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Immunovirological Evaluation of Triple Antiretroviral Therapy Tenofovir/Lamivudine/Dolutegravir (TLD) in HIV/HBV Co-Infected Patients in N'Djamena, Chad

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Abstract

HIV remains a major global public health problem. HIV infection alters the natural history of HBV and worsens the prognosis for patients with chronic hepatitis B. Since the advent of ARVs, chronic infection with the forgotten hepatitis B virus (HBV) has become relevant again in the population of people living with HIV (PLWH). Viral load plays a crucial role in patient classification and treatment initiation. This is a prospective longitudinal cohort; patient recruitment took place at the start of antiretroviral treatment (at treatment initiation), and the study lasted 6 months, from July 2025 to January 2026. The study population consisted of male and female patients over 18 years of age. It was carried out in patients co-infected with HIV/HBV and placed on TLD triple antiretroviral therapy. We included in the study, all Patients co-infected with HIV/HBV eligible for antiretroviral treatment of both sexes, followed at the CHU-RN, CHU-ATC and APMS. The VISITECT CD4 Advanced Disease rapid test is an immunochromatographic assay that estimates full length CD4 protein associated with CD4+ T cells in human whole blood, and is directly correlated with CD4+ T cells levels. For the viral loads of HIV RNA and HBV

DNA we used the BIOCENTRIC device. The extraction technique used was that recommended by BIOCENTRIC. For amplification, the main components of the Generic HIV viral loads and Generic HBV viral loads amplification Kits (BIOCENTRIC) were used. In total, 129 patients were included in the study, of whom 59.7% were women, i.e. a sex ratio of 0.67. The average age was 35 (±2) years (p = 0.036). Regarding the clinical stages, about 35.7% were at clinical stage I, 40.3% were at clinical stage II, and 20.2% were at clinical stage III. Regarding HBV DNA detectability, 97.7% were positive for detectable HBV DNA. The failure rate of CD4+ T lymphocytes ≤ 200 cells/µL was 65.9% at the start of antiretroviral treatment initiation at (M0); after 6 months (M6) of treatment the level of CD4 T Lymphocytes ≤ 200 cells/µL was 16.3%. The viral load RNA HIV was high (≥ 1000 copies/ml) at M0 (62.8%) followed by 13.2% after 3 months of treatment and 3.9% after 6 months of treatment in the population studied. The majority of participants in the study exhibited encouraging outcomes regarding viral load, with 6.2% viral loads below 1000 copies/ml at sixth month. The HBV DNA viral load is high ≥ 2000 IU/mL in 52.7% at the initiation of treatment at zero months (M0); after 3 months of treatment 7.0% and after 6 months of treatment 4.9%. The therapeutic regimen studied presents an efficacy profile and constitutes a relevant first-line option in the management of HIV/HBV co-infected patients in Chad. Nevertheless, prolonged longitudinal follow-up remains necessary in order to assess the durability of the virological response and the long-term evolution of hepatic parameters.

Keywords

Evaluation, Viral Loads, CD4, TLD, Co-Infection, HIV, HBV, N'Djamena, Chad

1. Introduction

HIV remains a major global public health problem, having killed an estimated 44.1 million people to date [1]. An estimated 40.8 million people were living with HIV at the end of 2024, 65% of them in the WHO African Region [1].

The abbreviation HIV stands for human immunodeficiency virus. It is a retrovirus that infects cells of the human immune system (particularly CD4+ T lymphocytes and macrophage cells essential to the cellular immune system) [2].

Hepatitis B constitutes a major public health problem. The WHO estimates that 254 million people were living with chronic hepatitis B in 2022 and there are 1.2 million new infections each year [3].

The 95-95-95 intermediate targets were put in place in 2020 to renew efforts to achieve the Sustainable Development Goals. These interim targets for HIV indicate that by 2025: 95% of people living with HIV know their HIV status; 95% of people living with HIV who know their HIV status are on treatment and 95% of people living with HIV on treatment have a suppressed viral load [4].

Antiretroviral treatment (ART) must be started as early as possible, as soon as

detected, as soon as treated, but the most appropriate moment must be assessed individually, taking into account the most beneficial combination for the patient and weighing the advantages and disadvantages. At the end of 2022, the treatment success rate among people living with HIV on ART was 71% [5].

