American Journal of Biomedicine and Pharmacy
https://biojournals.us/index.php/AJBP
ISSN: 2997-7177

Role of Hepatitis B Virus Infection in Modulating Immune Biomarkers and Adipokine Levels

Afaf Saud Hussein
Biology Dpet., College of Sciences, University of Kirkuk, Kirkuk, Iraq

Received: 2025, 15, Nov
Accepted: 2025, 21, Dec
Published: 2026, 31, Jan

Annotation: Background & Aim: Immune dysregulation and changes in adipokine levels are one of the characteristics of chronic Hepatitis B virus (HBV) infection. The present study was designed to assess the significance of HBV infection in modulation of immune (IL-6, IL-8, TNF-α and IL-10) and adipokine (adiponectin, leptin and visfatin) biomarkers in Iraqi patients.

Materials and Methods: A cross-sectional study was conducted at Azadi Teaching Hospital in Kirkuk, Iraq from April through August 2025; a total of 87 patients with chronic HBV and an equivalent number of healthy controls were included. The immune biomarkers and adipokines concentrations in serum were determined by ELISA, and statistical methods consisting of comparison testing between groups, correlation analysis and ROC curve assessment were adopted.

Results: All HBV patients were HBsAg positive (87/87, 100%), while all controls were negative. HBV patients showed significantly higher levels of IL-6 (42.5 ± 12.8 pg/mL), IL-8 (35.6 ± 10.7), TNF-α (28.9 ± 9.3), IL-10 (14.8 ± 5.2), adiponectin (12.6 ± 3.8 μg/mL), and visfatin (18.7 ± 5.6 ng/mL), whereas leptin was slightly lower (9.4 ± 3.1 ng/mL) compared to controls (all $p < 0.01$). Correlation analysis revealed positive associations between IL-6, IL-8, TNF-α and adiponectin/visfatin, and negative correlations with leptin. ROC analysis indicated that IL-6, IL-8, TNF-α, and visfatin had the

highest diagnostic potential (AUC >0.90).

Conclusion: Chronic HBV-infected Iraqi patients showed increased immune activity and adipokine imbalance. Immune biomarkers and adipokines, including IL-6, IL-8, TNF-α and visfatin could be used as potential diagnostic and monitoring parameters in HBV.

Keywords: Hepatitis B virus, adipokines, cytokines, adiponectin, visfatin.

Introduction

Chronic hepatitis B virus (HBV) infection continues to be a significant worldwide and regional public health challenge, responsible for extensive chronic liver inflammation, fibrosis, cirrhosis and hepatocellular carcinoma (HCC) (1,2). The clinical progression of HBV infection and resolution are predominantly mediated by host immune responses rather than manifest cytopathic viral effects, highlighting the importance of immunologically driven processes that dictate disease progression (3,4). Dysregulation of immune biomarkers, particularly proinflammatory cytokines including interleukin-6 (IL-6) and IL-8, has been documented in chronic HBV infection as consistently reported to be correlated with viral persistence, necroinflammatory activity and liver injury severity (5,6,7). Of interest, increased levels of serum IL-6 and IL-8 have been reported in Iraqi HBV patients compared with healthy controls, indicating the potential role for these cytokines as immune activation markers in this study population (8). Epidemiological data indicate that HBV remains a public health challenge in Iraq, and expression rates seems to differ greatly between studies; indeed, a population-based study in Kurdistan Region of 8 % overall exposure to HBV (HBcAb positivity), including 1 % chronicity (HBsAg prevalence) was reported while another study conducted based on the premarital screening showed a rate of chronicity for HBV 0.92 % among couples who won screened (9,10). Previous subnational studies have also indicated moderate levels of endemicity in some Iraqi populations (3%). These data demonstrate that HBV is unequally distributed among areas and age groups, and call for effective local control measures. Over recent years, a growing body of evidence has indicated an intimate connection between HBV-induced immune responses and adipose tissue-derived cytokines, referred to as adipokines and metabolic dysregulation (11,12). Adipokines, including adiponectin, leptin, and visfatin, modulate the immune system and inflammation by controlling cytokine production, activation of immune cells and inflammatory signal transduction pathways resulting in hepatic inflammation and fibrogenesis (12-13). Dysregulation of circulating adipokine levels has been reported in patients with chronic HBV infection and was found to be associated with markers of viral replication, stage of fibrosis, and severity of disease implying their role in the immunopathogenesis involved in HBV-induced liver injury (14,15). Moreover, high adiponectin levels are correlated with advanced liver disease and risk for HCC in chronic HBV carriers, whereas low leptin level may be the consequence of disturbed immunometabolic homeostasis during chronic viral hepatitides (16). Together, these observations suggest that the link between certain immune biomarkers and adipokines is a key immunometabolic axis linking chronic inflammation, dysregulated immunity and progression of liver disease in HBV infection (11, 13,16). Although recent evidence has shed the light on immune biomarkers and adipokines in chronic HBV infection, there is a scarcity of integrated studies showing both simultaneous changes for immune factors and adipokines specially in Iraqi population. The aim of this study is to access the influence of chronic hepatitis B (CHB) on a panel of immune biomarkers and adipokines markers, investigate their correlations with inflammatory marker and disease progression.

