ansah (21) found Male patients had a 1.3-fold higher likelihood of viral load suppression than female patients. Research from other African nations, such as Ethiopia, (27) Nigeria, (28) and Swaziland (29), were in line with these findings. According to published research, women may have been less likely to experience virological failure because of their propensity for seeking health care (29,30). Analyzing the gender disparity in the course of other viral diseases, such as COVID-19, also showed that male patients died at a higher rate than female patients, pointing to the protective effect of estrogen (31). The highest infection rate was for people aged 31-50 years in this study, can be explained by the increased social, sexual, and international travel that this age group engages in; these factors taken together may raise the risk of HIV exposure. This study was consistent with (32), as the highest infection rate was for the age group between 25-49 years. The results also agreed with another study in Iraq, where a higher percentage of HIV infections was among young people in the age group of 20–39 years (60.3%) (33). In addition, a study in Iraq conducted by (34) found that the percentage of adults (≥15 years) was 98.2%. It matches (35) she found that the highest infection rate is in the age group of 31-40 years, and the lowest infection age group is 11-20 years. This study not agree with (36) study in Nigeria who reported that age-specific distribution of HIV highest prevalence (7.1%) of HIV-related antibodies was found in the 10–20 age group, while the lowest prevalence (4.0%) was found in the 41–50 age group. These findings are consistent with previous reports from (37) and (38) who also found that HIV is more common in younger patients. Although the duration of treatment has an effect on the viral load, the rate of increase in the viral load is considered high compared to the fact that people are taking the treatment in this study. This may be due to non-adherence to the treatment or the lack of permanent availability of treatment due to the country's policy. HIV positive patients who got their infection from inside Iraq showed that after taking the treatment had a higher viral load than before taking it, this is may by the strains inside Iraq are more virulent and resistant to treatment compared with people who got the infection from Outside Iraq, who responded to treatment was better may

be this can be attributed to the old treatment methods followed in Iraq. The use of earliest treatment techniques increases treatment failure and hastens the emergence of HIV strains that are resistant to treatment, as government centers in Iraq do not offer quadruple therapy (35). Since the selection of antiretroviral therapy (ART) is primarily based on HIV genetic drug resistance testing, Sanger sequencing (SS) has been the standard test for genotype drug resistant test (GRT) for the past two decades (39). However, Sanger sequencing, due to its low sensitivity, generally fails to detect low-abundance drug-resistant HIV-1 variants (LA-DRVs), which are present at frequencies below 15–20% within the viral quasispecies (40). However, NGS particularly Illumina sequencing, is gradually replacing SS as the primary method for genotypic resistance testing (41). NGS has been found to be capable of detecting both high- and low-abundance drug resistance mutations and has proven effective in identifying HIV-1 drug resistance mutations (42). Furthermore, several studies have shown that NGS-GRT is highly concordant with Sanger sequencing at a threshold of 20% (43). For these reasons, NGS-GRT is gradually replacing Sanger sequencing. In this context, virology laboratories are making significant efforts to adapt their sequencing procedures to new technologies and interpret next-generation sequencing (NGS) data to provide clear clinical reporting (44). Unfortunately, one sample only can be analyzed in this study, while the rest 3 samples cannot may be due to transportation conditions, purification and storage conditions of extracted RNA, and other conditions related to the company implementing the sequencing. Previous research indicates that the genotype found in Iraq is HIV-1, subgroup M, subtype A1 (10). Ouyang found that next-generation sequencing facilitates a more sensitive detection of HIV-1 drug resistance than Sanger sequencing. The high prevalence of pretreatment HIV drug resistance emphasizes the importance of baseline resistance and assessing the threshold for optimal clinical detection using NGS (45).

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