# Evaluation of antiviral treatment for hepatitis B with tenofovir and entecavir at the viral hepatitis outpatient clinic of the tropical medicine research center in Rondônia

# Avaliação do tratamento antiviral da hepatite B com tenofovir e entecavir no ambulatório de hepatites virais do centro de pesquisa em medicina tropical de Rondônia

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**ABSTRACT**
The emergence of treatment-resistant strains has been an obstacle in antiviral treatment of hepatitis B, which is carried out when the disease evolves to the chronic phase. In 2017, the Ministry of Health limited the therapeutic arsenal to the use of Tenofovir and Entecavir, due to their greater effectiveness and genetic barrier. However, there are no comparative studies of the antiviral activity of these two drugs in the state of Rondônia; such studies would improve the treatment of this disease. Thus, the objective of this study is to evaluate the therapeutic response of hepatitis B patients treated with Entecavir and Tenofovir at state of Rondônia. Patient data were analyzed in medical records containing information about the treatment used and the evolution of each patient's clinical condition. The tests observed are those that detect HBV DNA to assess viral load, HBV serological markers and liver transaminases, to monitor the evolution of the disease. As a result, the samplings and correlations carried out in this study indicated that the efficacy of both Entecavir and Tenofovir was satisfactory in view of the different profiles of chronic HBV patients evaluated, since they reduced the viral load and normalized transaminases. The data obtained may help to understand the profile and therapeutic response of patients infected with the HBV virus who are treated with Tenofovir and Entecavir, as well as to disseminate pharmacoepidemiological data.

**Keywords**: hepatitis B treatment; Tenofovir; Entecavir.

**RESUMO**
A seleção de cepas resistentes tem sido um obstáculo no tratamento antiviral da hepatite B, sendo este realizado quando há evolução para a fase crônica da doença. Em 2017 o Ministério da Saúde limitou o arsenal terapêutico ao uso do Tenofovir e o Entecavir, pela sua maior eficácia e barreira genética. Contudo, não há no estado de Rondônia, estudos da atividade antiviral desses dois fármacos, resultado que aperfeiçoaria o tratamento dessa doença. Dessa forma, o objetivo deste trabalho é avaliar a resposta terapêutica de pacientes com hepatite B tratados com Entecavir e Tenofovir em Rondônia. Foram analisados dados de pacientes contidos em prontuários, com informações a respeito do tratamento empregado e da evolução do quadro clínico. Os exames observados são os de pesquisa de DNA-HBV para avaliar a carga viral, marcadores sorológicos do HBV e transaminases hepáticas, para acompanhamento da evolução da doença. Sendo assim, as amostragens e correlações realizadas neste estudo indicaram que tanto a eficácia do entecavir quanto do tenofovir foi satisfatória frente aos diferentes perfis de pacientes HBV crônicos avaliados, ao reduzir a carga viral e normalizar as transaminases. Os dados obtidos poderão auxiliar na compreensão do perfil e da resposta terapêutica dos pacientes

infectados pelo vírus HBV tratados com o Tenofovir e Entecavir, bem como, na divulgação de dados farmacoepidemiológicos.

**Palavras chaves**: tratamento da hepatite B; tenofovir; entecavir.

## 1 INTRODUCTION

The World Health Organization (WHO) estimates that there are about 257 million people with chronic hepatitis B virus infection and that one third of the world population has come into contact with its etiological agent, the hepatitis B virus (HBV) [1, 2]. However, these numbers may still be underestimated, since the existence of underreporting must be considered, given the fact that the disease triggers an asymptomatic course in most people, making early diagnosis difficult and favoring the spread of the disease [1]. In Brazil, between 2009 and 2019, detection rates in the Southern, Northern and Midwestern regions were higher than the national rate. In addition, it can be highlighted that between 2000 and 2018, in Brazil, 15, 912 deaths related to hepatitis B were recorded, and in 2018, the North region reached the highest mortality rate in the entire period, with 0.4 deaths per 100,000 inhabitants. Inserted in this region, Rondônia was among the twelve states that presented hepatitis B detection rates higher than those observed in their capital cities (Rondônia with 24.0 and Porto Velho with 23.0 cases/100,000 inhabitants) [3].