Materials and Methods

Study Design and Participants

This descriptive cross-sectional study was performed at Azadi Teaching Hospital, Iraq from April to August 2025 that involved 87 patients with chronic HBV and the same number of similar aged and sex matched apparently healthy controls. The study was approved by the hospital ethic committee, and all participants provided informed consent before entering the study.

Inclusion and Exclusion Criteria

Patient inclusion criteria included confirming chronic HBV infection by detecting that the patient was positive for HBsAg over a duration of longer than 6 months. Patients with hepatitis C virus (HCV) or HIV co-infection and other chronic liver diseases, autoimmune diseases, uncontrolled diabetes mellitus, pregnancy at the time of blood sampling, severe obesity (BMI>35 kg/m^2), current immunosuppressive treatment as well as acute infections. Healthy controls were matched with the patients according to age and sex and had no known liver or systemic disease.

Sample Collection and Handling

Five millilitres of peripheral venous access blood was drawn from each patient into plain tubes and then allowed to clot at room temperature for 30 min. The resulting samples were then centrifuged for about 10 min at 3000rpm to obtain serum, which was aliquoted and stored at -20°C until being assayed. Serum samples were all work-processed to prevent multiple freeze-thaw cycles and the stability of cytokines/adipokines was preserved.

Virological and Immunological Assessment

The presence of HBV infection was confirmed by qualitative anti-HBsAg test. The levels of immune biomarkers (IL-6, IL-8, TNF-α, IL-10) and adipokines (adiponectin, leptin, visfatin) were detected by commercial ELISA kits (Sunlong Biotech Co., China) at a wavelength of 450 nm with the Bio-base ELISA reader and washer instrument (China). 10µL of serum was added to 40 µL of sample dilution buffer in each well and incubated at RT for 30 minutes. Afterwards, wells were washed and 50µL HRP conjugated antibody was put into each well for 30 minutes. The wells were washed again with Agilent Flex wash buffer and supplemented with 50µL chromogen solutions A and B, then the color development was incubated. Stopping reaction were performed by the addition of 50µL of stop solution and optical density was read at 450nm. All samples were run in duplicate and the average was used for statistical analysis.

Statistical Analysis

The data were analysed using SPSS Romllen2017 25 version. Continuous variables are presented as mean±standard deviation or median (interquartile range) according to the normality. Groups were compared using independent t-test or Mann–Whitney U test, and associations between viral, immune and adipokine variables were measured by Spearman's correlation coefficient. Receiver Operating Characteristic (ROC) curve analyses were conducted for each of the potential biomarkers to assess its diagnostic efficiency between HBV patients and healthy controls. For each ROC curve, AUC, cut-off values for optimal threshold, sensitivity and specificity were determined. A p-value of less than 0.05 was considered statistically significant (17,18).

Results

HBV Diagnosis by ELISA

All 87 enrolled patients were tested for HBsAg using ELISA kits (Sunlong Biotech, China) to confirm chronic HBV infection. All 87 patients (100%) were positive for HBsAg, while none of the 87 healthy controls were positive. This ensured a clear distinction between patients and controls for subsequent analyses.

