Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol. (9) Issue (3) (2024), (239-244) **online: ISSN 2356–976x**
http://bjas.journals.ekb.eg

Treatment Response to Nucleoside Analogues Via MMP-9/NrF2 In Hepatitis B Egyptian Patients

Heba A.Ahmed[1], Mahmoud M.Amer[1], Ayman Hassan[2], Attia A. Attia[1]and Mohamed Nasr-Eldin[1]
[1.] Botany and Microbiology Dept., Faculty of Science, Benha University, Qalubiya Governorate 13511, Egypt.
[2.] Egyptian Liver Research Institute and Hospital (ELRIAH), Mansoura, Egypt
E-Mail: maznla06@gmail.com

Abstract
Background: Hepatitis B virus (HBV) infection is a serious worldwide health concern, especially in highly endemic areas like Egypt. Prolonged HBV infection causes serious liver problems that need efficient antiviral treatment. Although nucleoside analogs are often utilized in medicine, patient response differs for a variety of reasons. Improving outcomes requires an understanding of the molecular processes driving therapy response. Nuclear factor erythroid 2-related factor 2 (NrF2) and matrix metalloproteinase-9 (MMP-9) have been identified as important regulators of HBV pathogenesis and antiviral treatment response. **Methods:** In this outpatient clinic case-control research, 102 controls and 428 HBV patients participated. PCR was used to evaluate the genetic expression of NrF2 and MMP-9. Gene expression levels were used to assess therapy response in patients receiving nucleoside analogues. **Results:** Patients receiving various nucleoside analogues showed significant variations in MMP-9 and NrF2 expression. When compared to naïve or control groups, entecavir medication was linked to a substantial decrease in MMP-9 expression, indicating that it is effective in lowering MMP-9 levels. Comparatively speaking to MMP-9, NrF2 expression showed rather similar tendencies. **Conclusion**: our research sheds light on the molecular processes that underlie Egyptian HBV patients' responses to nucleoside analogue therapy via the MMP-9 and NrF2 pathways. In cases of chronic HBV infection, MMP-9 and NrF2 show potential as prognostic biomarkers for treatment outcomes. To further understand processes and investigate clinical consequences, more research is required. This might lead to the development of tailored treatment strategies that improve clinical results.

Keywords: HBV, MMP-9, NrF2, Nucleosides

1.Introduction

Millions of people worldwide are impacted by hepatitis B virus (HBV) infection, which is still a major public health concern, especially in areas where endemicity is high, such as Egypt [1]. Patients with chronic HBV infection are more likely to develop liver cirrhosis, hepatocellular carcinoma (HCC), and other serious liver-related problems, which has a significant negative impact on global economies and healthcare systems [2]. The majority of antiviral treatment for chronic HBV infection is nucleoside analogues, such as lamivudine, entecavir, and tenofovir, which are intended to reduce viral replication and stop the course of the illness [3]. However, patient responses to nucleoside analogues might differ, and therapy results can be influenced by host immunological response, viral mutations, and viral genotype [4].

For the purpose of enhancing therapeutic approaches and enhancing clinical results, it is essential to comprehend the molecular processes underpinning treatment responsiveness to nucleoside analogs in HBV-infected individuals. Nuclear factor erythroid 2-related factor 2 (NrF2) and matrix metalloproteinase-9 (MMP-9) have been identified as important regulators of major molecular pathways involved in HBV pathogenesis and antiviral treatment response [5].

One of the MMP family members involved in tissue remodeling and extracellular matrix breakdown is matrix metalloproteinase-9, or MMP-9. In chronic HBV infection, elevated MMP-9 levels have been linked to inflammation, liver fibrosis, and disease progression. MMP-9 may also be a predictive biomarker for treatment response since it has been linked to antiviral medication resistance [6].

A transcription factor NrF2 controls the production of detoxifying and antioxidant enzymes that are important in cellular defense against oxidative stress. A number of liver illnesses, including HBV infection, have been linked to dysregulation of NrF2 signaling, which may affect how well nucleoside analog treatments work [7]. In HBV-infected individuals, NrF2 signaling activation has been linked to better antiviral immune responses and better treatment results [8].

