

Association between serum Cystatin C and renal injury in patients with chronic hepatitis B

Hui Zheng, MD^a, Haidong Liu, MD^b, Anhua Hao, MD^c, Min Zhang, MD^b, Dexin Wang, MD^{d,*}

Abstract

To explore the association between serum cystatin C (Cys-C) and renal damage in patients with chronic hepatitis B.

We retrospectively analyzed the clinical data of 425 patients with chronic hepatitis B virus (HBV) infection. Liver stiffness measured by FibroScan was used to diagnosis liver fibrosis. Cys-C levels were detected via latex-enhanced immunoturbidimetric assay.

A total of 425 patients were enrolled. Among them, 217 were patients with CHB with an eGFR > 90 mL/min/1.73 m² and 208 with an eGFR ≤ 90 mL/min/1.73 m². Cys-C levels significantly differed in patients with eGFR > 90 mL/min/1.73 m² compared with patients with eGFR ≤ 90 mL/min/1.73 m² (0.81 ± 0.05 vs 1.05 ± 0.06 mg/L, *P* < .001). Moreover, the Cys-C levels were 0.82 ± 0.04 mg/L in patients without liver fibrosis, 0.98 ± 0.05 mg/L in patients with mild liver fibrosis, 1.05 ± 0.08 mg/L in patients with advanced liver fibrosis, and 1.12 ± 0.07 mg/L in patients with liver cirrhosis (*P* < .001). Multivariate analyses were conducted to explore the independent factors associated with a decreased eGFR. Multivariate analysis suggested that T2DM (*P* = .032), liver fibrosis (*P* = .013), and Cys-C level (*P* = .035) were the independent factors associated with the decreased eGFR in patients with CHB. While age (*P* = .020) and Cys-C level (*P* = .001) were the independent factors associated with the decreased eGFR in patients with CHB-related fibrosis.

The fibrosis group had significantly higher Cys-C levels than those without fibrosis. Routine monitoring of Cys-C levels is of positive significance in preventing the development of renal impairment of CHB patients.

Abbreviations: CHB = chronic hepatitis B, Cys-C = cystatin C, eGFR = estimated glomerular filtration rate, GFR = glomerular filtration rate, HBV = hepatitis B virus.

Keywords: chronic hepatitis B, cystatin C, kidney injury, liver cirrhosis

1. Introduction

Chronic hepatitis B virus (HBV) infection is an important cause of liver cirrhosis.^[1] Studies have reported that patients with chronic

Editor: Leyi Wang.

HZ and HL contributed equally to this work.

The Institutional Review Board of Qingdao Sixth People's Hospital had approved this study. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent was obtained from all patients for inclusion in the study. The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

^a Department of Clinical Laboratory, Qingdao Fifth People's Hospital,

^b Department of Gastroenterology, Qingdao Sixth People's Hospital, ^c Hepatology Department, Qingdao Chengyang People's Hospital, Qingdao, ^d Department of Hepatology, Qingdao Sixth People's Hospital, Qingdao, China.

* Correspondence: Dexin Wang, Department of Hepatology, Qingdao Sixth People's Hospital, No. 9 Fushun Road, Qingdao 266033, China (e-mail: xhkzhangmin@126.com).

Copyright © 2020 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Zheng H, Liu H, Hao A, Zhang M, Wang D. Association between serum Cystatin C and renal injury in patients with chronic hepatitis B. *Medicine* 2020;99:32(e21551).

Received: 15 February 2020 / Received in final form: 5 June 2020 / Accepted: 4 July 2020

<http://dx.doi.org/10.1097/MD.00000000000021551>

hepatitis B (CHB) and liver cirrhosis have different degrees of renal injury as the disease progresses.^[2,3] A variety of mechanisms can induce renal function injury in patients with CHB.^[4] Studies have reported that, after the hemodynamic changes, renal injury will further reduce the glomerular filtration rate (GFR) and therefore increase the mortality rate.^[5–7]

At present, the serum creatinine level and estimated GFR (eGFR) commonly used in clinical practice cannot accurately and sensitively reflect early renal damage.^[8,9] Serum cystatin C (Cys-C), as a biomarker of renal injury, is a more specific and sensitive endogenous indicator.^[10–12] However, no study has reported the association between Cys-C levels and renal damage among patients with CHB.

