BioBacta

Journal of Medical and Life Science
https://jmals.journals.ekb.eg/

Efficacy and safety of Tenofovir Disoproxil Fumarate in Egyptian patients with chronic hepatitis B virus infection

Othman Ali Othman [1], Sahar Sayed Kamal[2]

[1]Chemistry Department (Biochemistry Division), Faculty of Science Minia University, 61519 El-Minya, Egypt.

[2]Malawi Specialized Hospital (Laboratory Department) Malawi, Minia, Egypt.

Corresponding Author: -

Othman Ali Othman - Chemistry department (Biochemistry Division), Faculty of Science Minia University, 61519 El-Minya, Egypt- (Tel: 00201099632168)

e mail: osman.mouftah@mu.edu.eg-ORCID: http://orcid.org/0000-0003-4061-6929

DOI: 10.21608/jmals.2024.260653.1016

Abstract

Background: Hepatitis B virus (HBV) infection is a major global health problem leading to severe liver diseases such as cirrhosis and hepatocellular carcinoma. Tenofovir Disoproxil Fumarate (TDF) is an orally bioavailable prodrug of Tenofovir. This study aimed to evaluate the efficacy and safety of long-term TDF therapy in Egyptian Chronic Hepatitis B patients, **Objective:** Assessment of effectiveness and safety of TDF therapy in Egyptian HBV patients. **Materials and Methods:** HBV was confirmed by detection of both HBsAg and HBV DNA. All patients were given antiviral drug (300 mg TDF, oral, once a day for 3 months). **Results:** Before treatment; HBV Patients showed significant elevation in Ferritin, Albumin, S. Creatinine, AST, ALT, PO4, INR, and PT compared to the healthy volunteers' group. Additionally showed a decrease in AFP, DB, TB, PC, PLT, and WBCs again in comparison to the control group. After receiving HBV TDF, the same group of patients showed a significant increase in Ferritin, S. Creatinine, TB, PC, WBCs, and Hb% and a significant decrease in PO4, Albumin, INR, and PT compared to pre-treatment conditions. **Conclusion:** we show clear benefits of TDF therapy in ethnically diverse CHB populations, even among patients with only mild/moderate liver disease at baseline.

Keywords: Hepatitis B virus, Tenofovir Disoproxil Fumarate, Alfa fetoprotein, Ferritin, liver enzymes

---

Introduction

With an estimated global incidence of 500,000–1.2 million people die every year because of diseases attributable to chronic HBV infection such as liver failure and hepatocellular carcinoma (HCC) and approximately 2 billion people worldwide have been exposed to HBV infection and 350 million people have chronic HBV infection Hence HBV infection becomes a major global public health problem **[1].** The ninth leading cause of death is HBV **[2].** HBV infection remains common despite the presence of HBV vaccine. In susceptible individuals, primary HBV infection can be either symptomatic or asymptomatic **[3].** It was originally called serum hepatitis **[4].** HBV, which affects the liver, causes both acute and chronic infections. During the initial infection, many people have no symptoms whereas others develop a rapid onset of sickness with vomiting, tiredness, mucus membrane and yellowish skin, dark urine, and abdominal pain **[5].** The period

Received: November 10, 2023. Accepted: January 30, 2024. Published: February 5, 2024

of incubation for the symptoms to begin takes 3 to 6 months in 15 to 25% of those with chronic disease these complications may result in death **[6].** HBV is a partly double-stranded DNA virus that has 10 genotypes and more than 30 subtypes **[7].** Less than 10% of people at least five years old when infected and 90% of those contaminated around the time of conception develop CHB **[2].** The prevalence of HBV in Egypt was 1.4%, with low rates of HBV-HCV co-infection although an exceptionally high prevalence of hepatitis C virus (HCV) nationwide **[8].** The virus is also transmitted by exposure to contaminated blood or body fluids. By holding hands, sharing eating, utensils, kissing, hugging, coughing, sneezing, or breastfeeding, HBV cannot be spread **[9].** The most common modes of transmission of HBV are perinatal, percutaneous, and sexual routes **[2].**

In areas where the disease is common, infection during the time of birth or from contact with other people's blood during childhood is the most frequent method by which HBV can be acquired but in the areas where the disease is rare, intravenous drug use, sexual intercourse, infectious blood and body fluid are the most frequent routes of infection **[2].**

Treatment for acute HBV infection is generally supportive. However, there is antiviral therapy for chronic hepatitis B but these antiviral treatments do not eradicate HBV although it does not completely remove the virus. The goal of chronic HBV infection treatment is to lower the risk of chronic liver disease (CLD) by suppressing HBV replication in the liver for a long time. Antiviral therapy given for a long time has been proven to reduce up to 50% of the risk of disease progression and the development of HCC **[10].** The therapeutic goals of current antiviral treatment are mainly virologic and biochemical responses related to the improvement of clinical outcomes **[11].**

TDF a nucleotide reverse transcriptase inhibitor is a highly effective agent in the treatment of HBV and is considered by some clinicians to be a first-line antiviral agent due to the lack of any documented resistance to the drug and its ability to reverse liver fibrosis **[12, 13].**

Materials and methods

Study design:

This study is a pre-treatment, post-treatment study, and control case study, including 71 HBV patients (53 males and 18 females) (aged 21 to 69 years, mean age 39.06±10.54) and this study included 71 healthy volunteers group age-matched (32 males and 39 females). Patients were recruited from the internal medicine department, of Malawi General Hospital, Minia, Egypt during the period from March to June 2020.

