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Original Article

Alterations of Some Apolipoprotein Levels and ApoE RNA Expression in Iraqi Patients with Chronic Hepatitis B Viral Infection

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Abstract

Background: The high prevalence of hepatitis B virus (HBV) makes it a significant health concern, especially in Iraq. Clinical studies have found that chronic HBV infection affects the occurrence of cardiovascular disease (CVD) by regulating cholesterol metabolism in liver cells. **Objective:** This study aimed to determine alterations in some serum lipoproteins (ApoA, ApoB, and apolipoprotein E [ApoE]) and oxidized low-density lipoprotein (OxLDL), as well as ApoE gene expression in Iraqi patients with chronic HBV infection, as potential markers for increased cardiovascular risk. **Materials and Methods:** This case-control study involved fifty patients with chronic HBV admitted to the Gastroenterology and Hepatology Teaching Hospital in Medical city, Baghdad, besides 40 individuals serving as a healthy control group. **Results:** The current study showed significantly higher levels of ApoA, ApoB, ApoE, and OxLDL among patients compared to the control group. Additionally, HBV patients demonstrated lower expression of ApoE gene in HBV patients. The receiver operating characteristic curve analysis for all parameters showed high sensitivity and specificity, with area under the curve affirming their potential as biomarkers for increased CVD risk in patients with chronic HBV infection. **Conclusion:** The study reveals significant disparities in plasma levels of ApoA, ApoB, ApoE, and OxLDL between HBV patients and controls, along with reduced ApoE gene expression in these patients, suggesting a potential role of HBV in apolipoproteins dysfunction. Thus, serum ApoA, ApoB, ApoE, and OxLDL in CHB patients may help identify high-risk patients for CVD, thereby preventing the development of CVD or early diagnosis of (CVD) in these patients.

Keywords: ApoE gene expression, apolipoproteins, chronic HBV infection

Introduction

Hepatitis B virus (HBV) infection is a major global health concern, affecting millions of individuals worldwide. While the burden of HBV infection is significant worldwide, the situation in Iraq is particularly noteworthy due to the high prevalence of HBV infection in the country.[1]

Lipoproteins play crucial roles in the uptake and transportation of dietary lipids in the small intestine, as well as in the movement of lipids between the liver, peripheral tissues, and the liver and intestine in a process known as reverse cholesterol transport.[2] Additionally, lipoproteins have a secondary function of facilitating the transport of harmful hydrophobic and amphipathic substances from sites of invasion and infection, including bacterial endotoxin.[3]

Apolipoproteins are protein molecules that bind with lipids to create lipoproteins, which play a crucial role in the transportation of lipids. These proteins are essential for maintaining the structural integrity and solubility of lipoproteins, as well as for facilitating the recognition of lipoprotein receptors and regulating specific enzymes

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involved in lipoprotein metabolism.[4] There are six types of apolipoproteins, namely A, B, C, D, E, and H. Although specific disorders related to apolipoproteins are rare, their significance of apolipoproteins in various clinical conditions is increasingly being recognized and understood.[5]

It was shown that there are some differences or variations in the production of apolipoproteins, enzymes, and lipids in patients with liver failure. These variations can be linked to the severity of liver failure. When the production of apolipoproteins and lipids in the blood decreases, it indicates a more severe form of liver failure.[6]

Apolipoproteins serve as cofactors for enzymes and ligands for receptors. Except for free fatty acids, all lipids in the bloodstream are not soluble in water and therefore require transportation in the form of lipoproteins. These lipoproteins consist of a core composed of cholesterol and triglycerides, enclosed by a phospholipid coat that contains the apolipoproteins.[7] Apolipoprotein A1 (ApoA1) serves as the primary protein in high-density lipoprotein (HDL) cholesterol and can be used as a measure of HDL concentration, commonly referred to as "good cholesterol." It is important to note that while there may be multiple ApoA1-positive cells, it does not precisely indicate the number of particles since each HDL particle contains one molecule.[8]

On the other hand, apolipoprotein B (ApoB) plays a vital role in very low-density lipoprotein (LDL), intermediate-density lipoprotein, and LDL cholesterol, often referred to as "bad cholesterol." Measuring the number of these particles in plasma is challenging because there is only one ApoB molecule per particle.[9]

Oxidized LDL (OxLDL) is a modified form of LDL that is, formed through oxidative stress. Chronic HBV infection has been associated with increased levels of OxLDL, which may contribute to the development of atherosclerosis and cardiovascular disease (CVD) in patients with HBV. A study by Huang *et al.* (2018) found that patients with chronic HBV infection had significantly higher levels of OxLDL compared to healthy controls.[10]

Among other countries in the Middle East, Iraq has been grappling with the challenges posed by chronic HBV infection. Limited research has been conducted to explore the various aspects of HBV infection among Iraqi patients, particularly regarding the assessment of some plasma apolipoproteins, OxLDL, and ApoE gene expression.