A viral load is an indicator of how much Human Immunodeficiency Virus (HIV) is in the blood of an individual. Viral load gives an idea of how much of the HIV virus is in the patient's body. The test measures the number of HIV copies in a milliliter of blood [6]. Viral load also predicts how fast the disease progresses [7].

Next to viral load, an essential parameter to evaluate immunosuppression and monitor antiretroviral treatment (ART) response in people living with HIV (PLHIV) is the cluster of differentiation 4 (CD4) cell count. Advanced HIV disease (AHD, defined as a CD4 cell count of <200 cells/mL or meeting the criteria for WHO Stage 3 or 4 [8].

HIV infection alters the natural history of HBV and worsens the prognosis for patients with chronic hepatitis B. It is associated with an increased risk of acute hepatitis B becoming chronic, thereby accelerating the progression of lesions such as fibrosis and the risk of developing complications such as cirrhosis and hepatocellular carcinoma [9].

Patients were considered HIV/HBV co-infected if they had documented HIV-1 seropositivity and HBsAg positivity or, failing that, HBV DNA detectability. Baseline ART status was defined as naïve (no prior treatment) [10].

In N'Djamena, Chad, according to a study conducted at three HIV treatment centres (APMS, CHU-RN and CHU-ATC), the prevalence of HIV/HBV co-infection was 6% [11].

In cases of HIV/HBV co-infection in adults, adolescents, and children aged 3 years or older co-infected with HIV and HBV, the fixed-dose combination of Tenofovir + Lamivudine + Dolutegravir is the preferred option for starting triple antiretroviral therapy [12].

Since the advent of ARVs, chronic infection with the forgotten hepatitis B virus (HBV) has become relevant again in the population of people living with HIV (PLHIV). Coinfection with HBV in the latter involves reactivation of HBV, due to the alteration of immunity induced by HIV infection [13]. Virological success is defined by reduced viral load or complete non-detectability depending on the sensitivity of the detection method. Highly sensitive real-time PCR assays now provide reliable quantification of serum HBV DNA. Measurement of HBV DNA is essential in the management of chronic HBV infection [14].

The objective of this study is to evaluate the immuno-virological effectiveness of triple antiretroviral therapy based on TLD in HIV/HBV co-infected patients in N'Djamena, Chad.

2. Materials and Methods

2.1. Study Framework

The study was conducted in N'Djamena at three HIV and hepatitis treatment centers, including the Psycho-Medical-Social Support Center (APMS), the Chad-

China Friendship University Hospital Center (CHU-ATC), and the National Reference Hospital Center (CHU-RN). Laboratory analyses were carried out at the APMS Laboratory, which is the laboratory for the sectorial program to fight AIDS and hepatitis in Chad.

2.2. Type and Period of Study

This is a prospective longitudinal cohort, patient recruitment took place at the start of antiretroviral treatment ART (at treatment ART initiation), and the study lasted 6 months, from July 2025 to January 2026.

2.3. Study Population

The study population consisted of male and female patients over 18 years of age. All patients gave their consent after being informed of the objectives of the study and its importance. The study population consisted of patients suffering from HIV/HBV co-infection and followed in the three treatment sites (CHU-RN, CHU-ATC and APMS) for examinations of viral loads HIV-1, viral loads HBV and CD4+ T cells.

2.4. Inclusion Criteria

We included in the study, all Patients co-infected with HIV/HBV eligible for antiretroviral treatment of both sexes, followed at the CHU-RN, CHU-ATC and APMS. These patients were receiving on triple antiretroviral therapy based on Tenofovir + Lamivudine + Dolutegravir (TLD) and were followed for 6 months. All patients who agreed to participate in the study.

2.5. Exclusion Criteria

Children, and patients who are not willing or unable to take part in the study.

2.6. Variables Studied

The variables recorded in the data collection form include sociodemographic data (age, gender, marital status, educational level and occupation); stage HIV; immunological (CD4+ T Cells) and virological (virals loads HIV-1 RNA and HBV-DNA). A sample of venous blood was collected on EDTA two tubes of 4 ml each. Both tubes are agitated gently to mix the blood with the anticoagulant. The blood is centrifuged for 10 min at 2000 tg. Plasma was aliquoted into 3 cryo tubes of 2 ml (2 - 3 cryotubes were used according to the volume of plasma).