Ethical approval:

The study's protocol was approved by the Chemistry Department, Faculty of Science, Minia University, and by the Committee of "The Research Ethics Committee (P. No. HV 23/2020)".

Inclusion criteria included none of the patients had a history of habitual alcohol consumption or hepatocellular carcinoma. Moreover, all individuals were positive for HBsAg. All patients were negative test for anti-HIV antibodies. Exclusion criteria included patients with HCV infection.

Measurement of serum biomarkers

Blood samples were collected from all patients by vein puncture and a part of the blood was treated immediately with EDTA-K2 for Complete Blood Count, another part of the blood was immediately with sodium citrate for coagulation profile (prothrombin time, prothrombin concentration, and INR). The serum was separated from the rest of the blood samples by centrifugation at 3000 rpm for 5 min and frozen at −80°C until their analysis.

All serum samples were tested for HBsAg using the third-generation enzyme-linked immunosorbent assay (ELISA) testing (Murex HBsAg Version 3, DiaSorin, UK). Results were read using (Human Reader) universal micro-plate reader, (Human Instruments). All positive samples were retested using the same method (Double ELISA).

Serum was processed for the biochemical analysis of Albumin, Bilirubin (Direct and Total), Phosphorus (PO_4), Creatinine, Glucose, Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) (Automated photometer, StarDust MC 15, Germany). CBC is detected automatically by the Blood Cell Counter (Swelab, Sweden). Coagulation factors were detected by semi-automated with a PT kit (Stago) (Huma clot, Germany) according to the manufacturer's instructions Serum was processed for the chemiluminescence immunoassay of HBeAg, HCV, HIV, AFP, and Ferritin (Roche, Germany) by Cobas e 41(Germany) according to the manufacture instructions: Sandwich principle. HBV DNA was analyzed automatically by Qiagen Qiasymophony (DNA Extraction) and Rotorgene (DNA Detection).

Statistical analysis

All statistical analyses were done by a Statistical Package for Social Science (IBM SPSS) version 20 for Microsoft Windows and considered statistically significant at a two-sided $P < 0.05$. Numerical data were expressed as mean ± SD. When comparing the control group and pre-treatment patients group. We use an independent t-test. Additionally, when compared between the pre-treatment group and the post-treatment patients' group. We use paired t-test.

Result

Detailed clinical data of all studied groups are shown in Table (1).

As shown in Table (2) and Figure (1), the value of the tumor marker (AFP) was significantly decreased ($P<0.05$) in the pre-treatment group compared to the control group and also decreased in post-treatment group compared to pre-treatment group. Additionally, the level of ferritin (as a marker of inflammation) was very highly significant ($P <0.001$) in the pre-treatment group compared to the control group and in post-treatment very highly significantly ($P<0.001$) compared to pre-treatment group as shown in table (3) and figure (2).

The values of the functional liver enzymes (AST and ALT) were significantly ($P <0.05$) higher in the pre-treatment and post-treatment group compared to the control group. However, the value of AST in the post-treatment group was significantly decreased compared to the pre-treatment group as shown in Table (4) and Figure (3).

As shown in Table (2) and Figure (1), a significant elevation of serum Albumin (protein of the liver) was observed in the pre-treatment group compared to the control group ($P <0.001$). The levels of bilirubin (T.Bil and D.Bil) were decreased in the pre-treatment group compared to the control group. However, the value of T.Bil in the post-treatment group was slightly increased compared to the pre-treatment group as shown in Table (5) and Figure (4).

As shown in Table (5) and Figure (4), a significant elevation of serum creatinine was observed in pre-treatment and post-treatment group compared to the control group ($P<0.001$). Creatinine showed a significant decrease ($P<0.05$) in the post-treatment group compared to the pre-treatment group. However, the level of PO_4 was significantly increased ($P <0.05$) in the pre-treatment group compared to the control group and also significantly decreased in the post-treatment group compared to the pre-treatment group as shown in Table (2) and Figure (1).

No significant alternations were observed in the pre-treatment group when compared with the control group at the RBG level. Similarly, compared to the pre-treatment group, the post-treatment group developed no significant alternations in RBG level as shown in Table (3) and Figure (2).

As observed in Table (4) and Figure (3), a significant elevation of PT level was observed in a pre-treatment group compared to the control group ($P<0.05$), similarly, compared to the pre-treatment group, the post-treatment group developed a significant decrease in PT level. Additionally, a significant decrease in PC and INR was detected in the pre-treatment group compared to the post-

treatment group and a slight increase in the control group as shown in Tables (3&5) and Figures (2&4).