Understanding the expression and role of these biomarkers, including apolipoproteins A, B, E, and OxLDL in Iraqi patients with chronic HBV infection, is essential for gaining insights into the underlying mechanisms of the disease and potentially identifying new diagnostic and therapeutic targets.

The present study aimed to evaluate plasma levels of apolipoproteins A, B, E, and OxLDL, besides studying ApoE gene expression in a cohort of Iraqi patients suffering from chronic HBV infection. Our goal is to elucidate the potential link between these biomarkers and the occurrence of CVD in Iraqi patients with chronic HBV infection, and this work may enrich current knowledge and aid in devising targeted interventions and improved management strategies for the prevention of CVD in Iraq patients with chronic HBV infection.

Materials and Methods

This case-control study enrolled 90 participants, including 50 individuals with chronic HBV infection, aged 23–64 years (30 men and 20 women), admitted to the Gastroenterology and Hepatology Teaching Hospital in Medical City, Baghdad, during the period between December 2022 and May 2023. Patients were diagnosed by specialized physicians based on a patient's symptoms, clinical examination, medical history, and laboratory tests. Additionally, 40 apparently healthy individuals, age- and sex-matched individuals, served as the control group.

The inclusion criteria included a confirmed diagnosis of chronic HBV infection based on serological testing for positive HBsAg, specific viral load levels, and abnormal liver function tests. While exclusion criteria for HBV-infected patients included co-infection with other viral infections such as hepatitis C virus (HCV) or human immunodeficiency virus. Participants with liver diseases other than chronic hepatitis B, such as cirrhosis, fatty liver disease, liver cancer, or any other organic liver diseases, were also excluded.

Blood specimens were collected from participants using a standard venipuncture method, and sera were obtained and stored in Eppendorf tubes at recommended temperatures until analysis of apolipoproteins. Whole blood was used for RNA extraction.

ELISA was used for serum apolipoprotein A, B, E, and OxLDL analysis using commercially available kits according to the manufacturer's instructions (My BioSource, USA). ApoE RNA expression levels in patients with HBV were quantified using quantitative real-time polymerase chain reaction (qRT-PCR), following the protocol by Yadav *et al.* (2020).

ApoE RNA expression was normalized to the housekeeping gene GAPDH. The base sequences of ApoE (NM_000041.1) and GAPDH (NM_002046) were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/). Primers were designed using NCBI primer blast (https://www.ncbi.nlm.nih.gov/gene/) and synthesized.

RNA was extracted from blood samples using the TRIzol® reagent kit. The samples were homogenized and subjected to centrifugation, and the RNA pellet was dissolved in

Table 1: Primers used for quantitative polymerase chain reaction (qPCR)

Primer	Sequence	Reference
ApoE forward and reverse	GGGTCGCTTTTGGGATTACCTG CAACTCCTTCATGGTCTCGTCC	This study
GAPDH forward and reverse	TGCCACCCAGAAGACTGTGG TTCAGCTCAGGGATGACCTT	This study

nuclease-free water. The extracted RNA samples were stored at −80°C. The concentration of extracted total RNA was determined using a Quantus fluorometer system (Promega, USA) following the manufacturer's instructions.

qRT-PCR reaction mix was prepared with SYBR green master mix, forward and reverse primers for ApoE and GAPDH (listed in Table 1), and cDNA samples. The qRT-PCR was performed on an Applied Biosystems QuantStudio 5 system with the following thermocycling conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. Data were analyzed using the comparative Ct method ($2^{-\Delta\Delta Ct}$ method) to calculate the fold change in gene expression.

The statistical analysis was performed using GraphPad Prism version 9.2 (GraphPad Software Inc., LaJolla, CA, USA). Student *t* test and one-way analysis of variance were used to determine whether group variance was significant or not. Receiver operating characteristic (ROC) curve analysis was performed to determine AUC and the optimum parameter that best predicted the cutoff value. Statistical significance was determined at different levels: highly significant (**) with a $P < 0.01$, significant (*) with a $P < 0.05$, and insignificant with a $P > 0.05$.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki after gaining prior ethical clearance from the Iraqi Ministry of Health's Department of Medical Teaching City (no. 50562 on November 29, 2022). Verbal consent for participating in the study was obtained from each participant.

Results

The mean age of the patients was 40.90 ± 13.54 years compared to 39.15 ± 10.67 years for the control group. The age difference between the two groups was not significant ($P = 0.482$, data not shown).