2.6.1. Amplification Test for Quantification of HIV-1 RNA

1) Principle of the test: The GHIV-CV test is based on the principle of real-time RT-PCR, by the hydrolysis of an oligonucleotide detection probe labeled at 5' with an emitting fluorophore ("reporter") and at 3' with a non-fluorescent suppressor group ("quencher") [15].

2) Precautions for preparing reagents

a) Standards and controls: The 5 standards and the 2 controls can be refrozen after first use, thawed then used a second time without loss of activity [15]. After the first use, it is recommended to prepare 250 µL aliquots of the 5 standards and the 2 controls in sterile nuclease-free microtubes. Recap the tubes with the appropriate caps and label each tube with the reagent name, lot number and expiration date [15].

b) IC Reagents: IC components are supplied in lyophilized form and must be suspended before first use [15].

3) Preparation of proteinase K

Equilibrate proteinase K and its suspension buffer at +15˚C/+25˚C; a) Add 1 mL of buffer to the proteinase K tube; b) Close the tube. Gently shake the proteinase K suspension tube then vortex it for 5 to 6 seconds; c) Let stand for 5 minutes at room temperature; d) Store the proteinase K solution recommended by Biocentric (concentration 100 mg/mL) at −30˚C/−18˚C; e) After thawing, carefully check for cloudiness before pipetting [15].

4) Automated extraction of retroviral RNA: The GXT NA Extraction kit comes in the form of a 12-well cartridge allowing complete automation of the extraction of retroviral RNA from plasma samples, exclusively by the Arrow or GenoXtract machine [15].

a) Processing of plasma samples, standards and controls for nucleic acid extraction

Equilibrate the samples, the 5 standards, the 2 controls, the IC RNA and the proteinase K solution at +15˚C/+25˚C; Add 10 µL of proteinase K solution to a microtube (1.5 or 2 mL); Add 250 µL of plasma sample to be extracted; Vortex for 5 seconds; Incubate at +15˚C/+25˚C for 10 min [15].

b) Using IC RNA with GXT NA Extraction Cartridges

Load the GXT NA Extraction cartridges into the automatic extraction machine and pierce the cartridge cover using the perforator. Carefully add 2 µL of IC RNA Reagent to the first well (front) of each GXT NA Extraction cartridge used for extraction of nucleic acids from standards, controls, and test samples [15].

c) Automated RNA extraction procedure

Assemble the pumps and the tips, then load the assembly into the automatic extraction machine; Load the GXT NA Extraction cartridges into the extraction machine and pierce the cartridge cover using the perforator; Place each sample tube and elution tube in the appropriate locations on the extraction machine; Close the lid of the automaton and launch the extraction program Generic250_NA_VN.0; At the end of the extraction, the retroviral RNA obtained must be stored at +2˚C/+8˚C if it is tested the same day, or stored at −80˚C/−60˚C if it is tested subsequently [15].

5) PCR amplification

a) Preparation of the reaction mixture

Thaw the reagents at +15˚C/+25˚C (**Table 1**). In a sterile 1.5 mL nuclease-free

microtube, prepare the reaction mixture for N samples. For N samples, multiply the volumes of reagents indicated below by "N + 4" [15]:

Table 1. Preparation of the reaction mixture (1).

Reagents (molecular biology quality)	Volume (µL)
H_2O without nuclease	3.0
Mix enzymatique 4×	5.0
Primers A	0.5
Primers B	0.5
Probe C	0.5
IC primers/probe-Cy5	0.5
Volume reaction total	10.0

b) Preparation of the PCR microplate

Homogenize the reaction mixture using a vortex mixer. Eliminate droplets on the walls of the tube by centrifuging briefly; i) Distribute 10 µL of the prepared reaction mixture into a PCR microplate; ii) Vortex, for at least 5 seconds, the eluates of the standards, controls and patient samples; iii) Distribute 10 µL of eluate or water into each well of the microplate; iv) Carefully cover the PCR microplate with a suitable adhesive or heat-sealable film; v) Remove air bubbles by centrifuging the microplate for 10 seconds [15].

c) Running real-time PCR from "open" thermal cyclers

Load the sealed microplate into the real-time PCR instrument. Perform RT-PCR using the following amplification program (**Table 2**) [15].