As observed in Table (4) and Figure (3), no significant alternations in Hb% level were observed in the pre-treatment group when compared with the control group. However, the level of Hb% was significantly increased (P<0.05) in the post-treatment group compared to the pre-treatment group. A significant decrease in WBCs was detected in the pre-treatment group compared to the control group (P <0.05). A significant elevation of WBCs level was observed in a post-treatment group compared to the pre-treatment group (P <0.05) as shown in Table (2) and Figure (1). As observed in Table (3) and Figure (2), the value of the PLT was significantly decreased (P<0.01) in the pre-treatment group compared to the control group and post-treatment group.

The results showed an improvement in some biochemical parameters after treatment by comparing them to infected samples, for example, creatinine, alpha-fetoprotein, phosphate, white blood cells, and albumin.

Tenofovir Disoproxil Fumarate is used to treat a certain type of chronic hepatitis B infection. It helps to decrease the amount of hepatitis B virus in the infected body by interfering with virus growth. TDF treatment is useful in improving liver function and tumor markers such as alpha-fetoprotein. It also led to improvement in some other factors such as creatinine, albumin, and ferritin.

Table 1: Detailed clinical data of all studied groups.

		Healthy volunteers group		**Patients**	
		NO.	%	NO.	%
Age	20-35	39	54.9	30	43.2
	>35	32	45.1	41	57.8
Gender	Male	32	45.1	53	74.6
	Female	39	54.9	18	25.4

Comparison between laboratory biomarkers of HBV patients (n=71) and healthy volunteers (n=71).

Table 2: Determination of AFP, ALB, PO_4 and WBCs

Groups	AFP (ng/mL)	ALB (g/dl)	PO_4 (mg/dl)	WBCs (10^3/ul)
Group 1 Control group (n=71)	2.83 ± 1.18	3.80 ± 0.27	3.40 ± 0.42	6.50±1.46
Group 2 HBV patients (pre-treatment) n=71	2.65 ± 1.12	4.10 ± 0.46*	3.92 ± 0.73 *	5.83±1.75*
Group 3 HBV patients (post-treatment) n=71	2.37 ± 1.04	3.60 ± 0.59 #	3.35 ± 0.79 #	6.53±2.07#

(*): Denotes significant difference in comparison to the control group,
(#): Denotes significant difference in comparison to the pre-treatment group.
AFP: Alpha-fetoprotein, PO_4: Phosphorus, ALB: Albumin, WBCs: White Blood Cells

Table 3: Determination of Ferritin, RBG, PC and PLT

Groups	Ferritin (ng/mL)	RBG (mg/dl)	PC (%)	PLT (10^3/ul)
Group 1 Control group (n=71)	47 ± 27.5	88.5 ± 9.5	100 ± 0	263.8 ± 62.9
Group 2 HBV patients (pre-treatment) n=71	71.7 ± 40.6 *	98.3 ± 31.6	90 ± 9.8 *	199.3 ± 70.0*
Group 3 HBV patients (post-treatment) n=71	122 ± 69.8 #	92.6 ± 18.3	97.4 ± 5.35 #	209.5 ± 45.0

(*): Denotes significant difference in comparison to the control group.
(#): Denotes significant difference in comparison to the pre-treatment group.
R.B.G: Random Blood Glucose, PC: Prothrombin Concentration, PLT: Platelet

Table 4: Determination of ALT, AST, PT, and Hb%

Groups	ALT (U/L)	AST (U/L)	PT (Sec)	Hb % (g/dl)
Group 1 Control group (n=71)	21.3 ± 5.3	23.5 ± 4.9	12.4 ± 0	13.4±1.21
Group 2 HBV patients (pre-treatment) n=71	26.6 ± 11.1 *	27.0 ± 7.7 *	13.3 ± 0.88*	13.5±1.49
Group 3 HBV patients (post-treatment) n=71	25 ± 6.7	25.7 ± 5.7	12.65 ± 0.54#	14.1±1.99 #

(*): Denotes significant difference in comparison to the control group.
(#): Denotes significant difference in comparison to the pre-treatment group.
AST: Aspartate transaminase, ALT: Alanine aminotransferase, PT: Prothrombin Time, Hb%: Hemoglobin

Table 5: Determination of Creatinine, INR, T. Bil and D. Bil

Groups	Creatinine (mg/dl)	INR	T. Bil. (mg/dl)	D.Bil. (mg/dl)
Group 1 Control group (n=71)	0.75 ±0.16	1 ± 0	0.77 ± 0.1	0.14 ± 0.05
Group 2 HBV patients (pre-treatment) n=71	0.92 ± 0.15 *	1.08 ±0.09 *	0.68 ± 0.17*	0.11 ± 0.04*
Group 3 HBV patients (post-treatment) n=71	0.84 ± 0.07 #	1.02 ± 0.05 #	0.70 ± 0.16	0.11 ± 0.04