The therapeutic response to nucleoside analogues via MMP-9/NrF2 pathways in Egyptian individuals infected with the hepatitis

B virus is still not well known. Clarifying the functions of these molecular markers in the response to antiviral medication may provide important new information about individualized therapeutic approaches and predictive biomarkers for enhancing clinical outcomes in this group [9]. In order to help create specialized approaches for the management of chronic HBV infection, this research intends to examine how Egyptian individuals infected with the hepatitis B virus respond to therapy via the MMP-9/NrF2 pathways.

2.Materials and Methods

Study design

This case-control study was performed after Ethical approval for the study by the Ethics Committee of Faculty of science, Benha University, Egypt and obtaining adequate consent. The present study was carried out on 428 HBV patients *vs*. control group (n=102); attending the outpatient clinic during the period from April 2022 to June 2023. Inclusion Criteria of the selected patients were as follows; Patients diagnosed with chronic HBV infection. Age 18 years or older. Patients attending the outpatient clinic of the study institution. Willingness to participate in the study and provide informed consent. Availability of clinical and demographic data for analysis. Adequate medical records documenting HBV infection status and treatment history. The Exclusion Criteria were Patients with acute hepatitis B virus infection. Age younger than 18 years. Patients with co-infections such as hepatitis C virus (HCV) or human immunodeficiency virus (HIV). Patients with significant comorbidities affecting liver function, such as cirrhosis or hepatocellular carcinoma. Patients who have received liver transplantation. Patients with a history of other chronic liver diseases, such as autoimmune hepatitis or alcoholic liver disease. Patients with incomplete medical records or missing data necessary for analysis. Pregnant or lactating women. Patients were unwilling or unable to provide informed consent for participation in the study.

Genetic expression of the selected genes

To determine the genetic expression of MMP-9 and Nrf2, polymerase chain reaction (PCR) can be utilized. PCR is a widely used molecular biology technique that amplifies specific DNA sequences, allowing for the detection and quantification of gene expression levels. Total RNA is extracted from patient blood samples or liver tissue using a commercially available RNA extraction kit following the manufacturer's protocol. The extracted RNA is then reverse transcribed into complementary DNA (cDNA) using a reverse transcription kit. This step converts RNA into a stable form of DNA, which can be used as a template for PCR amplification. Specific primers targeting the MMP-9 and Nrf2 genes are designed. These primers should flank the regions of interest and be specific to the target genes to ensure accurate amplification [10].

Thermal Cycling

The PCR reaction mixture is subjected to a series of temperature cycles in a thermal cycler. The cycling conditions typically include denaturation, annealing, and extension steps: Denaturation: DNA strands are separated by heating to 95°C. Annealing: Primers anneal to complementary sequences in the DNA template at a temperature specific to the primer sequences. Extension: DNA polymerase extends the primers along the template strand, synthesizing new DNA strands at a temperature optimal for enzyme activity. PCR products are analyzed by gel electrophoresis or quantitative real-time PCR (qPCR) to visualize and quantify the amplified DNA fragments. Gel electrophoresis separates DNA fragments based on size, allowing for qualitative analysis of PCR products. qPCR provides quantitative data on gene expression levels by measuring the fluorescence emitted during amplification [11].

Data Analysis of genetic expression

The expression levels of MMP-9 and Nrf2 are determined based on the intensity of PCR bands or the cycle threshold (Ct) values obtained from qPCR. The relative expression levels of the target genes are normalized to an internal control gene (housekeeping gene) and compared between patient groups to assess differences in gene expression.

Statistical Analysis

Statistical analyses were done with GraphPad prism Version 9.2 and GraphPad software program. Kolmogorov Smirnov test used to evaluate normally distributed continuous variables. Student’s t test used to compare variables in two in-dependent groups with normal distribution, Mann Whitney U-Test used to compare those with abnormal distribution. Qualitative data were presented as frequency and percentage. Chi square and Fisher’s exact tests were used to compare groups. Quantitative data were presented by mean, SD, median and range or mean and SD. Comparisons between two groups were done using t-test or Man Whitney (for non-parametric).