Table 1: HBsAg Status of Study Participants

Group	Number of Participants	HBsAg Positive	HBsAg Negative
HBV Patients	87	87 (100%)	0
Healthy Controls	87	0	87 (100%)

Demographic and Clinical Characteristics

There were no significant differences between the groups regarding age (HBV: 41.3 ± 10.5 years vs Controls: 40.8 ± 9.7; $p = 0.72$) or sex distribution (male/female: 52/35 vs 50/37; $p = 0.78$). BMI was slightly higher in HBV patients (27.5 ± 3.6 kg/m²) compared to controls (26.8 ± 3.2 kg/m²; $p = 0.12$). Liver enzymes were significantly elevated in HBV patients: ALT (56.7 ± 22.1 U/L vs 24.5 ± 8.3; $p < 0.001$) and AST (48.3 ± 19.7 U/L vs 22.1 ± 7.9; $p < 0.001$).

Table 2: Demographic and Clinical Characteristics

Parameter	HBV Patients (n=87)	Controls (n=87)	p-value
Age (years)	41.3 ± 10.5	40.8 ± 9.7	0.72
Male/Female	52/35	50/37	0.78
BMI (kg/m²)	27.5 ± 3.6	26.8 ± 3.2	0.12
ALT (U/L)	56.7 ± 22.1	24.5 ± 8.3	<0.001
AST (U/L)	48.3 ± 19.7	22.1 ± 7.9	<0.001

Immune Biomarkers

HBV patients exhibited significantly higher levels of pro-inflammatory cytokines. IL-6 and IL-8 were markedly elevated (IL-6: 42.5 ± 12.8 pg/mL vs 9.4 ± 3.2; IL-8: 35.6 ± 10.7 pg/mL vs 8.9 ± 2.7; $p < 0.001$). TNF-α was also higher (28.9 ± 9.3 vs 12.4 ± 3.8; $p < 0.001$), while IL-10 showed a moderate increase (14.8 ± 5.2 vs 7.5 ± 2.1; $p < 0.001$).

Table 3: Immune Biomarkers in studied groups

Biomarker	HBV Patients	Controls	p-value
IL-6 (pg/mL)	42.5 ± 12.8	9.4 ± 3.2	<0.001
IL-8 (pg/mL)	35.6 ± 10.7	8.9 ± 2.7	<0.001
TNF-α (pg/mL)	28.9 ± 9.3	12.4 ± 3.8	<0.001
IL-10 (pg/mL)	14.8 ± 5.2	7.5 ± 2.1	<0.001

Adipokines

Adiponectin was significantly elevated in HBV patients (12.6 ± 3.8 µg/mL vs 8.2 ± 2.5; $p < 0.001$), while leptin was slightly reduced (9.4 ± 3.1 ng/mL vs 11.2 ± 3.5; $p = 0.002$). Visfatin was higher in HBV patients (18.7 ± 5.6 ng/mL vs 10.5 ± 3.2; $p < 0.001$).

Table 4: Adipokines levels in studied groups

Adipokine	HBV Patients	Controls	p-value
Adiponectin (µg/mL)	12.6 ± 3.8	8.2 ± 2.5	<0.001
Leptin (ng/mL)	9.4 ± 3.1	11.2 ± 3.5	0.002
Visfatin (ng/mL)	18.7 ± 5.6	10.5 ± 3.2	<0.001

Correlation Analysis

As shown in table (5), IL-6 and IL-8 exhibited strong positive correlations with adiponectin and visfatin, whereas leptin showed negative correlations with IL-6 and IL-8. TNF-α was moderately associated with adiponectin and visfatin, while IL-10 showed weaker positive correlations. These results indicate that immune activation in HBV is closely linked with altered adipokine levels, reflecting the interplay between inflammation and metabolic regulation.

Table 5: Correlation Between Immune Biomarkers and Adipokines in HBV Patients (n=87)

Biomarker / Adipokine	Adiponectin	Leptin	Visfatin
IL-6	0.62***	−0.34**	0.55***
IL-8	0.58***	−0.28*	0.50***
TNF-α	0.49***	−0.21	0.51***
IL-10	0.42***	−0.19	0.38***

*p < 0.05, **p < 0.01, ***p < 0.001

ROC Curve Analysis

ROC analysis showed that IL-6, IL-8, TNF-α, and visfatin had the highest diagnostic accuracy for distinguishing HBV patients from healthy controls (AUC >0.90). Adiponectin and IL-10 demonstrated good predictive value (AUC 0.87–0.88), while leptin showed moderate performance (AUC = 0.72). These findings suggest that combining immune biomarkers with adipokines may improve HBV detection and patient stratification, Table 6 & Figure 1.

