Objectives: Previous studies have revealed inconsistent results regarding the association between occult hepatitis B virus (HBV) infection and chronic kidney disease (CKD). Therefore, we conducted a prospective cohort study to evaluate the association between occult HBV infection and CKD.

Methods: A total of 4329 adults, aged 46.2 ± 13.7 years, without CKD at baseline were enrolled while undergoing physical examinations. Occult HBV infection was defined as seropositivity for antibody to HBV core antigen. CKD was defined as decreased estimated glomerular filtration rate (eGFR < 60 ml·min⁻¹·1.73 m²) or presence of proteinuria ≥1+, assessed using a repeated dipstick method. eGFR was computed using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Results: The prevalence of occult HBV infection was 8.1% (352/4329). During 5 years of follow-up, 165 patients (3.8%) developed CKD. Univariate Logistic regression analysis showed that occult HBV infection was positively associated with decreased eGFR, with an odds ratio (OR) of 2.15 (95% confidence interval (CI): 1.05–4.11). In contrast, occult HBV infection was not associated with either proteinuria or CKD (P > 0.05). After adjustment for potential confounders in the multivariate Logistic regression analysis, age, hypertension, diabetes, and the highest quartile of uric acid were associated with CKD, with ORs of 1.04 (95% CI: 1.02–1.05), 2.1 (95% CI: 1.46–3.01), 2.02 (95% CI: 1.36–2.99), and 1.86 (95% CI: 1.17–2.95), respectively. However, occult HBV infection was not associated with CKD, with an OR of 1.12 (95% CI: 0.65–1.95).

Conclusions: This study did not find an association between occult HBV infection and CKD. However, high-risk patients infected with HBV should still be targeted for monitoring for the development of CKD.

Keywords: Chronic kidney disease; Proteinuria; Estimated glomerular filtration rate; Hepatitis B virus
Introduction

Chronic kidney disease (CKD) has become a major worldwide public health problem over the past few decades, with increasing incidence and prevalence, high management costs, and poor treatment outcomes. A recent national survey in China has indicated that the prevalence of CKD is 10.8%, and the number of patients with CKD is estimated to be 119.5 million. The rapid increases in diabetes and hypertension are predicted to drive epidemics of CKD. However, this increased risk for CKD cannot be fully explained by traditional factors, including age, hypertension, diabetes, and dyslipidemia. Hepatitis B virus (HBV) infection, a common cause of liver disease and liver cancer, infects more than 400 million people worldwide. The prevalence of HBV infection varies significantly in different geographic areas of the world. In the mainland of China, the prevalence of seropositivity for HBV surface antigen (HBsAg) was found to be 7.18% and the number of patients with chronic HBV infection is estimated at 93 million.

It has been known for the past few decades that chronic HBV infection is associated with renal disease. Renal involvement is among the most common extrahepatic manifestations and is usually associated with membranous and membranoproliferative glomerulonephritis. The overall prevalence of HBV infection associated with nephropathy is not known. A study reported that about 3% of HBV-infected patients had glomerulonephritis. A cohort study of Chinese subjects with type 2 diabetes reported that chronic HBV infection was associated with an increased risk of end-stage renal disease (ESRD). In Taiwan of China, a cohort study also suggested that chronic HBV infection increased the risk of ESRD. However, several cross-sectional observational studies have shown inconsistent results regarding the association between HBV infection and CKD. They found that HBV infection was not associated with CKD in Chinese and Japanese adults. It has been observed that HBV infection is associated with nephropathy, but the association between occult HBV infection and CKD remains unclear. To help clarify this problem, we conducted a prospective cohort study to evaluate the association between occult HBV infection and CKD.