Table 2. Amplification program.

Temperature	Duration	Stage	Amplification
50˚C	10 minutes	Reverse transcription	50 cycles
95˚C	5 minutes	Enzyme activation	
95˚C	15 seconds	Denaturation	
60˚C	1 minute	Hybridation	

Edit for each well of the microplate the "reporter" fluorophores (FAM and Cy5 for the HIV-1 target and the IC respectively), the identification of the sample, the number of replicates and finally the concentration for each of the 5 HIV-1 standards, i.e. in copies/mL. Depending on the unit chosen for the standards, the viral load results will be expressed either in copies/mL [15].

2.6.2. Nucleic Acid Amplification Test for HBV Quantification

1) Principle of the test: The GHBV-CV v2.0 test is based on the principle of real-time quantitative PCR with the use of an oligonucleotide detection hydrolysis

probe labeled at 5' with an emitting fluorophore ("reporter"), and at 3' with a non-fluorescent suppressor group ("quencher") [16].

2) Processing of plasma samples, standards and controls for nucleic acid extraction

Equilibrate the samples, the four standards, the two controls, the IC DNA and the proteinase K solution at +15˚C/+25˚C. Prepare a proteinase K-IC DNA mixture for NEXT extractions. For NEXT extractions, multiply the volumes of reagents indicated below by "NEXT + 4" [16] (**Tables 3-5**).

Table 3. Preparation of the proteinase K-IC DNA.

Reagents	Volume (µL)
Proteinase K	10
IC DNA	2
Volume total	12

3) Preparation of the reaction mixture: Thaw the "5× Enzyme Mix" and "Mix Probe Primers" reagents at +15˚C/+25˚C. In a 1.5 mL nuclease-free microtube, prepare the reaction mixture for NPCR PCR assays. For NPCR, multiply the reagent volumes indicated below by "NPCR + 4" [16]:

Table 4. Preparation of the reaction mixture (2).

Reagents (molecular biology quality)	Volume (µL)
H_2O without nuclease	3.0
Enzyme mix 5×	4.0
Mixed probe primers	3.0
Total reaction volume	10.0

4) Running real-time PCR from "open" thermal

Load the sealed microplate into the real-time PCR instrument. Perform real-time PCR using the following amplification program [16]:

Table 5. Amplification program.

Temperature	Duration	Stage	Amplification
50˚C	15 minutes	-	50 cycles
95˚C	10 minutes	Enzyme activation	
95˚C	15 seconds	Denaturation	
60˚C	1 minute	Hybridation	

2.6.3. VISITECT CD4 Advanced Disease

Principle of the test: The VISITECT CD4 Advanced Disease rapid test is an im-

munochromatographic assay that estimates full length CD4 protein associated with CD4+ T cells in human whole blood, and is directly correlated with CD4+ Tcell levels [17]. A capture monoclonal antibody (MAb) specific for the cytoplasmic domain of CD4 is applied as a line on the nitrocellulose membrane [17]. These complexes are visualized as a pink/purple line. A Reference line (200 line) is include to allow estimation of CD4 levels by comparison to a set cut-off (equivalent to the signal level generated by specimens containing 200 CD4+ T cells/μL). The control line, located in the results window marked "C" [17]. The Reference line, located in the results window marked "200" exhibits an intensity approximately equal to the seen whit a specimen containing 200 CD4+ T cells/μL. The line, located in the results window marked "T" exhibits an intensity that correlates to the number of CD4+ cells in the specimen. The test results interpreted by comparing the intensity of the test (T) line with the reference (200) line. If the (T) line has equalor weaker intensity than the Reference (200) line, the test result is "Below Reference" (≤200 CD4+ T cells/μL) [17]. If the (T) line has stronger intensity than the reference (200) line, the test result is "Above Reference" (>200 CD4+ T cells/μL) [17].