With broad clinical aspects, HBV infection can present in acute (rarely fulminant), chronic and occult forms [4]. In advanced stages, liver failure, digestive hemorrhages, liver cirrhosis, hepatocellular carcinoma (HCC) and even death can occur [4]. Antiviral therapy is primarily aimed at suppressing viral replication before irreversible liver damage or patient death occurs [5]. In addition, a low-fat diet and suspension of the use of hepatotoxic substances and alcohol intake should be employed [6]. Treatment is not recommended for patients with hepatitis B in the acute phase, since in about 90% to 95% of immunocompetent adult patients the disease is resolved without progression to chronicity [6] . However, in the chronic phase of the disease, if deemed necessary based on clinical evaluations, the patient should start antiviral treatment as soon as possible [7, 8, 9]. The main laboratory markers that constitute a criterion for treatment adherence are the levels of viral load, HBsAg, HBeAg and transaminases present in the bloodstream [7, 9, 10].

Until 2016, in Brazil, the immunomodulators and nucleoside (or nucleotide) analogues lamivudine (3TC), adefovirdipivoxil (ADV), Entecavir (ETV), and telbivudine (LdT) and Tenofovir (TDF) were utilized as antiviral drugs for the treatment of hepatitis B (5). These agents are well tolerated by the body, but the effectiveness of these drugs has been affected by the emergence of strains with mutations associated with resistance [10, 11, 12, 13]. In 2017, the Brazilian Ministry of Health published a new decree that restricted therapeutic choice for chronic hepatitis B to the use of the drugs Tenofovir and Entecavir, due to their high efficacy and genetic barrier [5, 14]. However, even so, the effectiveness of this restricted and/or limited therapeutic arsenal may be compromised with the eventual rise of resistance due to selective pressure, for example. In this context, this study aimed to take an initial step in monitoring and evaluating the effectiveness of chronic hepatitis B therapy with Tenofovir and Entecavir in a specialized care unit in Rondônia, Northern Brazil, as well as to collect data that, in part, represents the current scenario in this region of the country.

## 2 MATERIALS AND METHODS

This study consists of data collection at the Specialized Outpatient Clinic for Viral Hepatitis of the Tropical Medicine Research Center of Rondônia (CEPEM-RO), in Porto Velho - RO. Data from the medical records of patients with chronic hepatitis B who were referred for treatment and admitted to the clinic in 2015 and 2016, were included. The study population consisted of 45 patients; the population was limited to this quantity due to missing data from other patients' records because of evasion of treatment or death, the inclusion of which would have made the performance of analyses impossible.

The medical records of patients seen by the medical team at the CEPEM/RO viral hepatitis outpatient clinic were analyzed based on the following information: age, sex, weight, ethnicity, month and year of beginning and end of treatment (if completed), therapeutic regimen adopted, and the results of clinical and laboratory tests performed to monitor the disease. The laboratory data analyzed included the dosage of AST (aspartate aminotransferase) and ALT (alanine aminotransferase) transaminases, serological tests (HBsAg, total Anti-HBc, HBeAg, Anti-HBe and Anti-HBs) and the determination/quantification of HBV DNA.

The extracted data with the results of laboratory tests performed were organized for standardization purposes, arranging them in such a way as to enable the reading and analysis of the elements in question. Thus, the data were temporally categorized: First,

data from pre-treatment, a phase that includes the results of exams requested at the beginning of treatment in patients and which serve, therefore, as a useful comparison in relation to later assessments. These, in turn, are usually requested by the outpatient medical team within 6 to 12 months after the start of treatment and are performed annually after this period. These assessments constitute the temporal categorizations of the data, and the analysis ended with the assessment after 2 years of treatment, considering that this is the most relevant time interval for studies to assess viral load clearance after therapy.

Data collected from medical records were analyzed in order to assess the response of patients to the treatment adopted, based on the assessment of serological changes that were presented by patients during the pre- and post-treatment phases, obtained with results of laboratory tests. At this stage, patients were organized into two groups, the first consisting of patients treated with Entecavir and the other with Tenofovir. These groups were compared to each other to assess the patient's prognosis and therapeutic response to chronic hepatitis B. All data from the medical records were entered into a database built using the software EpiData 3.1 (Center for Disease Control and Prevention, USA) and data analysis was distributed in mean and standard deviation; the construction of graphs and tables was performed using Microsoft Excel 2010. This project was approved by the Human Research Ethics Committee of Centro Universitário Aparício Carvalho, under protocol number: 626,947.