(*): Denotes significant difference in comparison to the control group,
(#): Denotes significant difference in comparison to the pre-treatment group,
T.B: Total Bilirubin, D.B: Direct Bilirubin, INR: International Normalized Ratio

Fig 1

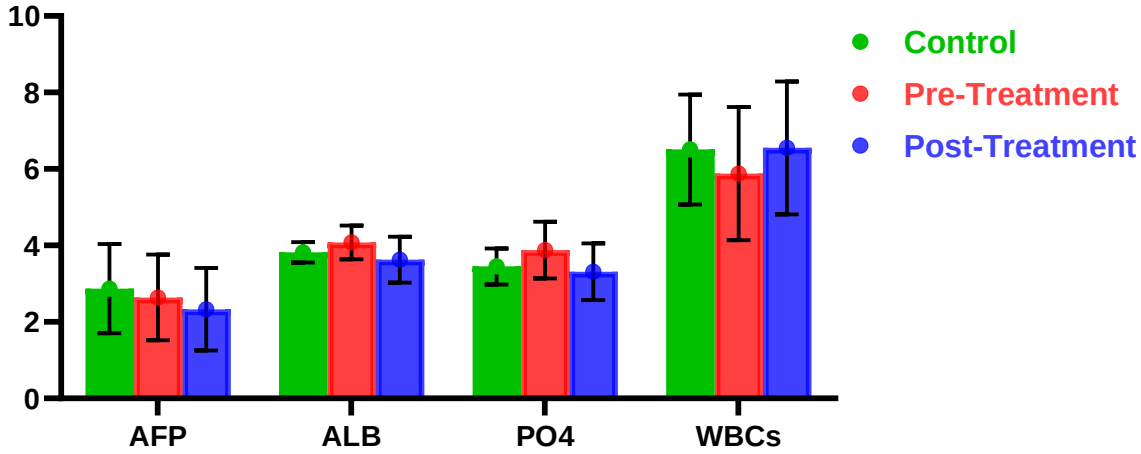

10
8
6
4
2
0
AFP
ALB
PO4
WBCs
Control
Pre-Treatment
Post-Treatment

Fig 2

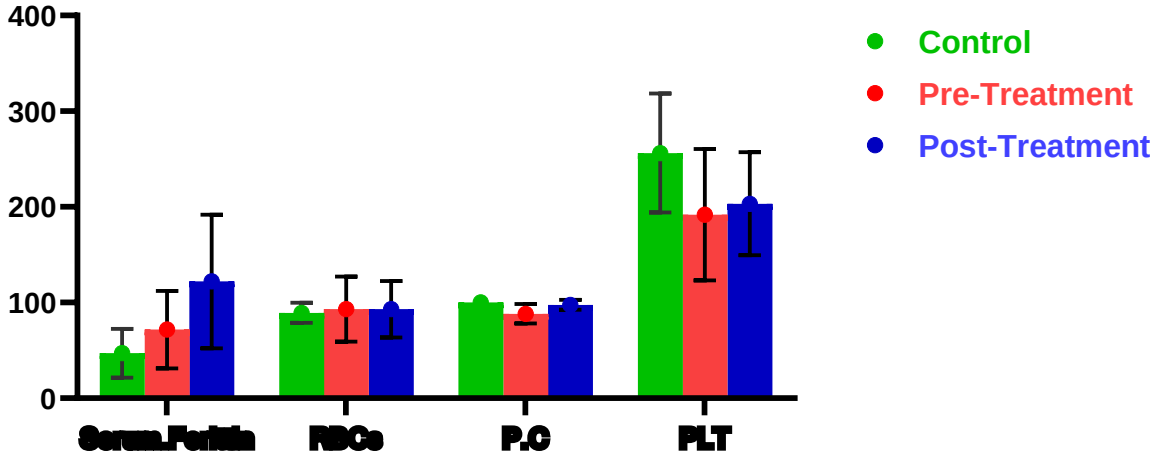

400
300
200
100
0
Serum Ferritin
RBCs
P.C
PLT
Control
Pre-Treatment
Post-Treatment