The Procedure: Touch the center of well A lightly and squeeze the bulb of the sampling device/depress the pipette plunger gently to ensure the full 30 μL specimen is released into well A [17]. Discard the sampling device/disposable tip a sharps/biohazard bin. Wait for 3 minutes. Hold the buffer bottle vertically 1 cm above well A. Add 1 drop of buffer to well A where the blood has been added. Wait for 17 minutes. Hold the buffer bottle vertically 1 cm well B [17]. Carefully add 3 drops of buffer to well B allowing each drop to absorb into the well before adding the next drop. Wait for 20 minutes. After the test is complete, interpret the results within 5 minutes [17].

Quality control: There is no quality control standard available, however it is recommended that a specimen greater than 200 CD4+ T cells/ μL (characterized by flow cytometry) is run and the pink/purple lines are visible. It is recommended that a control is run on a regular basis according to local guidelines [17].

2.7. Sampling

This was a cohort study with consecutive non-probability sampling including all HIV-HBV co-infected patients followed in the three treatment sites during the study period.

2.8. Data Analysis

The data were coded, entered, and cleaned were recorded in Excel 2016, exported, and analyzed using IBM SPSS 26 software for analysis. Descriptive statistics, including means and frequency tables, were used to describe the characteristics of the study participants. A difference of $p < 0.05$ was considered significant.

3. Results

A total of 129 HIV-HBV co-infected patients were included during the study pe-

riod. They were aged 18 to 65, the average age was 35 (±2) years. Women predominated with 59.7%, the sex ratio was 0.67. The majority of patients were housewives 30.2% followed by other jobs 20.2%. Married people had 39.5% followed by singles 25.6%. Educational backgrounds varied, with 29.5% of participants not in school and write, 15.5% completing primary education, 30.2% having a secondary school education and 24.8% completing Higher education (Table 6).

Table 6. Summary of sociodemographic characteristics of the patients.

Variable	Workforce (n)	Percentage (95% CI)
Sex		
Male	52	40.3
Female	77	59.7
Age Group (years)		
[18 - 25[	32	24.8
[26 - 35[	49	38.0
[36 - 45[	36	27.9
[46 - 55[	8	6.2
[56 - 65]	4	3.1
Marital Status		
Married	51	39.5
Single	33	25.6
Divorced	29	22.5
Widowed	16	12.4
Educational Level		
Higher	32	24.8
Secondary	39	30.2
Primary	20	15.5
Not in school	38	29.5
Occupation		
Student	8	6.2
Pupil	2	1.6
Civil servant	13	10.1
Military personnel	9	7.0
Merchant	19	14.7
Housewife	39	30.2
Unemployed	13	10.1
Other	26	20.2

Continued

Stage HIV		
I	46	35.7
II	52	40.3
III	26	20.2
IV	5	3.9

Regarding the clinical stages, about 35.7% were at clinical stage I, 40.3% were at clinical stage II, 20.2% were at clinical stage III, and the rest were at clinical stage IV, 3.9% (Table 6).

As indicated in Table 7 presented the frequency of the age group according to sex of which 30 women are in the age group of 26 - 35 years old followed by 25 women between 18 - 25 years old while 19 men are aged between 26 - 35 years old and 19 others between 36 - 45 years old.

Table 7. Range of age within the sex.

Range of age	Female	Male	Total
[18 - 25[	25	7	32
[26 - 35[	30	19	49
[36 - 45[	17	19	36
[46 - 55[	4	4	8
[56 - 65]	1	3	4
Total	77	52	129

Immunologically, the failure rate of CD4 T lymphocytes ≤ 200 cells/μL was 65.9% at the start of treatment at zero months (M0); on the other hand after 6 months (M6) of treatment with Tenofovir/Lamivudine/Dolutegravir (TLD), the level of CD4 T Lymphocytes ≤ 200 cells/μL was 16.3% (Table 8).

Table 8. Values of lymphocyte T CD4+.

CD4 Values (cells/μL)	1st Values M0	2nd Values M6
≤200 cells/μL	85 (65.9%)	21 (16.3 %)
>200 cells/μL	44 (34.1%)	108 (83.7%)
Total	100%	100%

Table 9 shows the variations in HIV RNA viral loads at the initiation of treatment at zero months (M0), after third month (M3) and after sixth month (M6) of treatment based on Tenofovir/Lamivudine/Dolutegravir (TLD). The criterion of

undetectability of HIV RNA if the viral load is less than 40 copies/ml according to the laboratory protocol.