Hence, this study analyzed the level of Cys-C and liver fibrosis in patients with chronic HBV infection and explored the association of Cys-C with renal injury in patients with CHB to improve early identification of renal damage in this population.

2. Subjects and methods

2.1. Subjects

This study used a retrospective analysis. We included patients with chronic HBV infection from January 1, 2017 to December 31, 2018. Patients were included if they were 18 years of age or older and tested positive for hepatitis B surface antigen > 6 months. Patients with kidney stones, kidney cysts, and other diseases causing kidney damage were excluded. This research was approved by the Ethics Committee of Qingdao Sixth People's Hospital, and all patients signed the informed consent.

2.2. Data collection

We used a questionnaire to collect data, including demographic data (sex, age, height, and weight) and laboratory test data. Liver stiffness measured by FibroScan was used to diagnosis liver fibrosis. HBV serological markers, HBV DNA quantification, and kidney-related detection markers, including creatinine and Cys-C, were also evaluated. According to FibroScan, the degree of liver fibrosis is classified as no fibrosis (liver stiffness <7.3 kPa), mild fibrosis (liver stiffness from 7.3 to 9.7 kPa), advanced fibrosis, (liver stiffness from 9.8 to 17.5 kPa), and liver cirrhosis (liver stiffness >17.5 kPa).^[13] GFR was estimated according to the MDRD formula: $eGFR \text{ (mL/min/1.73 m}^2\text{)} = 175 \times \text{blood creatinine}^{-1.234} \times \text{age}^{-0.179} \times 0.79$ (if female).^[14,15] Cys-C levels were detected via latex-enhanced immunoturbidimetric assay according to manufacturer's protocol (Shanghai Junrui Biotechnology Co., Ltd.). According to the international guidelines recommended by the KDIGO organization, we define $eGFR \geq 90 \text{ mL/min/1.73 m}^2$ as normal renal function.^[16]

2.3. Statistical methods

Data were analyzed using SPSS 13.0 software. Normally distributed data are expressed as mean \pm standard deviation. Comparisons between 2 groups were conducted using 2 independent samples *t* test. Multivariate forward Logistic Regression was conducted to evaluate risk factors associated with renal impairment. $P < .05$ was considered statistically significant.

3. Results

3.1. Patient demographics

A total of 425 patients were enrolled. Among them, 217 were patients with CHB with an $eGFR > 90 \text{ mL/min/1.73 m}^2$ and 208 with an $eGFR \leq 90 \text{ mL/min/1.73 m}^2$. The sex, age, alanine aminotransferase level, HBV DNA level, urea level, and proportion of hyperlipidaemia were similar for the two groups. There was a significantly higher proportion of patients diagnosed with type 2 diabetes mellitus (T2DM) in the group with $eGFR \leq 90 \text{ mL/min/1.73 m}^2$ (Table 1).

3.2. Renal function differs among patients with CHB

Cys-C levels significantly differed in patients with $eGFR > 90 \text{ mL/min/1.73 m}^2$ compared with patients with $eGFR \leq 90 \text{ mL/min/1.73 m}^2$ (0.81 ± 0.05 vs $1.05 \pm 0.06 \text{ mg/L}$, $P < .001$) (Fig. 1). To

Table 1
Baseline demographic and clinical characteristics by groups.

Variables	Chronic hepatitis B		P
	e-GFR > 90 ml/min	e-GFR ≤ 90 ml/min	
Sample size	217	208	
Sex, M/F	145/72	141/67	.832
Age, years	40.17 \pm 10.58	41.32 \pm 11.87	.292
Urea, mmol/L	4.42 \pm 0.23	4.49 \pm 0.67	.147
ALT	125.54 \pm 131.72	127.72 \pm 126.25	.862
HBV DNA log IU/mL	5.74 \pm 2.92	5.77 \pm 2.80	.914
T2DM, Y/N	25/192	39/169	.037
NAFLD, Y/N	63/154	53/155	.411
HBeAg, +/-	133/84	119/89	.392
Hyperlipidemia, Y/N	35/182	31/177	.727