## 3 RESULTS

A total of 45 individuals were included in the study, all of whom started treatment in 2015 and 2016, following the inclusion and exclusion criteria described in the methodology. The population included has a minimum age of 24 years old, a maximum age of 82 years old and an average age of 49 years old with a standard deviation of 13.8 years. Of these, 73.3% (33) are male while 26.7% (12) are female. The individuals presented pre-treatment Body Mass Indexes (BMI) that varied between 17.2 (Underweight) and 31.3 (Obesity Grade I), with a mean of 26.5 and standard deviation of 2.5 (Table I). As for ethnicity, 68.8% of the study population identified themselves as Mulatto, 20% as White, 4.5% as Black and 6.7% were not identified. Regarding the drugs included in the study used by the population, 60% had been or were being treated with Entecavir and 40% with Tenofovir. Most of the study population had non-reactive

serology for HBeAg (66.7%) and reactive serology for Anti-HBe during pre-treatment. All these data are shown in table I.

Table I. The table represents the epidemiological analysis of the study population. The sample is represented by N while the standard deviation is represented by SD.

| | **n: 45** |
|---|---|
| **Male** – n (%) | 33 (73,3) |
| **Mean Age** – years (SD) | 49 (13,8) |
| **Ethnicity** – n (%)<br>Mulatto<br>White<br>Black<br>Not identified | 31 (68,8)<br>9 (20)<br>2 (4,5)<br>3 (6,7) |
| **Average BMI** – BMI (SD) | 26,5 (2,5) |
| **Drug Used** – n (%)<br>Entecavir<br>Tenofovir | <br>27 (60)<br>18 (40) |
| **HBeAg Negative** – % | 66,7% |

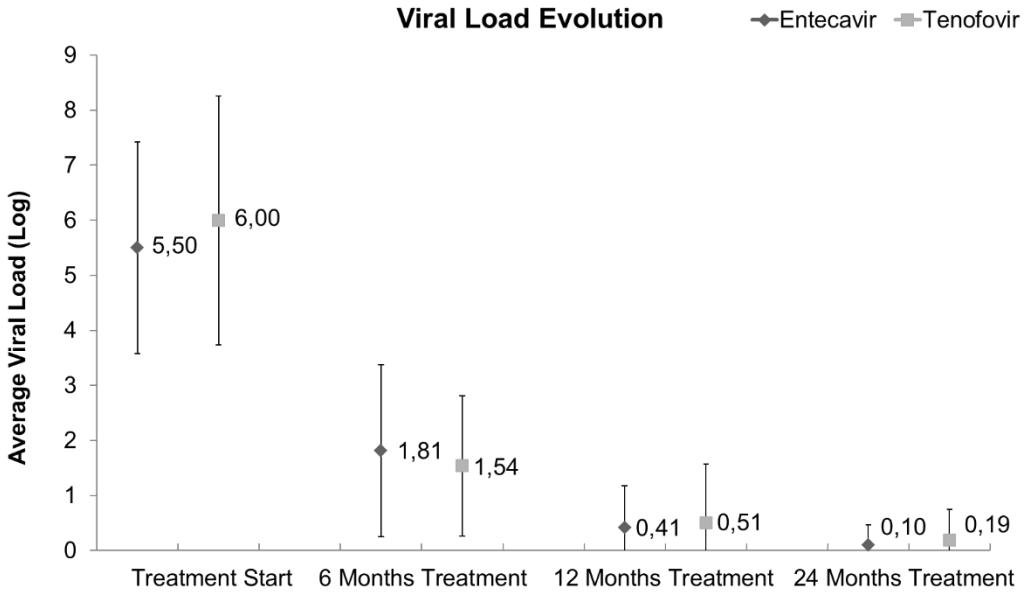
Throughout the observation period of the present study, the entire study population presented reactive serology for the HBV surface antigen, HBsAg, and non-reactive serology for its respective antibody marker, Anti-HBs. This indicates that during this period the infection was active in 100% of patients. Additionally, all individuals included presented serology that was reactive for Total Anti-HBc and non-reactive for Anti-HBc IgM during the same period that was evaluated, indicating that they were classified as chronic carriers of hepatitis B.

By dividing the study population into two distinct groups (those treated with Entecavir and those treated with Tenofovir), the relationship between the therapy used and evolution of the viral load (assessed by the means and standard deviations and measured in log base 10) was evaluated and obtained through Real-time Polymerase Chain Reaction (RT-qPCR) presented by patients during the observation period, in order to assess the response of different approaches through viral load clearance.