Table 9. Distribution according to HIV RNA viral load.

HIV Viral Load (copy of RNA/mL)	1st Values M0	2nd Values M3	3rd Values M6
<1000 copies/ml	29 (22.5%)	9 (7.0%)	8 (6.2%)
≥1000 copies/ml	81 (62.8%)	17 (13.2%)	5 (3.9%)
Undetectable	19 (14.7%)	103 (79.8%)	116 (89.9%)
Total	100%	100%	100%

The viral load was high (≥ 1000 copies/ml) at M0, i.e. 62.8%, followed by 13.2% after 3 months of treatment and 3.9% after 6 months of treatment in the population studied. The immunovirological failure rate with TLD was 3.9% after 6 months of treatment, this shows the effectiveness of Tenofovir/Lamivudine/Dolutegravir (TLD) in HIV/HBV co-infected patients.

The viral load is undetectable or suppressed in 19.4% in zero months (M0) of patients; subsequently, the percentage of suppression increases after 3 months and 6 months of treatment, respectively 79.8% and 89.9%. The majority of participants in the study exhibited encouraging outcomes regarding viral load, with 6.2% viral loads below 1000 copies/ml at sixth month.

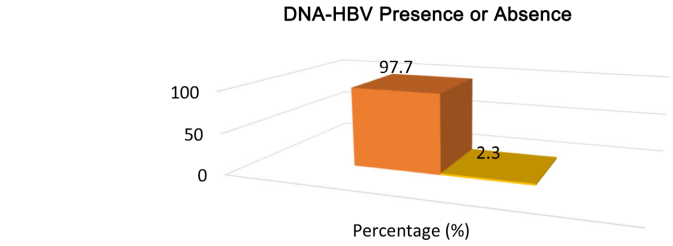
Table 10 shows us that the HBV DNA viral load is highly elevated in zero months (M0) at the initiation of treatment ≥ 2000 IU/mL 52.7%. The criterion of undetectability of HBV DNA if the viral load is less than 50 copies/mL according to the laboratory protocol.

Table 10. Distribution according to HBV DNA viral load.

HBV DNA Viral Load (UI/mL)	1st Values M0	2nd Values M3	3rd Values M6
≥2000 UI/mL	68 (52.7%)	9 (7.0%)	6 (4.7%)
<2000 UI/mL	31 (24.0%)	7 (5.4%)	4 (3.1%)
Undetectable	30 (23.3%)	113 (87.6%)	119 (92.2%)
Total	100%	100%	100%

Subsequently the number decreases after 3 months (M3) and 6 months (M6) of treatment based on Tenofovir/Lamivudine/Dolutegravir (TLD) respectively 5.4% and 3.1%. The immunovirological failure rate was 3.1% after 6 months of treatment of the population studied.

Regarding HBV DNA detectability, among the 129 samples analyzed, 126 (97.7%) were presence for detectable HBV DNA, while 3 samples (2.3%) showed absence DNA (Figure 1).



	Percentage (%)
Presence DNA	97.7
Absence DNA	2.3

Presence DNA Absence DNA

Figure 1. Distribution of patients according to DNA-HBV detectability (Presence or absence).

Figure 2 shows the distribution of CD4 T lymphocytes according to sex with the numbers of CD4 T lymphocytes ≤ 200 cells/µL being high in females compared to males.