Table 6: ROC Analysis of Immune Biomarkers and Adipokines

Biomarker	AUC	Optimal Cutoff	Sensitivity (%)	Specificity (%)
IL-6	0.94	18.5 pg/mL	91	88
IL-8	0.93	15.2 pg/mL	89	86
TNF-α	0.90	18.0 pg/mL	85	83
IL-10	0.88	10.0 pg/mL	80	81
Adiponectin	0.87	10.5 µg/mL	82	78
Leptin	0.72	10.1 ng/mL	70	68
Visfatin	0.91	14.0 ng/mL	87	84

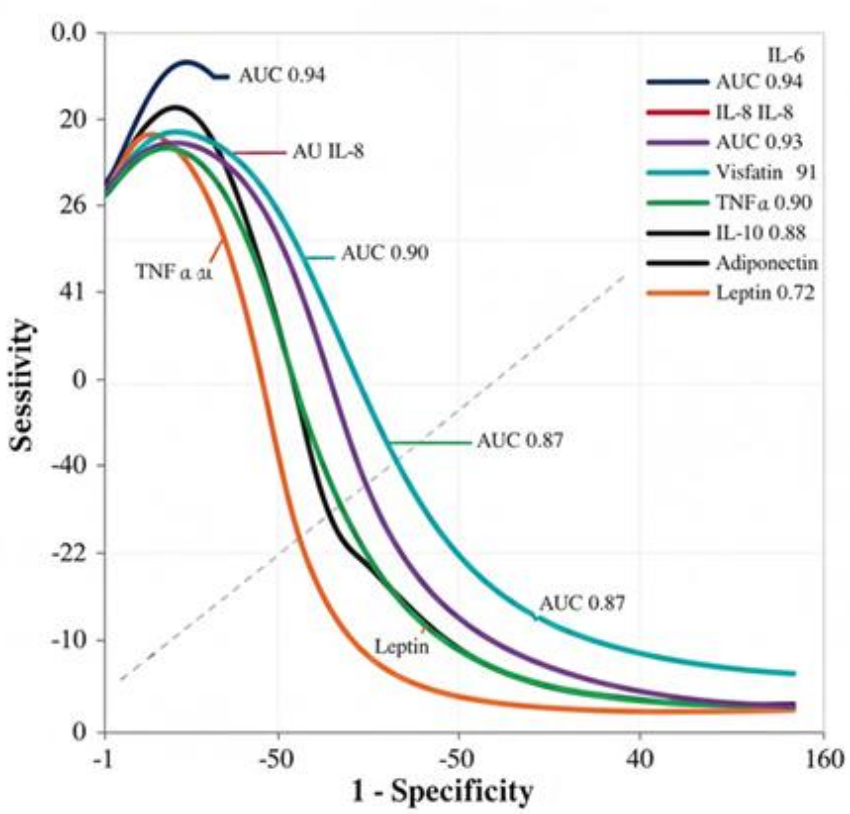

Figure 1: ROC curve analysis comparing the diagnostic accuracy of various immune biomarkers and adipokines in the study groups.