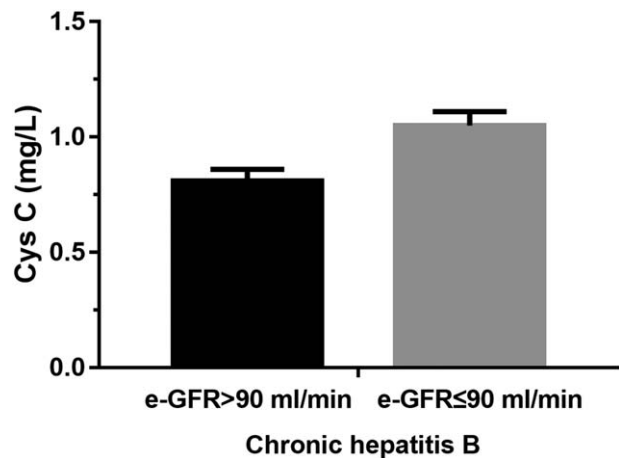


Figure 1. Cys-C levels in CHB patients with different eGFR level. Cys-C significantly differed in patients with $eGFR > 90 \text{ mL/min/1.73 m}^2$ compared with patients with $eGFR \leq 90 \text{ mL/min/1.73 m}^2$ (0.81 ± 0.05 vs $1.05 \pm 0.06 \text{ mg/L}$, $P < .001$).

further explore renal function in patients with CHB, we compared Cys-C level and eGFR in patients with different degrees of liver fibrosis. The results are shown in Figure 2. According liver stiffness values, 204, 124, 52, and 48 patients were diagnosed with no liver fibrosis, mild liver fibrosis, advanced liver fibrosis, and liver cirrhosis, respectively. The eGFR levels were, respectively, 105.3 ± 3.81 , 104.4 ± 3.57 , 97.17 ± 6.78 , and $91.6 \pm 5.15 \text{ mL/min/1.73 m}^2$ ($P < .001$). The eGFR decreased with the severity of liver fibrosis. Similar results were observed for serum Cys-C levels. The Cys-C levels were $0.82 \pm 0.04 \text{ mg/L}$ in patients without liver fibrosis, $0.98 \pm 0.05 \text{ mg/L}$ in patients with mild liver fibrosis, $1.05 \pm 0.08 \text{ mg/L}$ in patients with advanced liver fibrosis, and $1.12 \pm 0.07 \text{ mg/L}$ in patients with liver cirrhosis ($P < .001$).

3.3. Risk factors related to impaired renal function in all patients

Univariate and multivariate analyses were conducted to explore the independent factors associated with a decreased eGFR. The univariate analysis results showed that liver fibrosis, T2DM, and Cys-C level were the factors associated with the decrease in eGFR in patients with CHB. Multivariate analysis suggested that T2DM ($P = .032$), liver fibrosis ($P = .013$), and Cys-C level ($P = .035$) were the independent factors associated with the decreased eGFR in patients with CHB (Table 2).

3.4. Risk factors related to impaired renal function in patients with liver fibrosis

Univariate and multivariate analyses were conducted to explore the independent factors associated with decreased eGFR in patients with CHB-related fibrosis. The multivariate analysis suggested that age ($P = .020$) and Cys-C level ($P = .001$) were the independent factors associated with the decreased eGFR in patients with CHB-related fibrosis (Table 3).

4. Discussion

Chronic HBV infections not only lead to chronic liver damage but also can cause kidney damage through various mechanisms.^[17]