The study population that used Entecavir had a mean log viral load of 5.50 (Standard Deviation - SD 1.92) during the pretreatment period, while the group that used Tenofovir had a mean of 6.00 (SD 2.26) in the same period. Six months after the start of treatment, the group that used Entecavir had a mean log viral load of 1.81 (SD 1.56) while

the group that used Tenofovir had a mean viral load of 1.54 (SD 1.27). At 1 year from the start of treatment, the mean viral load in the Entecavir-treated group was 0.41 (SD 0.76), while that of the Tenofovir-treated group was 0.51 (SD 1.06). At the end of the study, 2 years after starting antiviral treatment, the group that used Entecavir had a mean viral load of 0.10 (SD 0.36), while the group that used Tenofovir had a mean rate of 0.19 (SD 0.56) (Figure 1).

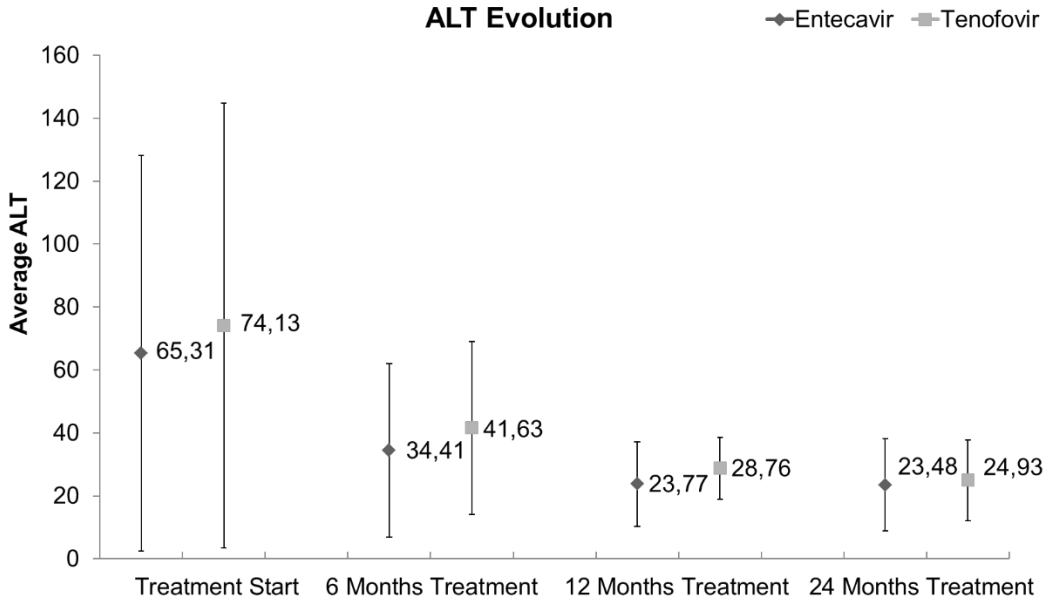
Figure 1. In the graph, the relationship between the axes shows the equivalent to the viral load mean expressed in logarithms of base 10 (y-axis) and the chronological time expressed in months (x-axis). The geometric figure in diamond shape represents the group of patients who used the drug Entecavir while the geometric figure in square shape represents the group of patients who used the drug Tenofovir. The black line around the geometric figure represents the standard deviation.




The relationship between the drug used and the liver damage profile obtained through the measurement of transaminases (ALT and AST), altered by the disease during the period of observation of the treatment, was studied. Previously, it is noteworthy that the values of transaminases considered to be “altered” were those greater than 30 units per liter (U/L) for both ALT and AST, based on local reference values. During the pre-treatment period, the mean dose of Alanine Aminotransferase (ALT), measured in units per liter (U/L), presented by the study population that used Entecavir was 65.31 (SD 62.91), while that of the group that used Tenofovir was 74.13 (SD 70.57). Six months after starting treatment, the value of this variable was 34.41 (SD 27.49) for those treated with Entecavir and 41.63 (SD 27.47) for those treated with Tenofovir. One year after the start of treatment, the group that used Entecavir had a mean ALT dose of 23.77 (SD 13.43), and the group that used Tenofovir, 28.76 (SD 9.76). At the end of the study, 2