3.Results

Clinical presentation of the studied HBV cohort

Patients were divided into 110 females and 518 males based on their initial characteristics: In terms of HBV therapy, 144 patients were taking Entecavir, 106 patients were taking Tenofovir, and 102 patients were taking Lamivudine. Only 42 of the 318 patients with HBV DNA levels higher than 20,000 (IU/mL) had levels higher than 2000 (IU/mL). In the current study, only 7 patients experienced a breakthrough, while 312 individuals had seroclearance detected in 24 patients **(Figure 1a-d)**. This indicates that the HBV viral loads dropped significantly in the 318 patients with HBV DNA levels higher than 20,000 (IU/mL), and that the efficacy of the HBV antiviral drugs Entecavir, Tenofovir, and Lamivudine was effective in reducing HBV viral loads in these patients.

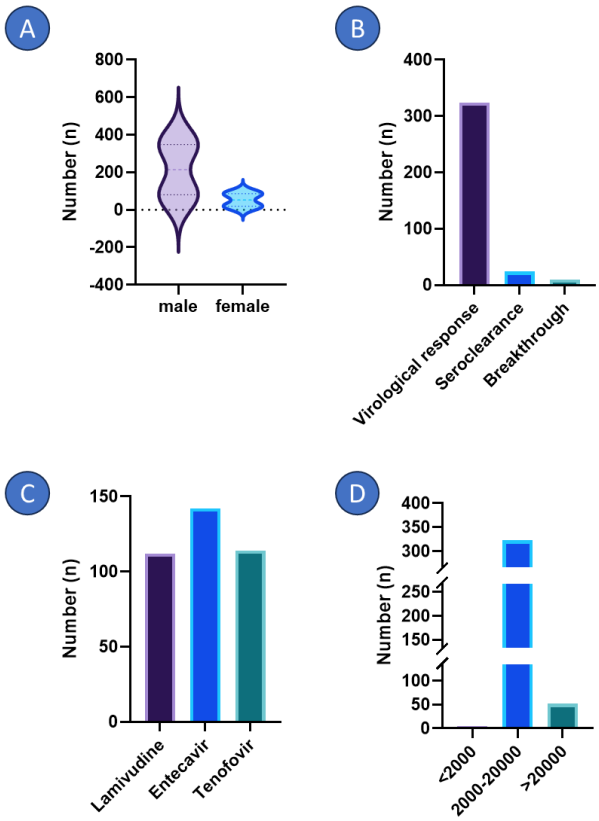

Fig. (1) Demographic data of Hepatitis B patients **(A)** gender distribution**; (B)** Response classification**; (C)** Treatment options**; (D)** Viral load**.** Data were expressed by number

Genetic expressions of MMP-9 and NrF2 in response to the treatment

The study's findings indicate that there were notable disparities in the downregulation of MMP-9 among patients treated with entecavir compared to those in the naïve or control groups. Entecavir demonstrated a substantial reduction in MMP-9 levels among the treated patients, although the naïve and control groups did not exhibit any notable changes, as seen in **Figure 2**. This indicates that entecavir has superior efficacy in decreasing MMP-9 levels compared to other therapies. These findings provide evidence for the efficacy of entecavir as a viable therapy choice for those suffering from chronic hepatitis B virus infection. Moreover, entecavir might potentially serve as a viable treatment choice for halting the advancement of fibrosis and cirrhosis linked to chronic hepatitis B virus infection.

Furthermore, the patients who received entecavir treatment had similar results in terms of NrF2 expression, but to a lesser extent than MMP-9 expression, as seen in **Figure 3**. This implies that entecavir may have a greater efficacy in decreasing the expression of MMP-9 compared to NrF2. Moreover, this implies that entecavir could have a greater efficacy in diminishing inflammation and fibrosis in those with chronic hepatitis B. Entecavir exerts its effects by suppressing the activity of MMP-9, a key factor involved in the inflammation and fibrosis associated with chronic HBV. In addition, entecavir may exert its effects via reducing the function of NrF2, a key factor involved in the inflammation and fibrosis associated with chronic HBV.