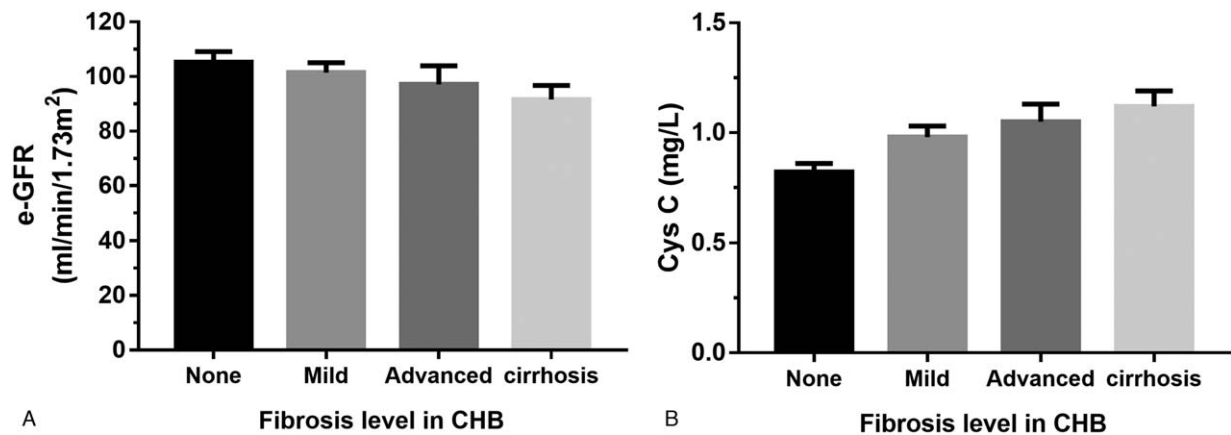


Figure 2. Cys-C levels in CHB patients with different degrees of liver fibrosis. A. The eGFR levels were 105.3 ± 3.81 mL/min/1.73 m² in patients without liver fibrosis (n = 204), 104.4 ± 3.57 mL/min/1.73 m² in patients with mild liver fibrosis (n = 124), 97.17 ± 6.78 mL/min/1.73 m² in patients with advanced liver fibrosis (n = 52), and 91.6 ± 5.15 mL/min/1.73 m² in liver cirrhosis (n = 48) (P < .001). B. The Cys-C levels were 0.82 ± 0.04 mg/L in patients without liver fibrosis, 0.98 ± 0.05 mg/L in patients with mild liver fibrosis, 1.05 ± 0.08 mg/L in patients with advanced liver fibrosis, and 1.12 ± 0.07 mg/L in patients with liver cirrhosis (P < .001).

Table 2
Risk factors for impaired renal function in CHB.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	P	OR	95% CI	P
Sex, M/F	0.987	0.467–1.195	.278			
Age, years	1.371	0.792–2.671	.189			
Urea, mmol/L	1.235	0.400–2.497	.840			
ALT	1.150	0.153–2.934	.403			
HBV DNA log IU/mL	0.740	0.307–1.512	.704			
T2DM, Y/N	1.348	1.081–2.308	.025	1.427	1.221–2.018	.032
NAFLD, Y/N	0.817	0.615–2.449	.631			
HBeAg, +/-	1.158	0.210–1.659	.459			
Hyperlipidemia, Y/N	0.640	0.572–1.248	.367			
Liver fibrosis, Y/N	1.235	1.119–2.716	.001	1.322	1.174–2.118	.013
Cys-C, mg/L	1.216	1.074–2.497	.020	1.343	1.015–2.470	.035

When chronic hepatitis caused by HBV progresses to the stage of end-stage liver cirrhosis, portal hypertension further causes systemic hemodynamic changes and kidney damage, while kidney damage further aggravates portal hypertension, forming a vicious cycle. Previous studies^[18,19] have shown that 29% of patients with HBV-related nephritis will develop renal failure, of which 10% need renal replacement therapy for end-stage renal

disease. Early kidney damage is not an organic change, and timely treatment can reverse kidney damage. A decreased GFR is considered the main manifestation of early kidney injury, but urea nitrogen and creatinine levels are susceptible to diet, body metabolic status, and the patient’s muscle condition. The eGFR calculated based on this is not sufficiently sensitive or accurate for clinical diagnosis of early kidney injury.

Table 3
Risk factors for impaired renal function in CHB with liver fibrosis.