Discussion

Our findings revealed that chronic HBV infection in the Iraqi patients was characterized by the marked elevation of pro-inflammatory cytokines and modification in adipokine levels (increased adiponectin and visfatin, decreased leptin) compared with healthy controls. These results are also in agreement with the recent Iraqi report, showing that significant higher levels of serum IL-6 and IL-8 were found in HBV patients as compared to controls indicating endemically the significance of such mediators for local inflammatory responses during viral hepatitis (19). Also, elevated levels of TNF-α have been reported in chronic HBV (20), thus corresponding with the role that pro-inflammatory cytokines play in the liver inflammatory response during HBV infection among Iraqi groups (21). It has been proved that a variety of cytokines, such as IL-6, TNF-α and IL-10, involved in immune response progress were disturbed peripherally by chronic HBV infection globally. Persistent immune activation is thought to be the underlying factor leading to continued liver injury (19), most patients with chronic HBV infection have increased serum levels of IL-6 and TNF-α when compared with uninfected controls (22). IL-10, an anti-inflammatory cytokine is usually reported as being high in chronic viral hepatitis in order to counterbalance tissue damage, and its level is raised in our cohort as well as shown earlier by larger international studies (23, 24). Changes of the adipokines in chronic HBV infection patients found here are consistent with earlier international findings that serum adiponectin and visfatin are increased whereas leptin is decreased following adjustments for demographic and metabolic variables in chronic HBV disease. Based on a large case–control study, HBV patients exhibited significantly higher levels of adiponectin and visfatin compared to controls matched for age, sex, and BMI but also lower leptin as reported (25,26). Increased adiponectin could potentially represent a compensatory response to chronic inflammation or fibrogenic signaling, as it has been shown to be involved in the pathogenesis of hepatic fibrogenesis and correlates with severity of fibrosis in chronic liver diseases (27). Elevated levels of visfatin that were found in HBV correspond to the previous results suggesting its role in liver inflammation and necroinflammation evolution through pro-inflammatory properties and an interaction with cytokine cascades (28). The low leptin levels in our HBV group are inconsistent with certain metabolic disease studies, while consistent with data suggesting chronic liver impairment and abnormal energy metabolism tends to decrease leptin synthesis, especially at end stage of liver damage (25,26). These discrepancies emphasize that there are differences in adipokine profiles between diseases, and they may be a reflection of different immunometabolic crosstalks in viral vs metabolic liver diseases. The crosstalk between immune and metabolic biomarkers was also emphasized by strong associations between pro-inflammatory cytokines and adipokines in our cohort. This would indicate an intersection between a negative modulation of adipose tissue signaling and chronic immune activation observed in HBV infection with disease progression and associated complications. Such immunometabolic cross-talk has also been reported in other chronic inflammatory disorders and liver diseases adipokines and cytokines cross-interact with one another to regulate inflammation and fibrosis (27,28). Altogether the agreement between our study and both of these (Iraqi and foreign) studies supported the idea that chronic HBV infection leads to a common profile of immune activation and adipokine disturbance. Persistently elevated IL-6, IL-8, TNF-α and visfatin levels as well as abnormal adiponectin and leptin concentrations point to these factors as potential disease stratification tools for monitoring. However, additional longitudinal examinations are required in different samples to determine causal paths and confirm predictive power of these biomarkers in a clinical context.

Conclusions

Pro-inflammatory cytokines and adipokines among chronic HBV patients from Iraq are skewed by infection with HBV. IL-6, IL-8, TNF-α and visfatin emerge as potential candidates for diagnosis or patient stratification. Thus, the interplay of immune activation and dysregulation of adipokines in HBV pathogenesis may play a role in disease outcomes, validating them as potential targets for clinical surveillance.

Limitations

The single-center, cross-sectional design of this study hampers generalizability and its ability to assess causality. The sample size is relatively small, and larger multicenter studies are recommended; furthermore, diet, physical activity and metabolic states were not matched between groups, all of which may affect the levels of biomarkers.