Fig 3

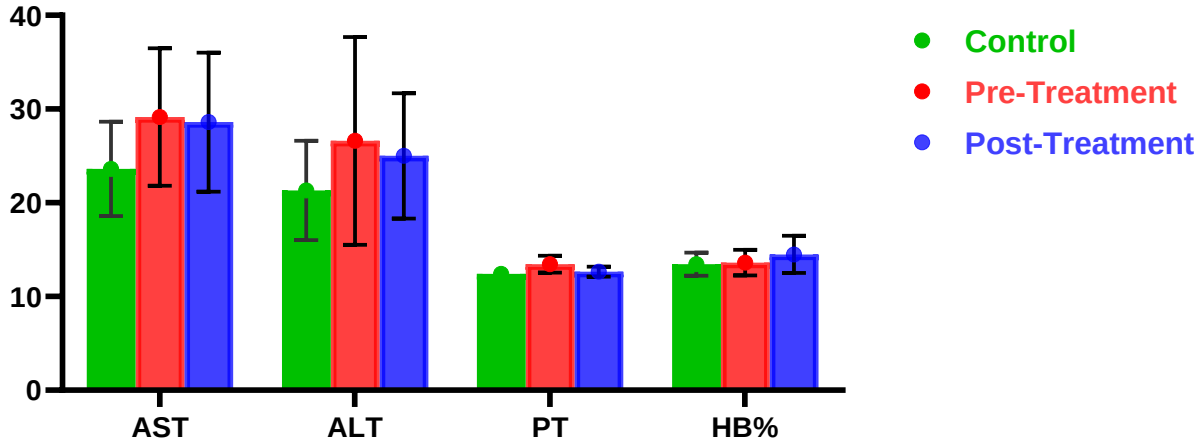
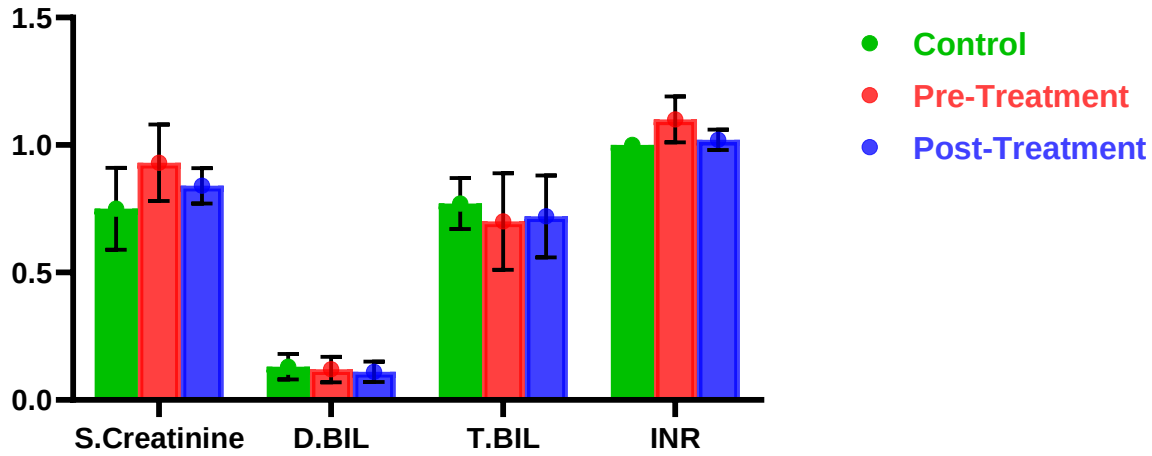

40
30
20
10
0
AST
ALT
PT
HB%
Control
Pre-Treatment
Post-Treatment

Fig 4


1.5
1.0
0.5
0.0
S.Creatinine
D.BIL
T.BIL
INR
Control
Pre-Treatment
Post-Treatment

Discussion

HBV infection is still prevalent worldwide and accounts for significant morbidity and mortality through the existence of hepatitis B vaccination. Most patients who have acute infection recover but those who have chronic disease are at high risk of having complications such as HCC, cirrhosis, and liver failure. Many factors can influence HBV infection such as the host's immune status, age at infection, and level of viral replication **[3]**. HBV which affects the liver that an infectious disease caused by HBV that causes both acute and chronic infections **[5]**, this is the most common cause of HCC **[14]**.

Treatment for acute HBV infection is generally supportive. However, there is antiviral therapy for CHB, but these antiviral treatments do not eradicate HBV although it does not completely remove the virus. The goal of chronic HBV infection treatment is to lower the risk of CLD by suppressing HBV replication in the liver for a long time. Antiviral therapy given for a long time has been proven to reduce up to 50% of the risk of disease progression and the development of HCC **[10]**. The therapeutic goals of current antiviral treatment are mainly virologic and biochemical responses related to the improvement of clinical outcomes **[11].**

TDF is a potent antiviral therapy for CHB and is a nucleotide analogue (NA) recommended as first-line treatment for CHB, tenofovir was first developed as an antiviral for the treatment of HIV **[15]**.

Demographic data of study patients showed no statistically significant difference between sex and age distribution in HBV-infected patients and healthy volunteers. Also, BMI was shown that none of our patients was obese.