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	P	OR	95% CI	P
Sex, M/F	0.561	0.183–1.622	.544			
Age, years	1.753	1.085–2.720	.012	1.812	1.241–2.027	.020
Urea, mmol/L	1.462	0.403–1.561	.335			
ALT	1.085	0.335–1.594	.447			
HBV DNA log IU/mL	0.777	0.437–1.840	.451			
T2DM, Y/N	1.048	0.987–1.687	.008			
NAFLD, Y/N	0.742	0.831–2.766	.258			
HBeAg, +/-	1.065	0.273–2.490	.792			
Hyperlipidemia, Y/N	0.467	0.129–1.348	.387			
Cys-C, mg/L	1.371	1.119–2.658	.003	1.028	1.105–2.274	.024

Cys-C is a secreted protein whose amino acid sequence is stably expressed in most human tissues, and its production rate is stable in the body.^[20–22] It is not affected by factors such as eating status, disease factors, or muscle content and is almost completely filtered by the glomeruli. It is not reabsorbed by the renal tubules and is a new index for evaluating renal function.^[23–25] Previous research^[26–29] showed that Cys-C is significantly better than creatinine and urea nitrogen, and it can better reflect the change of GFR. This study analyzed Cys-C levels in patients with chronic HBV infection. As a result, eGFR levels in patients with cirrhosis were significantly lower than in those without fibrosis. The fibrosis group and liver cirrhosis group had significantly higher Cys-C levels than those without fibrosis. Moreover, T2DM, liver fibrosis and Cys-C level were the independent factors associated with the decreased eGFR in patients with CHB. While age and Cys-C level were the independent factors associated with the decreased eGFR in patients with CHB-related fibrosis.

It is suggested that with the progress of liver disease, renal damage in patients worsens.^[17,30] Cys-C level can detect renal damage earlier than eGFR, creatinine, or urea nitrogen. It was found that the level of Cys-C increased significantly in patients with liver cirrhosis. It is suggested that Cys-C may have early diagnostic significance for both chronic HBV infection. However, it needs further study to confirm. Previous studies have reported that CHB patients have impaired renal function compared to healthy controls.^[17,31] In our study, we further found that the renal function of CHB patients deteriorated further with the progression of liver fibrosis. Moreover, as eGFR decreases, the level of Cys-C will increase. Our research suggests that for patients with CHB, especially those with liver cirrhosis, detection of Cys-C and eGFR helps early warning of renal impairment.

This study had some limitations. All the patients were from one medical center, a prospective multicenter study is thus necessary. In our study, we failed to explore further the reasons why Cys-C and liver cirrhosis contributed to the decreased eGFR. We believe that a well-designed controlled study is needed to answer these questions.

In summary, our study found that eGFR levels in patients with cirrhosis were significantly lower than in those without fibrosis. The fibrosis group and liver cirrhosis group had significantly higher Cys-C levels than those without fibrosis. Moreover, T2DM, liver fibrosis and Cys-C level were the independent factors associated with the decreased eGFR in patients with CHB. While age and Cys-C level were the independent factors associated with the decreased eGFR in patients with CHB-related fibrosis. Routine monitoring of Cys-C levels is of positive significance in preventing the development of renal impairment of CHB patients.

Author contributions

HZ and DW designed and guided this study, HZ and HL conducted experiments and wrote the main manuscript text, AH and MZ prepared all figures and data analysis. All authors reviewed the manuscript.