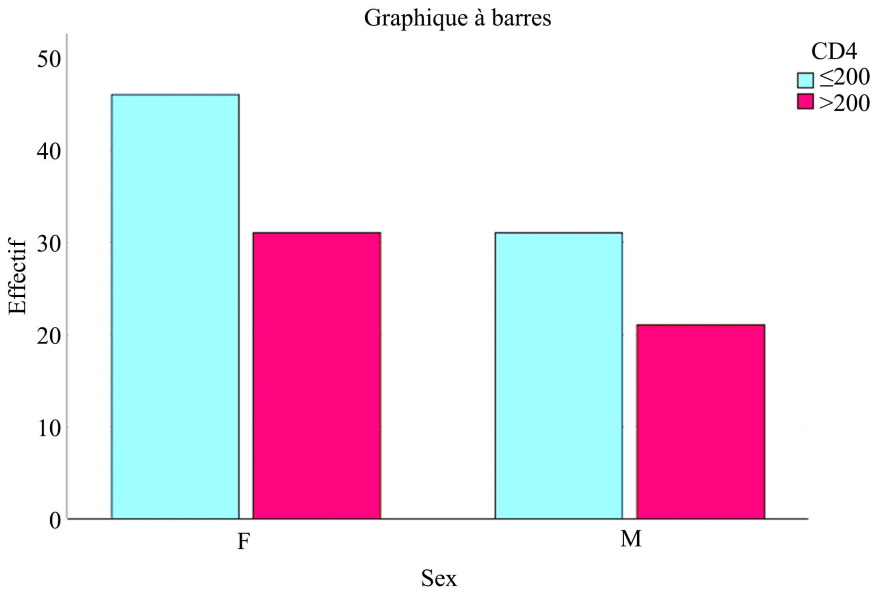


Figure 2. Distribution of CD4 T lymphocytes according to sex.

4. Discussion

In our study, women predominated with 59.7%, the sex ratio was 0.67. Similar trends were observed in studies conducted in Burkina Faso by T. M. Zohoncon *et al.*, a female predominance of 53.08% [18]; in Cameroon, the predominance is female [19]; in Sénégal, Ndiaye M *et al.*, found that women predominated, accounting for 78.13% [20]. This female over-representation could be explained by women's better access to health care or an increased propensity to seek medical care.

The average age of our patients was 35 years, ranging from 18 to 65; this can be

explained by the fact that HIV infections and other viruses with the same modes of transmission are frequently observed in young adults who are physically and sexually active. Our findings are comparable to those of Kumar *et al.*, who reported a mean participant age of 36.35 ± 14.76 years, with the most represented group being young sexually active adults aged 21 - 40 years [21]; with that of Traoré *et al.*, in Burkina Faso who found 83.49% of patients were adults (≥25 years old) [22].

Young adults represent the main target of HIV infection, due to sexual transmission, which is the predominant mode of contamination. Being part of the active population, these subjects are also more frequently exposed to risky behaviors [23].

Among our patients, married individuals accounted for 39.5%, a result similar to those found by MA Bolti *et al.*, with 46.6% married [24]. Married individuals are most at risk. This increase among married individuals can be explained by the refusal to disclose HIV status to a spouse for fear of stigmatization and breakup of the couple [24].

Regarding the clinical stages, about 35.7% were at clinical stage I, 40.3% were at clinical stage II, 20.2% were at clinical stage III, and the rest were at clinical stage IV, 3.9%. Our results are contrary to those of Essomeyo NM Magalie *et al.*, in Libreville who found in HIV/HBV co-infected patients the WHO clinical stages stage I, 24.3%; stage II, 48.6%; stage III, 7.1% and stage IV, 20.0% [23]. The difference between these results and those in our study is related to the methodological approach (often a retrospective study; the inclusion criterion; and also the study period, which has an influence).

Regarding HBV DNA presence, 97.7% were positive for detectable HBV DNA, while 2.3% showed absence DNA. Our results are consistent with those of P. S. Diawara *et al.*, who reported 92.48% detectable (presence) HBV DNA and 7.52% undetectable DNA (absence DNA) among the quantified patient samples [25].

The immunological failure rate of CD4+ T lymphocytes is ≤200 cells/μL in 65.9% at the initiation of treatment, and 16.3% after 6 months of treatment. This result is similar to that of Petra de Haas *et al.*, in Ethiopia most VISITECT-CD4-AD test results were read as ≤200 cells/μL 74.3% [26]. This upward trend in CD4 counts is found in Zhong *et al.*, who evaluated the immunological effectiveness of ARV treatment in naive PLHIV in China CD4+ T-cell 15.0% [27].

The results of our study show a significant improvement in immunological parameters in HIV/HBV co-infected patients under Tenofovir/Dolutegravir/Lamivudine triple therapy. The increase in the level of CD4 lymphocytes observed after initiation of treatment reflects a progressive immune restoration, consistent with integrase such as Dolutegravir.