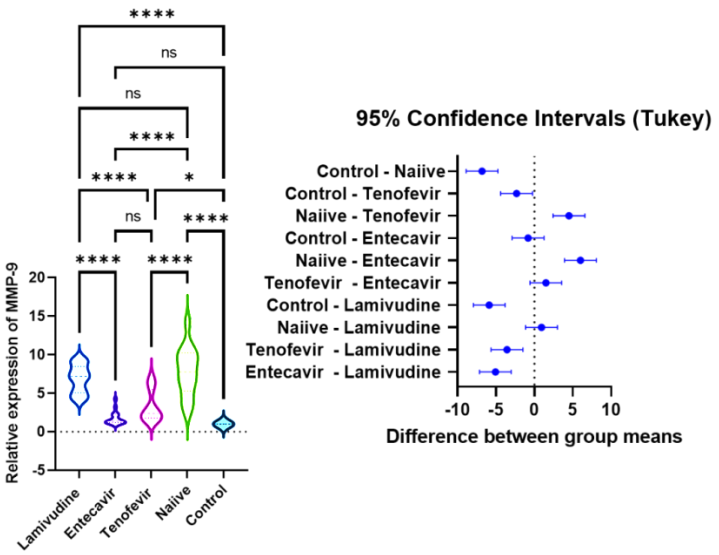

Fig. (2) mRNA expression of MMP-9 in all studied groups.

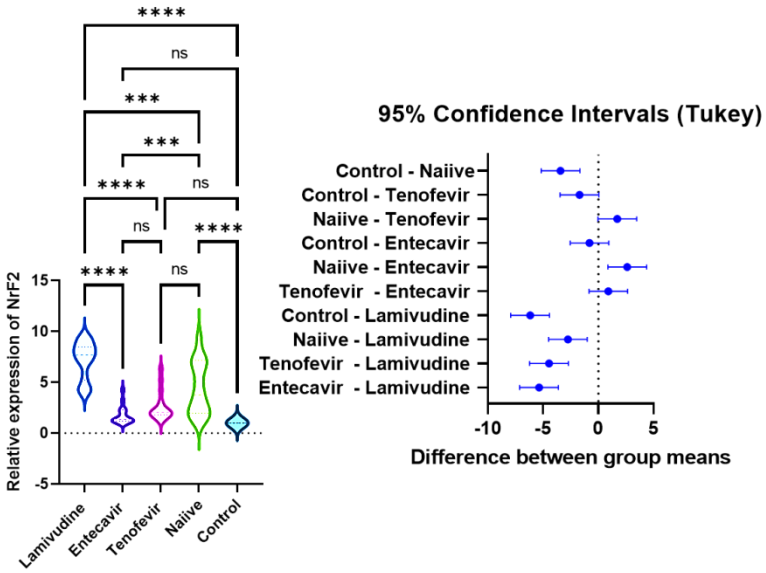

Fig. (3) mRNA expression of NrF2 in all studied groups.

4.Discussion

With individual differences in treatment results, the response to nucleoside analogues is an important part of managing patients with chronic hepatitis B virus (HBV) infection [12]. Optimizing therapy approaches and enhancing clinical outcomes need an understanding of the molecular processes driving treatment response [13]. In this work, we examined the response of Egyptian patients with hepatitis B virus to nucleoside analogues via the pathways of matrix metalloproteinase-9 (MMP-9) and nuclear factor erythroid 2-related factor 2 (NrF2).

Infection with HBV is still a major worldwide public health concern, especially in areas where the virus is highly prevalent, such as Egypt [14]. Prolonged HBV infection may cause serious liver-related problems, such as HCC and liver cirrhosis, which place a significant financial strain on healthcare systems and economies throughout the globe. The mainstay of antiviral treatment for chronic HBV infection is nucleoside analogues, which include entecavir, tenofovir, and lamivudine [15]. These drugs reduce viral replication and stop the course of the illness. However, patient responses to nucleoside analogue therapy might differ according to host immunological response, viral alterations, and viral genotype [4].