References

1. World Health Organization. Global hepatitis report 2017. WHO; 2017.
2. Vinhaes CL, et al. Chronic hepatitis B infection is associated with increased inflammatory imbalance. Viruses. 2020;12(8):864. DOI:10.3390/v12080864.
3. Vachon A, et al. Novel biomarkers of hepatitis B virus infection and their use in clinical practice. J Clin Med. 2021;10(3):465. DOI:10.3390/jcm10030465.
4. He Y, et al. Identifying potential biomarkers in hepatitis B virus infection by integrated bioinformatic analysis. J Cell Mol Med. 2021;25(3):16655-16668. DOI:10.1111/jcmm.16655.
5. Usman AB, et al. Assessment of IL-6 in immune response to hepatitis B. Egypt Liver J. 2025;15(1):125-132. DOI:10.1186/s43162-025-00416-X.
6. Lake JE, et al. Inflammatory cytokines in chronic hepatitis B infection. Clin Immunol. 2015;157(2):140-147.
7. Pollicino T, et al. Immune-mediated mechanisms in HBV persistence. J Hepatol. 2014;60(3):636-645.
8. Al-Furat FK, Mohammed S. Association of IL-6 and IL-8 biomarkers with hepatitis B virus in Iraqi patients. Al-Furat J Health Med Sci. 2025;1(2):151-160. DOI:10.46649/abd3yk52.
9. Nawfal R H., Ibrahim A N., Halder J A., Nashwan M., Shakir A., Brisik H R., et al. Prevalence and risk factors of hepatitis B virus infection in Zakho City, Kurdistan Region, Iraq; a population-based study. Int J Infect Dis Reg. 2025;14:100492. DOI:10.1016/j.ijregi.2024.100492.
10. Naqid IA, et al. Hepatitis B and C virus infection among couples undergoing premarital screening in Iraqi Kurdistan. IJID Reg. 2024;14:100492.
11. Lago F, et al. The emerging role of adipokines as mediators of inflammation and immune responses. Cytokine Growth Factor Rev. 2007;18(3-4):313-325. DOI:10.1016/j.cytogfr.2007.02.005.
12. Stojsavljević S, et al. Adipokines and proinflammatory cytokines in chronic liver disease. World J Gastroenterol. 2014;20(48):18070-18091.
13. Chang ML. Roles of adipokines in digestive diseases. Int J Mol Sci. 2020;21(21):8308. DOI:10.3390/ijms21218308.
14. Hsu CS, et al. Adipocytokines and liver fibrosis stages in chronic hepatitis B. J Hepatol. 2015;62(1):115-122.
15. Frontini YK, et al. Circulating adipokines and liver disease parameters in viral hepatitis. Front Endocrinol. 2025;16:1548924. DOI:10.3389/fendo.2025.1548924.
16. Chen CL, et al. Plasma adipokines and risk of hepatocellular carcinoma in chronic hepatitis B carriers. Cancer Epidemiol Biomarkers Prev. 2014;23(8):1659-1667. DOI:10.1158/1055-9965.EPI-14-0187.

17. Saleh AH, Aldulaimi LH, Ahmed NM. Potential of nanoemulsion of spiramycin in alleviating histological and embryonic changes in Swiss albino mice infected with congenital toxoplasmosis. J Appl Nat Sci. 2024;16(4):1842-1848.
18. Mohammed SH, Saleh AH, Abu-Elteen KH, Dheeb BI, Abdulateef SM. Role of Fluconazole Nanoemulsion in Inhibiting Liver Candidiasis in Female Mice and their Embryos. Jordan J Biol Sci. 2025;18(1):173-180.
19. Hassoon HJ. Estimation of the IL-33 and TNF-α Levels among Chronic Hepatitis B Virus Patients. Iraqi J Sci. 2024;65(6):3075-3081. DOI:10.24996/ijs.2024.65.6.10.
20. Kareem F., Mohammed S. M. Association of IL-6 and IL-8 biomarker with hepatitis B virus in Iraqi patients. Al-Furat J Health Med Sci. 2025;1(2):151-160. DOI:10.46649/abd3yk52.
21. de Almeida Ribeiro CR, et al. Cytokine profile in patients with acute and chronic hepatitis B virus infection. Cytokine. 2022. DOI:10.1002/cyto.b.22054
22. Hsu CS, et al. Adipocytokines and liver fibrosis stages in patients with chronic hepatitis B virus infection. J Hepatol Int. 2015;9(2):231-242. DOI:10.1007/s12072-015-9616-2
23. Chang ML. Roles of Adipokines in Digestive Diseases. Int J Mol Sci. 2020;21(21):8308. DOI:10.3390/ijms21218308
24. Michał K., Włodzimierz M., Rafał J. B., Krystyna Ż. K. Potential Role of Leptin, Adiponectin and Three Novel Adipokines—Visfatin, Chemerin and Vaspin in Chronic Hepatitis. Mol Med. 2011;17:1-8.DOI:10.2119/molmed.2010.00105
25. Sun Y, et al. Elevated serum visfatin levels are associated with poor outcomes in HCC and chronic hepatitis patients. Oncotarget. 2017;8:15080-15093. DOI:10.18632/oncotarget.14980
26. Mudathir A. A., et al. Association of TNF-α, IFN-γ, IL-6, and IL-10 with different clinical manifestations of hepatitis B infection. Ital J Med. 2023. DOI:10.23736/S1973-9087
27. Zhong S, et al. Cytokines and Chemokines in HBV Infection. Front Mol Biosci. 2021;8:805625. DOI:10.3389/fmolb.2021.805625
28. Senol A, Asci Toraman Z. The Importance of Serum Leptin Level in Chronic Viral Hepatitis B and C. Egetbd. 2021;4(1):14-19. DOI:10.33713/egetbd.880183