In the present study, the results of apolipoprotein levels compared to the control group showed the following: the mean of ApoA was 9.236 ± 3.037 in patients and 5.934 ± 0.7192 in the control group; the mean of ApoB was 1.703 ± 0.3598 in patients and 1.035 ± 0.1444 in the control group; the mean ApoE was 26.65 ± 9.031 in patients and 16.20 ± 1.279 in the control group. For OxLDL, the mean was 58.25 ± 8.255 in the patient group and 22.54 ± 2.956 in the control group. A highly significant difference was observed with $P > 0.001$ among the patients group in comparison with the control group [Table 2].

Table 2: Level of apolipoproteins and OxLDL among study groups

Parameter (ng/mL)	Studied groups		*P* value
	Patients group	Control group	
ApoA	9.236 ± 3.037	5.934 ± 0.719	0.0001
ApoB	1.703 ± 0.359	1.035 ± 0.144	0.0001
ApoE	26.65 ± 9.031	16.20 ± 1.279	0.0001
OxLDL	58.25 ± 8.255	22.54 ± 2.956	0.0001

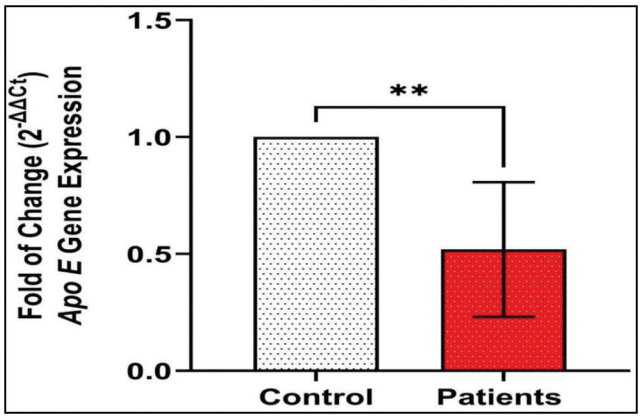

Figure 1: Fold change in ApoE gene expression among studied groups

Measuring the expression of ApoE using RT-PCR demonstrated that the control group had a higher mean expression level of ApoE compared to the patient group ($P = 0.0002$), as illustrated in Figure 1.

The ROC curve was used to analyze the investigated parameters among the studied groups, as seen in Figure 2. For ApoA, the sensitivity was 100%, the specificity was 90%, and the AUC was 0.988 ($P = 0.0001$), indicating a highly significant result. In the case of ApoB, sensitivity and specificity were 100% and 95%, respectively, while the AUC was 0.999 ($P < 0.0001$). As for ApoE, the sensitivity and specificity were 100% and 97.6% respectively, and the AUC was 1.0 ($P < 0.0001$). Finally, for OxLDL, sensitivity was 100% and specificity was 97.6%, with an AUC of 1.0 ($P < 0.0001$).

Discussion

HBV infection is a major cause of liver-associated morbidity and mortality, with a diverse spectrum of diseases affecting millions of people worldwide, cutting across genders and all age groups.[11] The current study