In this work, we primarily examined MMP-9 and NrF2 as important regulators connected to the pathophysiology of HBV and the response

to antiviral therapy. MMP-9, a member of the MMP family, is essential for tissue remodeling and the breakdown of the extracellular matrix [16]. In chronic HBV infection, elevated MMP-9 levels have been linked to inflammation, liver fibrosis, and disease progression. MMP-9 may also be a predictive biomarker for treatment response since it has also been linked to antiviral medication resistance [17].

The transcription factor NrF2, on the other hand, controls the expression of detoxifying and antioxidant enzymes that are involved in cellular defense against oxidative stress. A number of liver illnesses, including HBV infection, have been linked to dysregulation of NrF2 signaling, which may affect how well nucleoside analog treatments work [18]. In HBV-infected individuals, NrF2 signaling activation has been linked to better antiviral immune responses and better treatment results [8].

428 HBV patients and 102 control participants who were seen at the outpatient clinic were included in our research. To ascertain the genetic expression of MMP-9 and NrF2 in patient samples, we used PCR. The patients treated with various nucleoside analogues showed substantial changes in MMP-9 and NrF2 expression levels, according to the data [19]. In particular, entecavir medication was linked to a noteworthy decrease in MMP-9 expression in contrast to the naïve or control groups, suggesting that it is effective in lowering MMP-9 levels. Additionally, while to a lower degree than MMP-9, entecavir therapy demonstrated a similar tendency to diminish NrF2 expression [20]. According to these results, entecavir may lessen the inflammation and fibrosis brought on by persistent HBV infection by regulating the activities of MMP-9 and NrF2.

5.Conclusion

Conclusively, our research offers significant understanding of the molecular processes that underlie the responsiveness of Egyptian HBV patients to nucleoside analogues via the MMP-9 and NrF2 pathways. The correlations between the levels of gene expression and treatment response that have been found underscore the potential of MMP-9 and NrF2 as prognostic biomarkers for the treatment outcomes of chronic HBV infection. To fully understand the specific mechanisms of action and investigate the therapeutic consequences of inhibiting the NrF2 and MMP-9 pathways in patients with HBV infection, further study is necessary. In the end, these discoveries could aid in the creation of tailored treatment plans to maximize clinical results in this group.

Conflict of interest: None

References

[1] S. Madihi, H. Syed, F. Lazar, A. Zyad, A. Benani, A systematic review of the current hepatitis B viral infection and hepatocellular carcinoma situation in Mediterranean countries, Biomed Res. Int. 2020 (2020).

[2] C. Chu, Natural history of chronic hepatitis B virus infection in adults with emphasis on the occurrence of cirrhosis and hepatocellular carcinoma, J. Gastroenterol. Hepatol. 15 (2000) E25–E30.

[3] J. Fung, C.-L. Lai, W.-K. Seto, M.-F. Yuen, Nucleoside/nucleotide analogues in the treatment of chronic hepatitis B, J. Antimicrob. Chemother. 66 (2011) 2715–2725.

[4] D. Ramesh, B.G. Vijayakumar, T. Kannan, Advances in nucleoside and nucleotide analogues in tackling human immunodeficiency virus and hepatitis virus infections, ChemMedChem. 16 (2021) 1403–1419.

[5] A. Ramezani, M.P. Nahad, E. Faghihloo, The role of Nrf2 transcription factor in viral infection, J. Cell. Biochem. 119 (2018) 6366–6382.

[6] G. V Halade, Y.-F. Jin, M.L. Lindsey, Matrix metalloproteinase (MMP)-9: a proximal biomarker for cardiac remodeling and a distal biomarker for inflammation, Pharmacol. Ther. 139 (2013) 32–40.