In the study population, the HIV RNA viral load was high (≥ 1000 copies/ml) in 62.8% at the initiation of M0 treatment, 13.2% after 3 months of treatment and 3.9% after 6 months of treatment. Our results are close to those of Essomeyo NM Magalie *et al.*, in Libreville who found the viral load was ≥1000 copies/ml in 82.1%

at the initiation of treatment [23]. The association might be because HIV patients who have HBV/HIV co-infection have weaker immunity than those who have not HBV/HIV co-infected HIV patients which will make them more prone to increased viral load [28].

Some patients skip drug intakes, forgetfulness or neglect, which can promote the emergence of HIV resistance to treatment. Others go to traditional healers and marabouts who believe that healing is possible through traditional medicine. It should be noted that this practice is not without consequences as it contributes to the deterioration of the health status of patients who are often subject to many opportunistic infections [29]. The rest of the medications were checked during the visits to calculate the percentage of doses actually taken and an analysis of the regularity of prescription renewals at the pharmacy was made in all patients.

The HBV DNA viral load is high ≥ 2000 IU/mL in 52.7% at the initiation of treatment (M0), after 3 months of treatment 5.4% and after 6 months of treatment 3.1%. The viral load rate ≥ 2000 IU/mL of 3.1% at 6 months of treatment could be explained by poor compliance with treatment in some patients. Our results differ from those of Nan-arabe Lodoum *et al.*, who found a low viral load in the study population, with 74.41% showing HBV DNA levels below 2000 UI/mL, compared to 23.59% above this threshold [30]. The difference between these viral load HBV and those in our study is related to the methodological approach.

Indeed, the viral load of HBV DNA is suppressed only in 23.3% at the initiation of M0 treatment, on the other hand the frequency of suppression of HBV DNA is high after 3 months (M3) and 6 months (M6) of treatment respectively 87.6% and 92.2%. However, our results at M3 and M6 were similar to those of a study in Bouaké (Côte d'Ivoire), the proportion of patients in whom the viral load was undetectable increased over time from 55.5% to 64.4% from M6 to M24 [31]. This demonstrated virological effectiveness in relation to the duration of follow-up and also good compliance. Prolonged use of therapeutic combinations based on Tenofovir/Dolutegravir/Lamivudine in HIV/HBV co-infected patients considerably reduces the viral load when compliance is good.

The significant suppression of the HIV-1 viral load in the majority of patients confirms the antiviral power of the regimen studied. Dolutegravir, recognized for its high genetic barrier to resistance, plays a central role in this effectiveness. Furthermore, the presence of Tenofovir and Lamivudine, active against both HIV and the hepatitis B virus, constitutes a major therapeutic advantage in the context of co-infection. However, the possible absence of systematic monitoring of HBV viral load or viral replication markers may represent a limitation in the complete evaluation of the hepatic response.

5. Conclusion

In conclusion, the immunovirological evaluation of the triple therapy combining Tenofovir/Lamivudine/Dolutegravir in HIV/HBV co-infected patients in Chad highlights significant virological suppression of HIV accompanied by progressive

immune restoration. The therapeutic regimen studied presents an efficacy profile and constitutes a relevant first-line option in the management of HIV/HBV co-infected patients in Chad. Nevertheless, prolonged longitudinal follow-up remains necessary in order to assess the durability of the virological response and the long-term evolution of hepatic parameters.

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Authors' Contributions

All authors contributed to data acquisition, analysis, and interpretation; writing of the paper; critical review of its intellectual content; and final approval of the version to be published. All authors contributed to the conduct of this work. All authors also declare that they have read and approved the final version of the manuscript.

Ethical Considerations

The study was conducted in strict compliance with confidentiality requirements, and anonymity was ensured through the use of patient registration numbers. We obtained ethical clearance from the National Bioethics Committee of Chad under number N°056/MESRS/SE/SG/CBNT/SG/2025. Research authorizations were obtained from the various health centers in N'Djamena. In addition, all participants provided specific written consent for the utilization of their demographic information in this research study. This consent was obtained after explaining in a clear and understandable manner the objectives, risks, potential benefits, and rights of participants, including their freedom to withdraw at any time without prejudice.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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