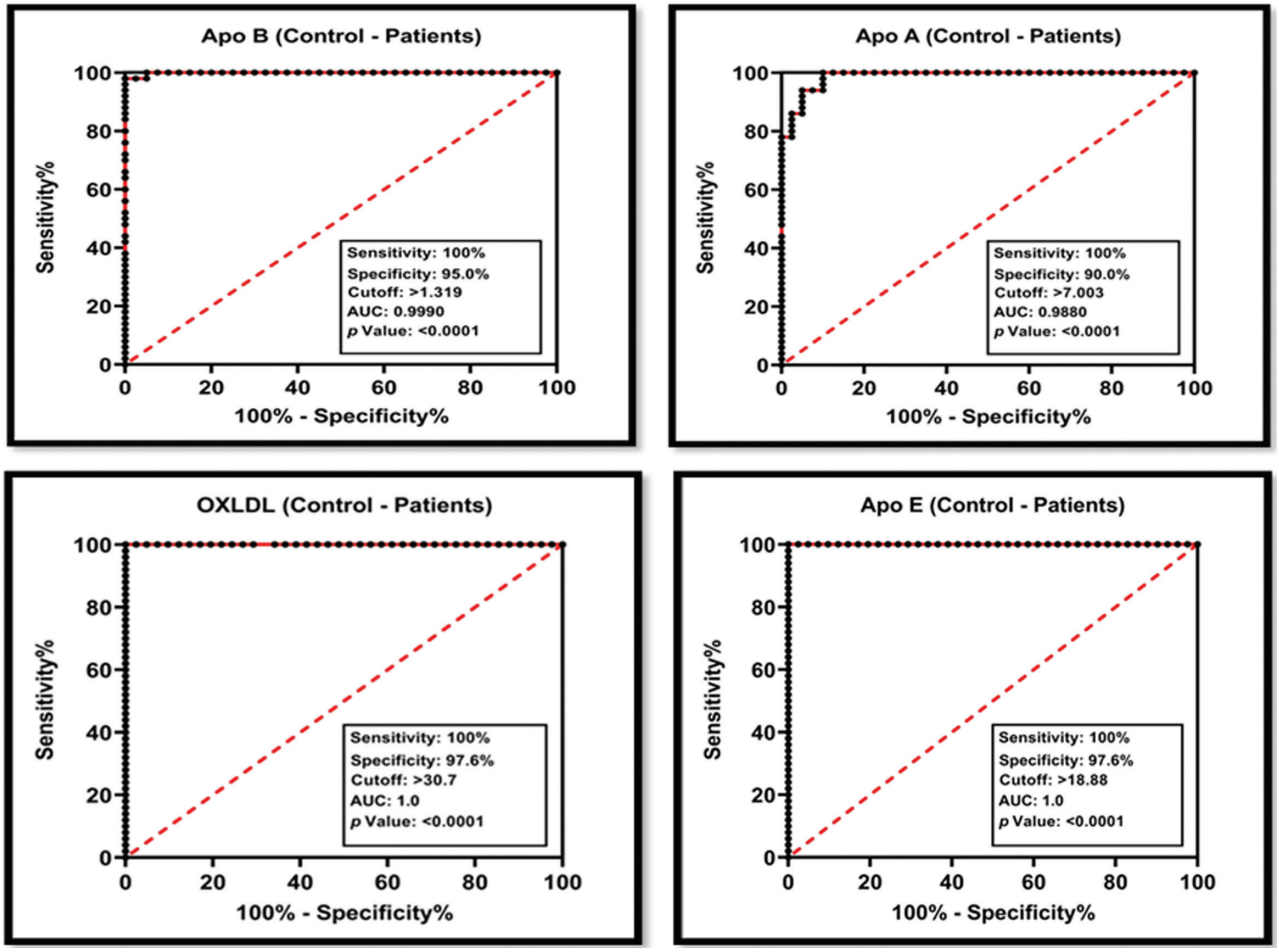

Figure 2: Receiver operating characteristic curve analysis of apolipoproteins and OxLDL across the study groups

revealed that the mean age of patients was 40.90 years, which is in agreement with previous studies[12,13] in Japan, where the majority of CHB patients were older, with a mean age of 65 years. These outcomes disagree somewhat with our study, and this variation may be related to the sample size and geographic distribution from country to country. The connection to care for CHB patients is suboptimal, even in high-income countries.[14]

The highly significant increase in ApoA levels among the patient group compared to the control group is in line with another study.[15] In this respect, some clinical studies in the HBV-infected population have found significantly decreased levels of plasma ApoA1.[16] These results contrast with our findings which may be attributed to differences in sample size besides individual differences in the subjects. The duration of hepatitis B infection, viral load, and the effect of antiviral therapy in these patients may be other factors that could have impacted the outcome of lipid levels.

It has been proposed that HBV can modulate ApoA1 function through HBx, leading to ApoA1 dysfunction, such as decreased self-association ability, increased carbonyl levels, and impaired lipid binding ability.[17]

The highly significant levels of ApoB and ApoE among patients, compared to the control group, are compatible with previous results[18] which reported that ApoB and ApoE levels were higher in both HBV and HCV patients than in healthy controls.

Furthermore,[19] a significant increase in ApoB levels in CHB patients was reported by another study, which is in concordance with the present study. In addition, a highly significant level of OxLDL was observed in the patient group compared to the control group, resembling published results (Huang *et al.* 2018). It has been found that excessive ApoB-containing particles are the main trigger in the atherogenic process.[20] Studies have shown that the higher the ApoB/ApoA1 ratio, the more cholesterol is deposited on the arterial wall, which triggers atherogenesis and thus increases CVD risk.[21]