Methods

Study population

A total of 4874 adults who consecutively visited the Health Checkup Clinic in a large tertiary-care university hospital were enrolled from April 2008 to December 2008. Those participants came from all over Jinan to receive a regular paid health examination. Of these, 48 participants with proteinuria, 151 with hematuria, and 62 with decreased eGFR at baseline were excluded. Thus, 4613 participants were followed for 5 years; 198 were lost to follow-up. Patients with cirrhosis, hepatic carcinoma, HIV infection, and Hepatitis C virus infection were not included. We also excluded subjects who took nucleoside or nucleotide analogues (lamivudine, adefovir, entecavir, telbivudine) before the CKD event occurred. The ethics committee of Qianfoshan Hospital approved the study. All participants gave written informed consent prior to data collection.

Blood biochemistry measurements and biometric parameters at baseline

Blood was collected by venipuncture after an overnight fast of at least 10 h. Routine serum and urinary chemistry analyses were performed by standard automated techniques. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) two-level race equation. Serum creatinine level was measured using the Roche enzymatic method on an automatic biochemistry analyzer (Roche-Hitachi P-Module instrument with Roche Creatininase Plus assay, Hoffman-La Roche, Basel, Switzerland). Proteinuria was measured on a morning urine sample by using a repeated dipstick method. Participants with pyuria were excluded from the analysis of proteinuria due to possible urinary tract infection. Menstruating women were asked to delay the urine test until 3 days after menstruation. A dipstick result of trace or more urine protein was defined as proteinuria.

Serum sample was tested for the presence of HBV surface antigen (HBsAg) and antibodies to HBV core antigen (anti-HBc) and HBV e antigen (anti-HBe) by using enzyme-linked immunosorbent assay (Roche Diagnostics, Basel, Switzerland). Occult HBV infection was defined as seropositive for antibody to HBV core antigen. Fasting blood glucose, alanine transaminase (ALT), aspartate aminotransferase (AST), hemoglobin, serum uric acid (UA), serum total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were also measured using an automatic biochemistry analyzer. Total plasma alkaline phosphatase (ALP) level was assessed using colorimetric determination.
Sociodemographic characteristics, such as health history (e.g., hypertension and diabetes), were obtained by means of a questionnaire. The body mass index (BMI) was calculated as weight (in kilograms) divided by height squared (in square meters). Diabetes was defined as fasting blood glucose level $\geq 7.0$ mmol/L, the use of hypoglycemic agents, or self-reported history of diabetes. Blood pressure was measured using a sphygmomanometer, and 3 measurements were obtained at 5-min intervals. The mean of the 3 readings was calculated, unless the difference between the readings was greater than 10 mmHg, in which case the mean of the 2 closest measurements was used. Hypertension was defined as systolic blood pressure of more than 140 mmHg or diastolic blood pressure of more than 90 mmHg, or both, or the use of antihypertensive medication.

All of the investigators and staff members completed a training program to learn the methods and procedures of the study.

**Outcomes**

The eGFR was reevaluated using the same strategy after 5 years of follow-up. CKD was defined as decreased eGFR <60 ml·min$^{-1}·1.73$ m$^{-2}$ or presence of proteinuria (urine protein $\geq 1+$). A total of 86 participants were excluded from the analysis because of insufficient blood or urine results, and 4329 participants were included in the final analysis.

**Statistical analysis**

Data were presented as proportions for categorical variables and mean ± standard deviation (SD) or median [interquartile range (IQR)] for continuous variables. The significance of the differences in continuous variables between groups was tested using one-way analysis of variance. The difference in the distribution of categorical variables was tested using the Chi-square or Fisher's exact test. Univariate and multivariate Logistic regression analysis were used to estimate the association between HBV infection and CKD. Independent variables included age (continuous), gender, hypertension (yes/no), diabetes (yes/no), BMI (continuous), chronic carrier (yes/no) or occult HBV infection (yes/no), and levels of serum UA, total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol. Serum UA levels were analyzed in gender-specific quartiles. The 25th, 50th, and 75th percentiles for males were 300.0, 344.0, and 386.0 μmol/L, respectively. The 25th, 50th, and 75th percentiles for females were 224.0, 255.0, and 293.0 μmol/L, respectively.