[7] M.L. Pall, S. Levine, Nrf2, a master regulator of detoxification and also antioxidant, anti-inflammatory and other cytoprotective mechanisms, is raised by health promoting factors, Sheng Li Xue Bao. 67 (2015) 1–18.

[8] D. Bender, E. Hildt, Effect of hepatitis viruses on the Nrf2/Keap1-signaling pathway and its impact on viral replication and pathogenesis, Int. J. Mol. Sci. 20 (2019) 4659.

[9] A.A. Attia, A. Elmetwalli, Screening of Occult Hepatitis B Virus Infection among Egyptian Blood Donors., Med. J. Viral Hepat. 5 (2021) 27–31.

[10] A.A. El-Shehawy, A. Elmetwalli, A.H. El-Far, S.A.E.-R. Mosallam, A.F. Salama, A.O. Babalghith, M.A.

Mahmoud, H. Mohany, M. Gaber, T. El-Sewedy, Thymoquinone, piperine, and sorafenib combinations attenuate liver and breast cancers progression: epigenetic and molecular docking approaches, BMC Complement. Med. Ther. 23 (2023) 1–21.

[11] A. Elmetwalli, S.M. Hashish, M.G. Hassan, M.A. El-Magd, S.A. El-Naggar, A.M. Tolba, A.F. Salama, Modulation of the oxidative damage, inflammation, and apoptosis-related genes by dicinnamoyl-L-tartaric acid in liver cancer, Naunyn. Schmiedebergs. Arch. Pharmacol. (2023) 1–13.

[12] J.L. Dienstag, Benefits and risks of nucleoside analog therapy for hepatitis B, Hepatology. 49 (2009) S112–S121.

[13] J.M. Llovet, R. Montal, D. Sia, R.S. Finn, Molecular therapies and precision medicine for hepatocellular carcinoma, Nat. Rev. Clin. Oncol. 15 (2018) 599–616.

[14] A. Elbahrawy, M.K. Ibrahim, A. Eliwa, M. Alboraie, A. Madian, H.H. Aly, Current situation of viral hepatitis in Egypt, Microbiol. Immunol. 65 (2021) 352–372.

[15] J. Fung, W. Seto, C. Lai, M. Yuen, Extrahepatic effects of nucleoside and nucleotide analogues in chronic hepatitis B treatment, J. Gastroenterol. Hepatol. 29 (2014) 428–434.

[16] P.E. Van den Steen, B. Dubois, I. Nelissen, P.M. Rudd, R.A. Dwek, G. Opdenakker, Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9), Crit. Rev. Biochem. Mol. Biol. 37 (2002) 375–536.

[17] T. Medeiros, G.N. Saraiva, L.A. Moraes, A.C. Gomes, G.S. Lacerda, P.E.C. Leite, E.B.C. Esberard, T.G. Andrade, A.R. Xavier, T. Quírico-Santos, Liver fibrosis improvement in chronic hepatitis C after direct acting-antivirals is accompanied by reduced profibrogenic biomarkers–a role for MMP-9/TIMP-1, Dig. Liver Dis. 52 (2020) 1170–1177.

[18] M. Galicia-Moreno, S. Lucano-Landeros, H.C. Monroy-Ramirez, J. Silva-Gomez, J. Gutierrez-Cuevas, A. Santos, J. Armendariz-Borunda, Roles of Nrf2 in liver diseases: molecular, pharmacological, and epigenetic aspects, Antioxidants. 9 (2020) 980.

[19] Y.I. Yang, E.Y. Estrada, J.F. Thompson, W. Liu, G.A. Rosenberg, Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat, J. Cereb. Blood Flow Metab. 27 (2007) 697–709.

[20] X. Cao, N. Zhang, H. Chen, W. Wang, Y. Liang, J. Zhang, R. Liu, S. Li, Y. Yao, Q. Jin, Exploring the mechanism of JiGuCao capsule formula on treating hepatitis B virus infection via network pharmacology analysis and in vivo/vitro experiment verification, Front. Pharmacol. 14 (2023) 1159094.