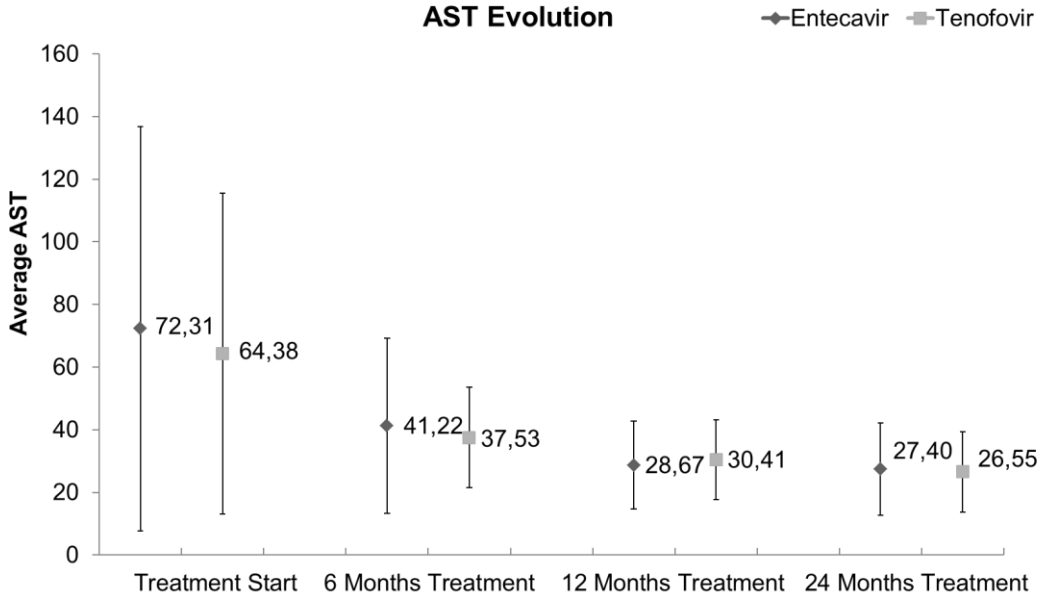
years after starting antiviral treatment, the group that used Entecavir had a mean ALT of 23.48 (SD 14.65) while those that used Tenofovir had a mean ALT of 24.93 (SD 12.85) (Figure 2).

Figure 2. In the graph, the relationship between the axes shows the equivalent to the ALT (alanine aminotransferase) mean (y-axis) and the chronological time expressed in months (x-axis). The geometric figure in diamond shape represents the group of patients who used the drug Entecavir while the geometric figure in square shape represents the group of patients who used the drug Tenofovir. The black line around the geometric figure represents the standard deviation.




During the pre-treatment period, the mean dosage of Aspartate Aminotransferase (AST), measured in units per liter (U/L), presented by the study population that used Entecavir was 72.31 (SD 64.54), while that of the group that used Tenofovir was 64.38 (SD 51.23). Six months after starting treatment, the value of this variable was 41.22 (SD 28.0) for those treated with Entecavir and 37.53 (SD 16.10) for those treated with Tenofovir. One year after the start of treatment, the group that used Entecavir had a mean AST dose of 28.67 (SD 14.06), and the group that used Tenofovir, 30.41 (SD 12.68). At the end of the study, 2 years after starting antiviral treatment, the group that used Entecavir had a mean AST of 27.4 (SD 14.74) while those that used Tenofovir had a mean AST of 26.55 (SD 12.79) (Figure 3).

Figure 3. In the graph, the relationship between the axes shows the equivalent to the AST (aspartate aminotransferase) mean (y-axis) and the chronological time expressed in months (x-axis). The geometric figure in diamond shape represents the group of patients who used the drug Entecavir while the geometric figure in square shape represents the group of patients who used the drug Tenofovir. The black line around the geometric figure represents the standard deviation.




## 4 DISCUSSION

Most of the population in the present study had non-reactive serology for the HBeAg antigen (66.7%) and reactive serology for Anti-HBe during pre-treatment, but the entire population presented the virus in active replication with detectable HBV DNA. The presence of HBeAg in serum is related to the high rate of replication and contagiousness of the virus; however, studies indicate that patients with negative HBeAg and detectable HBV DNA are frequently present a mutation in the pre-core G1896A region. This viral profile, as a consequence, brings with it great potential for infectivity and viral replication, even though it does not produce the HBeAg antigen, giving the HBV carrier greater chances of progression to advanced liver diseases resulting from the chronicity of the disease, such as fibrosis and hepatocarcinoma [15, 16, 17] In addition, it is a profile that can goes unnoticed in healthcare units that cannot detect HBV DNA, increasing the risk of a poor prognosis of liver disease.