Crude and adjusted odds ratios (ORs) with 95% confidence interval (CI) were reported. All analyses were performed using SPSS statistical package, version 16.0 (SPSS Inc, Chicago, IL, USA). All P-values were two-tailed. A P value of less than 0.05 was considered statistically significant.

**Results**

Among 4329 participants in the study, the mean age was 46.2 ± 13.7 years (range 18–84 years), and 67.2% (2907/4329) were men. The prevalence of occult HBV infection was 8.1% (352/4329). The mean eGFR was (97.0 ± 14.6) ml·min$^{-1}·1.73$ m$^{-2}$. Baseline characteristics of the participants stratified according to HBV status are shown in Table 1.

During 5 years of follow-up, the median change in eGFR was $-2.4$ ml·min$^{-1}·1.73$ m$^{-2}$ (IQR, $-7.4$–$2.9$ ml·min$^{-1}·1.73$ m$^{-2}$). A total of 165 patients (3.8%) developed CKD, with median change in eGFR of $-5.3$ ml·min$^{-1}·1.73$ m$^{-2}$ (IQR, $-14.0$–$4.0$ ml·min$^{-1}·1.73$ m$^{-2}$). The incidence of kidney damage according to HBV status is shown in Table 2. The percentages of decreased eGFR and incidence of CKD in patients with occult HBV infection were higher than in participants with no exposure to HBV, i.e., 2.6% vs. 1.2% ($P = 0.04$) and 4.8% vs. 3.7% ($P = 0.31$), respectively.

We analyzed the ORs of variables associated with CKD. Univariate Logistic regression analysis showed that occult HBV infection was positively associated with decreased eGFR, with an OR of 2.15 (95% CI: 1.17–3.97). In contrast, occult HBV infection was not associated with either proteinuria or CKD ($P > 0.05$) (Table 3). After adjustment for potential confounders in the multivariate Logistic regression analysis, age, hypertension, diabetes and the highest quartile of UA were associated with CKD, with ORs of 1.04 (95% CI: 1.02–1.05), 2.1 (95% CI: 1.46–3.01), 2.02 (95% CI: 1.36–2.99), and 1.86 (95% CI: 1.17–2.95), respectively. However, occult HBV infection was not associated with CKD, with an OR of 1.12 (95% CI: 0.65–1.95) (Table 4).

**Discussion**

Although renal involvement in HBV infection was first reported decades ago6–7, knowledge of the association between occult HBV infection and CKD remains limited. The current cohort study analyzed data
HBV infection associated with membranous nephropathy is well established, and there also have been reports of HBV association with membranoproliferative or proliferative glomerulonephritis. The association between chronic HBV infection and the development of CKD remains controversial. A 2-year multicenter cross-sectional study reported that CKD was highly prevalent in treatment-naive patients with chronic HBV infection. A cross-sectional study reported that HBV was not associated with CKD or proteinuria in 5424 HBV-infected adults. Similarly, a cross-sectional study of 328 HBV-infected adults in China reported that HBV was not associated with CKD or albuminuria. On the other hand, a cross-sectional Japanese study of 130 HBV-infected adults showed...
Table 4
Multivariate Logistic regression analysis for associations with CKD.

| Variables                | Multivariable adjusted OR (95% CI) | P   |
|--------------------------|------------------------------------|-----|
| Age                      | 1.04 (1.02–1.05)                   | 0.00|
| Gender                   | 0.67 (0.44–1.02)                   | 0.06|
| Hypertension             | 2.1 (1.46–3.01)                    | 0.00|
| Diabetes                 | 2.02 (1.36–2.99)                   | 0.00|
| BMI                      | 0.99 (0.94–1.05)                   | 0.88|
| UA (gender-specific quartiles)\(^b\) |                     |     |
| Quartile 1, Reference    |                                    |     |
| Quartile 2               | 0.95 (0.57–1.58)                   | 0.84|
| Quartile 3               | 0.99 (0.59–1.66)                   | 0.98|
| Quartile 4               | 1.86 (1.17–2.95)                   | 0.01|
| Occult HBV infection     | 1.12 (0.65–1.95)                   | 0.68|
| Total cholesterol        | 1.3 (0.83–2.04)                    | 0.25|
| Triglycerides            | 1.05 (0.88–1.26)                   | 0.58|
| LDL cholesterol          | 0.61 (0.37–1.01)                   | 0.05|
| HDL cholesterol          | 0.8 (0.39–1.61)                    | 0.53|