Based on previous background, in the last few years, there are limited data that describe the safety and efficacy of TDF in the world. The few studies that have been published describe populations in North America and Europe **[15-17].**

Increased levels of AFP are seen in inflammation of the liver (such as in chronic hepatitis), When AFP is used as a monitoring tool, decreasing levels indicate a response to treatment. This indicated that TDF has shown efficacy in reducing AFP levels, which agrees with a previous study **[18].**

Ferritin is an iron-containing protein with a highly symmetrical and stable structure **[19].** Hepatocellular necrosis is triggered by excessive iron deposition in the liver, which leads to additional damage **[20],** inflammation **[21]**, fibrosis **[22, 23],** and even carcinoma **[24].** Previous studies that increased serum ferritin levels are associated with an increased risk of primary hepatocellular carcinoma (PHC) **[25].** Iron overload, which is a significant risk factor for the development of HCC, is frequently linked to chronic viral hepatitis. This might be connected to iron's capacity to cause oxidative stress, which causes liver tissue damage and chronic inflammation**.** In our study, HBV patients before treatment show an increase in the level of ferritin, this is supported with done by Wang et al **[26]** and agrees with previous study **[27-29].**

When body tissue or an organ such as the liver or heart is diseased or damaged, additional AST and ALT are released into the bloodstream, causing levels of the enzyme to rise. Therefore, the amount of AST and ALT in the blood is directly related to the extent of the tissue damage**.** CHB infection is a common etiology of elevated ALT values worldwide. After treatment with TDF, we found that ALT and AST levels decreased, this indicates that hepatocytes excrete a minimal amount of ALT and AST So that reflecting the effectiveness of TDF on the disease progression of CHB patients **[30-33].**

The most prevalent protein in blood plasma is albumin **[34].** The osmotic pressure of blood is kept constant by albumin, an important protein for transporting and binding drugs for numerous compounds in plasma **[35].** A relatively small, negatively charged protein called albumin is created by liver cells is the most prevalent protein in

extracellular fluid and is responsible for over 70% of the plasma colloid osmotic pressure **[36].** Treatment of HBV that causes hypoalbuminemia can decrease the level of albumin back to normal. TDF has shown efficacy in reducing ALB levels, this indicates that TDF improves the progression of CHB patients.

Bilirubin is the final by-product of hemoglobin breakdown and is used to diagnose liver and blood diseases **[37].** Relevant studies in recent years have focused on the impact of bilirubin on various hepatic diseases. D- Bil may have a unique risk-reducing effect for non-alcoholic fatty liver disease (NAFLD), according to a recent study **[38].**

Creatinine is a by-product of creatine and phosphocreatine and it serves as an indicator of renal function **[39].** TDF treatment was associated with a higher incidence of acute kidney injury (AKI) **[40].** Renal function was decreased from baseline in CHB patients receiving TDF therapy, which indicates that the renal function of patients undergoing treatment with TDF should be monitored regularly **[41].**

Measurement of phosphorus in serum and urine is mainly performed to detect disorders of kidneys, bones, and parathyroid glands. Increased concentrations are found in renal failure, hypoparathyroidism, pseudo-hyperparathyroidism, and loss of calcium phosphate in bones and cells. Decreased values occur in malabsorption, hyperparathyroidism, and vitamin D deficiency **[42, 43].**

In the present study, HBV patients before treatment did not affect RBG levels compared to the control group. When patients were given TDF, we found that TDF has shown no effect on RBG levels.

Prothrombin time is dependent on factor VII, which declines faster than other liver-derived clotting factors for the most sensitive measurement of hepatic synthesis of clotting factors **[44].** International normalized ratio (INR) is the preferred test of choice for patients taking vitamin K antagonists (VKA). It can also be used to assess the risk of bleeding or the coagulation status of the patients **[45].** HBV, like other viral hepatitis, affects the liver, an organ with high metabolic activities, and reduces the synthesis of coagulation factors resulting in prolonged clotting time, and hypo fibrinogenemia.

INR above the therapeutic range is associated with an increased risk of bleeding among which the most concerning condition is an intracranial hemorrhage. Patients can also present with gastrointestinal bleeding, hematuria, or bleeding from any other site. This agrees with the previous study **[46].**

Marked anemia is an infrequent finding in patients with acute viral hepatitis **[47].** HBV patients before treatment showed a decrease in Hb levels but when patients were given TDF, we found that TDF has shown an increase in Hb levels. This agrees with a previous study **[48].** When white blood cells are abnormally high, this usually indicates that the immune system is fighting a disease or infection. In general, the symptoms associated with high white blood cells are primarily related to the problem behind their elevation. For example, high white blood cells are caused by Infection with B virus. In pre-treatment patients, show a decrease in the level of WBCs compared to the control group. After treatment with TDF, we found that TDF showed an increase in WBCs levels. Platelet count decrease may be the first indication of and is often directly proportional to the severity of liver failure **[49, 50].** HBV patients before treatment showed a decrease in the level of PLT, when patients were given TDF, PLT counts gradually increased.

Acknowledgments

We thank Dr Mohammed F. Abdelrahman, Malawi Specialized Hospital (Laboratory Department) Malawi. Minia, Egypt for facilitating the sample and diagnosis.

Conflict of interest: The authors have not disclosed any conflicts of interest.

Funding: This research received no external funding.