The present results extend the current understanding of the effect of chronic HBV on the levels of apolipoprotein A, B, E, and OxLDL in blood specimens by providing evidence that there were significant differences in the levels of these hepatic biomarkers between the control and patient groups. Our findings were in line with previous studies which showed significant differences between the

control and patient groups and offer new insights into the effect of chronic HBV on the level of lipoproteins. These previous studies evaluated levels of lipoprotein(a), ApoA1, ApoB, and LDL in the plasma of individuals with acute coronary syndrome (ACS) compared to control individuals without ACS.[22] In a study conducted by Ruth Frikke-Schmidt, the author reviewed the literature on plasma levels of ApoE and the risk of dementia and ischemic heart disease and found significant differences in ApoE levels among different groups of patients and controls. While these reports do not provide a direct comparison of all the apolipoproteins we are interested in, they provide strong evidence that levels of apolipoproteins can significantly differ between patient and control groups, and these differences may have clinical implications.[23]

Several factors are known to influence apolipoprotein levels in patients with chronic HBV infection. These include viral load, disease duration, and individual metabolic factors.[24]

Recent research into the lifecycle of HBV has shown that the virus, along with its subviral envelope particles, also includes ApoE. ApoE is beneficial for the entry of HBV and is essential in the formation of infectious particles by interacting with the envelope glycoproteins of HBV.[25]

ApoE is known to play a critical role in lipid metabolism, and alterations in its expression might contribute to dysregulated lipid homeostasis observed in the patients. Furthermore, since ApoE is also implicated in inflammatory responses and immune regulation, its reduced expression may have implications for the disease pathogenesis or progression. Despite the high serum level of ApoE seen in CHB patients, lower ApoE RNA expression was noted in these patients as compared to the control group, which could be attributed to the fact that some genes have reduced correlation between RNA expression and protein levels, resulting in high protein levels with low RNA expression but extremely high translation efficiency.[26]

However, the precise mechanisms linking reduced ApoE expression to the clinical outcomes in the patient group warrant further investigation. This study's findings, therefore, not only underline the potential role of ApoE as a biomarker in this disease context but also pave the way for future mechanistic studies to elucidate the exact role of ApoE in disease pathology. However, these findings may not fully represent ApoE activity within the liver, the primary site of HBV impact. While blood samples offer insights into systemic changes, direct analysis of liver tissue could reveal more specific alterations in gene expression related to HBV pathogenesis.[27,28] Thus, future investigations should consider employing liver tissue samples to substantiate our initial findings.

Despite the statistically significant difference observed, it is important to bear in mind potential limitations. For instance, variations in individual genetic backgrounds or other confounding factors could also affect ApoE expression. Further research, preferably with larger sample sizes and possibly including different patient subgroups, would be beneficial to confirm these results and fully understand the implications.

The analysis of the apolipoproteins and OxLDL in HBV patients using the ROC curve has yielded significant findings. The current results indicate high sensitivity and specificity for all tested parameters, including ApoA, ApoB, ApoE), and OxLDL. The high sensitivity values (100% for all parameters) suggest that these biomarkers are extremely effective in correctly identifying HBV patients. The specificity values, ranging from 90% to 97.6%, imply that these parameters are also good at correctly identifying non-HBV cases, though there might be a small room for false positives.

The optimal cutoff values for each parameter (ApoA at 7.003, ApoB at 1.31, ApoE at 18.8, and OxLDL at 30.7) can be effectively used to distinguish between HBV patients and non-HBV cases. These cutoff points are the thresholds at which the sensitivity and specificity are maximized.

The AUC values close to 1 (0.988 for ApoA, 0.999 for ApoB, and 1.0 for both ApoE and OxLDL) indicate that these biomarkers provide almost perfect discrimination between HBV and non-HBV patients. AUC is an aggregate measure of performance across all possible classification thresholds, and an AUC of 1 represents a perfect test.

These results highlight the potential of ApoA, ApoB, ApoE, and OxLDL as powerful biomarkers in predicting chronic HBV patients with cardiovascular risk. The high sensitivity, specificity, and AUC suggest that these parameters are reliable indicators of the disease. The present findings are in agreement with what has been reported by previous studies on this topic.[29]

Future research should validate these findings in larger and more diverse populations to contribute to the early treatment of high-risk groups. Moreover, it would be of interest to understand the underlying biological mechanisms connecting these apolipoproteins and OxLDL with HBV, which could open avenues for novel therapeutic targets.

Conclusion

In conclusion, the current study provides novel insights into the alterations of apolipoprotein plasma levels and ApoE RNA expression in Iraqi patients with chronic hepatitis B. These findings underscore the potential involvement of apolipoproteins and ApoE gene expression in the pathophysiology of chronic hepatitis B, highlighting them as possible biomarkers or therapeutic targets. To the best of our knowledge, the present study is the first

to investigate ApoE RNA expression in patients with chronic HBV in Iraq, expanding our understanding of the molecular impacts of this viral infection. Furthermore, it would be interesting to explore how these findings might vary in different populations, under different co-morbid conditions, or at different stages of disease.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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