CKD: chronic kidney disease; OR: odds ratio; CI: confidence interval; BMI: body mass index; UA: uric acid; LDL: low-density lipoprotein; HDL: high-density lipoprotein.

\(^a\) Adjusted for age, gender, hypertension, diabetes, BMI, UA, previous exposure, total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol.

\(^b\) The 25th, 50th, and 75th percentiles for males were 300.0, 344.0, and 386.0 µmol/L, respectively. The 25th, 50th, and 75th percentiles for females were 224.0, 255.0, and 293.0 µmol/L, respectively.

that HBV was not associated with albuminuria, but is inversely associated with CKD.\(^{15}\) A 13-year nationwide cohort study of CKD risk among treatment-naive patients with chronic HBV showed that untreated chronic HBV infection was associated with an increased risk of CKD.\(^{25}\) The discrepancies among these studies may be attributed to the differences among the specific populations. The present study used a cohort design with a large dataset, which afforded considerable statistical power and allowed tracking of the incidence of CKD events over 5 years, but we did not find that occult HBV infection was associated with CKD.

The pathogenetic mechanisms by which individuals with HBV infection may develop nephropathy are not clearly defined. The most widely accepted mechanism is the deposition of immune complexes of viral antigen and host antibody.\(^{18}\) Experiments in animals by Germuth et al\(^{26}\) demonstrated that when an animal was exposed to a foreign antigen, nephritis developed, depending on the circulation of different proportions of antibodies and antigens. In the absence of antibodies, with the antigen present only in the serum, there was no nephritis. When the antigen persisted in the serum with low levels of antibody, chronic nephritis with subepithelial deposits developed. HBV infection is also associated with increased insulin resistance\(^{15}\) and intensification of oxidative stress\(^{27}\), and these may contribute to renal injury.\(^{28}\) Other research has revealed that HBV infection increase apoptosis in renal tubular cells via upregulation of Fas gene expression, and these patients had a higher circulating level of transforming growth factor-β, which is implicated in the potentiation of apoptosis and renal fibrosis.\(^{29}\) In this study, the cumulative incidence rate of CKD following occult HBV infection was only 4.8%. This indicates that occult HBV infection by itself is insufficient for the development of nephropathy but requires interplay of genetic and environmental factors in specifically vulnerable individuals.\(^{18}\) Nevertheless, most adults (30%–50%) with HBV-associated nephropathy progress to ESRD.\(^{30}\) It is estimated that 50 million new cases of HBV infection are diagnosed annually, despite a marked increase in vaccination rates.\(^{31}\) Therefore, it is conceivable that the burden of CKD following HBV infection is increasing.

This study has limitations. First, the effect of viral load evaluated by HBV-DNA levels was not assessed. Second, we only had 2 measurements of eGFR. Although there may be variations in laboratory measurements, this type of misclassification would tend to bias our study toward not finding an association, and may have underestimated the true association. Third, we used a single morning spot urine sample to assess microalbuminuria instead of timed urine collections. Finally, since our study was observational, the possibility of residual confounding by some unmeasured covariate exists.

In conclusion, this study did not find that occult HBV infection was associated with CKD. However, high-risk patients infected with HBV should still be targeted for monitoring for the development of CKD.

Conflicts of interest

The authors declare that they have no competing interests.

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