References

1. **Lavanchy D**. *Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures*. Journal of viral hepatitis. 2004;**11**(2): p. 97-107.
2. **Parveen R, Gen BD and Qing LH.** *NewAwareness Review on Hepatitis B Prevalence Transmission and Related Complications*, 2019, **8**(2), p. 316-321
3. **Pan CQ and Zhang JX.** *Natural history and clinical consequences of hepatitis B virus infection. International journal of medical sciences.* 2005;**2**(1): p. 36-40
4. **Barker LF, Shulman NR, Murray R, Hirschman RJ, Ratner, Diefenbach WC and Geller HM** .*Transmission of serum hepatitis.* Jama. 1970;**211**(9): p.1509-1512.
5. **Walsh N, Verster A, Rodolph M, and Akl EA.** *WHO guidance on the prevention of viral hepatitis B and C among people who inject drugs.* International Journal of Drug Policy. 2014;**25**(3) : p. 363-371
6. **Bivigou-Mboumba B, François-Souquière S, et al.,**. *Broad range of hepatitis B virus (HBV) patterns, dual circulation of quasi-subgenotype A3 and HBV/E and heterogeneous HBV mutations in HIV-positive patients in Gabon.* PloS one. 2016;**11**(1): p. 1-16 .
7. **Lin C-L and Kao J-H.** *Natural history of acute and chronic hepatitis B: the role of HBV genotypes and mutants.* Best Practice & Research Clinical Gastroenterology. 2017;**31**(3): p. 249-255.
8. **Ismail SA, Cuadros DF and Benova L.** *Hepatitis B in Egypt: A cross-sectional analysis of prevalence and risk factors for active infection from a nationwide survey.* Liver international. 2017;**37**(12): p:1814-1822.
9. **Jagjit S. Yadav Ph.D. and Renuka Kapoor Ph.D,** *Bloodborne Pathogens in the Workplace.* Patty's Toxicology. 2001.**1**(1): p. 535-558.
10. **Lok AS and McMahon BJ.** *Chronic hepatitis B.* Hepatology. 2007;**45**(2): p. 507-539.
11. **Naggie, S. and A.S. Lok,** *New Therapeutics for Hepatitis B: The Road to Cure.* Annual Review of Medicine, 2021;**72**(1): p. 93-105.
12. **Wong, V. and H. Chan**. *Chronic hepatitis B: a treatment update*. in *Seminars in liver disease*. 2013; **33**(2): 122-129
13. **Marcellin, P., et al.**, *Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study*. 2013. **381**(9865): p. 468-475.
14. **Singal, A.G. and H.B. El-Serag,** *Hepatocellular carcinoma from epidemiology to prevention: translating knowledge into practice.* Clinical gastroenterology and hepatology, 2015. **13**(12): p. 2140-2151.
15. **Grace C Lovett, Tin Nguyen, David M Iser, et al.,** *Efficacy and safety of tenofovir in chronic hepatitis B: Australian real world experience.* 2017; **9**(1): p. 48-56.
16. **Carey, I., et al.,** *P47 De-novo antiviral therapy with nucleos (t) ide analogues in 'real-life' patients with chronic hepatitis B infection: comparison of virological responses between lamivudine+ adefovir vs entecavir vs tenofovir therapy.* 2011. **60** (2): p. 22-23.
17. **Lampertico, P., et al.**, *Oc. 07.1 2-Year Effectiveness and Safety of Tenofovir in 302 Nuc-Naive Patients with Chronic Hepatitis B: A Multicenter European Study in Clinical Practice*. 2012: **44**(2): p . S70-S71.
18. **Lee, C.S., et al.,** *O-008: Change in Alpha-fetoprotein Levels in Chronic Hepatitis B*

Patients on Tenofovir Therapy. 2016. **2016**(1): p. 22-22.