Treatment with ETV results in a faster response of declining HBV viral load in the first year of treatment, whereas TDF is more successful than Entecavir in total virological suppression. However, both approaches have very similar efficacy with regard to reducing viral load without major differences in the therapeutic evolution and limitation of liver damage with reduction of transaminases to normal levels [18, 19]. This is in line with the results obtained in the present study, as they showed no significant

differences in the therapeutic efficacy of both approaches, both being responsible for therapeutic responses considered desirable. Other studies and protocols also point out that ETV and TDF are comparable in efficacy and safety to support HBV DNA suppression with limited side effects, both being classified as 1st line of treatment of chronic hepatitis B, with no distinction in efficacy [7, 9, 12, 19, 20, 21] .

The Clinical Protocol and Therapeutic Guidelines for Hepatitis B and Coinfections [6] suggest using both drugs, Entecavir and Tenofovir, in the treatment of chronic hepatitis B, since they are the nucleoside analogues with the greatest efficacy and genetic barrier. Additionally, the document states that the choice of these drugs simplifies treatment for patients and healthcare teams, since both offer easier dosage and fewer adverse effects, increasing rates of correct adherence to treatment [6].

In a multicenter study carried out by Cai et al. [22], which compared the efficacy and safety of Entecavir and Tenofovir in previously untreated patients, treated for 144 weeks, no significant difference was observed between the groups treated with ETV and TDF including a lack of significant differences in serological and biochemical responses, similar to the results obtained in the present study. In addition, Zoulim [23] showed that treatment with ETV and TDF was quite effective and tolerable in patients who had failed to respond to previous therapies, achieving virological suppression in 85% of patients for 96 weeks with no emergence of resistant strains during therapy.

One of the factors that determines the decrease in the effectiveness of the aforementioned drugs is the appearance of resistant mutations. Research shows that no resistance has been reported in Tenofovir therapies for up to 3 years [24], although there are reports of mutations in the viral genome that provide resistance to Tenofovir [8, 13, 25, 26]. There are cases of intermediate resistance to Tenofovir, such as in the cases of rtA181T/V and rtN236T substitution mutations, which reduce the drug's efficacy, and may also reduce susceptibility by up to 10 times in the presence of other secondary mutations [26]. A study by Tenney et al. [27], which followed patients with chronic hepatitis B over the long-term, showed a low probability, 1.2% - 1.5%, of resistance even after 5 years of treatment with this drug. However, the study showed a resistance rate of up to 51% in individuals who had previously been treated with Lamivudine [27].

Given that in Brazil there is not ample availability of resources to perform molecular biology techniques, many centers use the serum levels of transaminases (ALT and AST) associated with serologies to try to assess the presence of viral replication and evolving liver damage [28]. However, research carried out in areas endemic for Hepatitis

B showed that the relationship between viral load and transaminases is quite weak and nonspecific, and the study of HBV DNA is absolutely necessary to assess viral replication activity [29, 30]. In the present study, this observation was reinforced by the large amplitude of the standard deviation of transaminases in the pre-treatment phase, when the mean dosage of ALT and AST of all groups presented a standard deviation of at least ±1.6 times the value of reference used, reaching up to ±2.3 times in one of the groups. This variation is quite important when considering that the mean of transaminases in the different groups is 2.3 times the normal value, demonstrating that even with all study participants having detectable HBV DNA, not all of the individuals had elevated levels of transaminases, converging with that which is in the guidelines that are currently available, which suggest that the analysis of HBV DNA and transaminases should be carried out in conjunction with multiple clinical evaluations for therapeutic decision-making and better management of the infected patient [6, 31, 32].

## 5 CONCLUSION

The samplings and correlations carried out from the analyses indicate that both the effectiveness of Entecavir and Tenofovir are satisfactory against the different profiles of patients with chronic hepatitis B in the state of Rondônia, Brazil, by reducing the viral load and normalizing liver transaminases in up to 2 years after the start of antiviral therapy. The similarity of the results obtained in this study between the two therapeutic approaches analyzed is noteworthy, since both, despite their particularities, proved to be efficient in the treatment of chronic HBV, especially in the reduction of HBV DNA. Therefore, in order to define the criteria for choice of therapy, it is necessary to include an evaluation of the various clinical aspects of HBV carriers and the pharmacological particularities, such as the nephrotoxicity of Tenofovir, for example. Finally, the authors emphasize the need for more research on the effectiveness of therapy and monitoring of resistance in the region, given the importance of the subject.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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