19. **Laufberger VJSCB.** Sur la cristallisation de la ferritine. 1937;**19**(1):p. 1575-1582
20. **Olynyk, J., et al.,** *A long-term study of the interaction between iron and alcohol in an animal model of iron overload.* Journal of hepatology, 1995. **22**(6): p. 671-676.
21. **Deugnier, Y.M., et al.,** *Liver pathology in genetic hemochromatosis: a review of 135 homozygous cases and their bioclinical correlations.* Gastroenterology, 1992. **102**(6): p. 2050-2059.
22. **Arezzini, B., et al.,** *Iron overload enhances the development of experimental liver cirrhosis in mice.* The international journal of biochemistry & cell biology, 2003. **35**(4): p. 486-495.
23. **Gardi, C., et al.**, *Effect of free iron on collagen synthesis, cell proliferation and MMP-2 expression in rat hepatic stellate cells.* Biochemical pharmacology, 2002. **64**(7): p. 1139-1145.
24. **Niederau, C., et al.,** *Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis.* New England Journal of Medicine, 1985. **313**(20): p. 1256-1262.
25. **Hann, H.W.L., et al.**, *Increased serum ferritin in chronic liver disease: a risk factor for primary hepatocellular carcinoma.* 1989. **43**(3): p. 376-379.
26. **Wang, X.-h., et al.,** *The effect of hepatitis B virus infection on hepcidin expression in hepatitis B patients.* 2013. **43**(2): p. 126-134.
27. **Elkhalifa A.H.M.,** Association *between Iron Profile and Hepatitis B Viral Load in Sudanese Patients.* Sudan University of Science and Technology 2020; **5**(7): p .1-53.
28. **Thierry Granier , Béatrice Langlois d'Estaintot, et al.,** *Structural description of the active sites of mouse L-chain ferritin at 1.2 Å resolution.* 2003; **8** (1-2): p.105-111
29. **Wahid, I.B.A. and A.A.J.A.J.o.M.S. Salman,** *Estimation of Serum Ferritin Concentration among Hepatitis B infected Sudanese Patients in Khartoum (Sudan).* 2018; **3**(1): p.1-4.
30. **Hafkenscheid, J. and C.J.C.c. Dijt,** *Determination of serum aminotransferases: activation by pyridoxal-5'-phosphate in relation to substrate concentration.* 1979. **25**(1): p. 55-59.
31. **Sampson, E.J., et al.**, *An interlaboratory evaluation of the IFCC method for aspartate aminotransferase with use of purified enzyme materials.* 1980. **26**(8): p. 1156-1164.
32. **Purcell, G.V., D.B. Behenna, and P.R.J.C.c. Walsh,** *Alanine aminotransferase and aspartate aminotransferase measurements with two automated analyzers, SMAC and the ABA-100, compared.* 1979. **25**(5): p. 780-782.
33. **Daudé, M., et al.,** *Tenofovir Therapy in Hepatitis B Virus-Positive Solid-Organ Transplant Recipients.* 2011. **91**(8): p. 916-920.
34. **Peters Jr TJAicc.** *Serum albumin.* 1970; **13**(1): p. 37-111.
35. **Rabbani, G., et al.,** *Binding of tolperisone hydrochloride with human serum albumin: effects on the conformation, thermodynamics, and activity of HSA.* 2018. **15**(4): p. 1445-1456.
36. **Bernardi, M., et al.,** *Role of human albumin in the management of complications of liver cirrhosis.* 2014. **4**(4): p. 302-311.
37. **Fevery JJLI.** *Bilirubin in clinical practice: a review.* 2008; **28**(5): p. 592-605
38. **Tian, J., et al.,** *Association between bilirubin and risk of Non-Alcoholic Fatty Liver Disease based on a prospective cohort study.* 2016. **6**(1): p. 1-9.

39. **Price, C.P. and H.J.C.c.a. Finney,** *Developments in the assessment of glomerular filtration rate.* 2000. **297**(1-2): p. 55-66.
40. **Min, I.S., et al.**, *Treatment outcome and renal safety of 3-year tenofovir disoproxil fumarate therapy in chronic hepatitis B patients with preserved glomerular filtration rate.* 2019. **13**(1): p. 93.
41. **Woo Jin Jung, Jae Young Jang, et al.**,.*Effect of tenofovir on renal function in patients with chronic hepatitis B.* Medicine (Baltimore), 2018; **97**(7): p. 1-7.
42. **Thomas L.** *Alanine aminotransferase (ALT), Aspartate aminotransferase (AST).* Clinical Laboratory Diagnostics 1st ed Frankfurt: TH-Books Verlagsgesellschaft. 1998;**2**(7): p. 55-65
43. **Burtis CA.** *Tietz textbook of clinical chemistry:* Saunders; 1999: **23**(35) p. 104-105 .
44. **CHAN, P.C., et al.,** *Factors affecting the mortality of pediatric fulminant hepatic failure in relation to hepatitis B virus infection.* 2005. **20**(8): p. 1223-1227.
45. **Shikdar, S., R. Vashisht and P.T. Bhattacharya,** *International Normalized Ratio (INR)*. 2022: StatPearls Publishing, Treasure Island (FL.2018; 1(1) : p. 1-5.
46. **Yi, Y., et al.,** *Does Hepatitis B Virus Affect the Coagulation Parameters for HBV-Related Decompensated Cirrhosis?* 2021:**1**(8) p 1883-1887.
47. **Laffan, M.A. and R. Manning,** *Investigation of haemostasis,* in *Dacie and Lewis practical haematology*. 2012,**5**(1). p. 393-445.
48. **Fasola, F.A., et al.,** *Haematological parameters in Nigerians with acute viral hepatitis.* 2009. **1**(1): p. 27-31.
49. **Xu, X., et al.**, *Relationship Between Platelet to White Blood Cell Ratio and 30-Daycte Prognosis of Patients with Acute-on-Chronic Liver Failure.* 2021. **21**(9). : p. 1-10.
50. **Rüdiger E. Scharf,** *Thrombocytopenia and hemostatic changes in acute and chronic liver disease: pathophysiology, clinical and laboratory features, and management.* 2021. **10**(7): p. 1530.