References

- [1] Sarin SK, Kumar M, Lau GK, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatology* 2016;10:1–98.
- [2] Liu B, Shen B, Mei M, et al. Potential effects of telbivudine versus entecavir on renal function in patients with chronic hepatitis B virus receiving glucocorticoids therapy. *Ther Apher Dial* 2020;24:56–63.
- [3] Vu V, Trinh S, Le A, et al. Hepatitis B and renal function: A matched study comparing non-hepatitis B, untreated, treated and cirrhotic hepatitis patients. *Liver Int* 2019;39:655–66.
- [4] Udompap P, Kim D, Ahmed A, et al. Longitudinal trends in renal function in chronic hepatitis B patients receiving oral antiviral treatment. *Aliment Pharmacol Ther* 2018;48:1282–9.
- [5] Jung WJ, Jang JY, Park WY, et al. Effect of tenofovir on renal function in patients with chronic hepatitis B. *Medicine (Baltimore)* 2018;97:e9756.
- [6] Park J, Jung KS, Lee HW, et al. Effects of entecavir and tenofovir on renal function in patients with hepatitis B virus-related compensated and decompensated cirrhosis. *Gut Liver* 2017;11:828–34.
- [7] Trinh S, Le AK, Chang ET, et al. Changes in renal function in patients with chronic HBV infection treated with tenofovir disoproxil fumarate vs entecavir. *Clin Gastroenterol Hepatol* 2019;17:948–56.
- [8] Luis-Lima S, Porrini E. An overview of errors and flaws of estimated GFR versus True GFR in patients with diabetes mellitus. *Nephron* 2017;136:287–91.
- [9] Porrini E, Ruggenenti P, Luis-Lima S, et al. Estimated GFR: time for a critical appraisal. *Nat Rev Nephrol* 2019;15:177–90.
- [10] Bargnoux AS, Barguil Y, Cavalier E, et al. Estimation of glomerular filtration rate using cystatin C. *Ann Biol Clin (Paris)* 2019;77:375–80.
- [11] Kar S, Paglialunga S, Islam R. Cystatin C is a more reliable biomarker for determining eGFR to support drug development studies. *J Clin Pharmacol* 2018;58:1239–47.
- [12] Wang Y, Li W, Yang J, et al. Association between cystatin c and the risk of ischemic stroke: a systematic review and meta-analysis. *J Mol Neurosci* 2019;69:444–9.
- [13] Zeng J, Cai S, Liu J, et al. Dynamic changes in liver stiffness measured by transient elastography predict clinical outcomes among patients with chronic hepatitis B. *J Ultrasound Med* 2017;36:261–8.
- [14] Zhang L, Wang F, Wang L, et al. Prevalence of chronic kidney disease in China: a cross-sectional survey. *Lancet* 2012;379:815–22.
- [15] K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002;39 (2 Suppl 1):S1–266.
- [16] Stevens PE, Levin A. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med* 2013;158:825–30.
- [17] Wu X, Cai S, Li Z, et al. Potential effects of telbivudine and entecavir on renal function: a systematic review and meta-analysis. *Virology* 2016;13:64.
- [18] Tang S, Lai FM, Lui YH, et al. Lamivudine in hepatitis B-associated membranous nephropathy. *Kidney Int* 2005;68:1750–8.
- [19] Tang S, Lai KN. Hepatitis B-related membranous nephropathy should be treated with a specific anti-viral agent. *Kidney Int* 2006;70:818.
- [20] Cabarkapa V. Cystatin C - more than the marker of the glomerular filtration rate. *Med Pregl* 2015;68:173–9.
- [21] Mathews PM, Levy E. Cystatin C in aging and in Alzheimer's disease. *Ageing Res Rev* 2016;32:38–50.
- [22] Zhou B, Zou H, Xu G. Clinical utility of serum Cystatin C in predicting diabetic nephropathy among patients with diabetes mellitus: a meta-analysis. *Kidney Blood Press Res* 2016;41:919–28.
- [23] Anila NP. Cystatin C: Its role in pathogenesis of OSMF. *J Oral Biol Craniofac Res* 2014;4:42–6.
- [24] Brou NA, Jacqz-Aigrain E, Zhao W. Cystatin C as a potential biomarker for dosing of renally excreted drugs. *Br J Clin Pharmacol* 2015;80:20–7.
- [25] Xu Y, Ding Y, Li X, et al. Cystatin C is a disease-associated protein subject to multiple regulation. *Immunol Cell Biol* 2015;93:442–51.
- [26] Andersen TB. Estimating renal function in children: a new GFR-model based on serum cystatin C and body cell mass. *Dan Med J* 2012;59: B4486.
- [27] Angelidis C, Deffereos S, Giannopoulos G, et al. Cystatin C: an emerging biomarker in cardiovascular disease. *Curr Top Med Chem* 2013;13:164–79.
- [28] Kandasamy Y, Smith R, Wright IM. Measuring cystatin C to determine renal function in neonates. *Pediatr Crit Care Med* 2013;14:318–22.
- [29] Odutayo A, Cherney D. Cystatin C and acute changes in glomerular filtration rate. *Clin Nephrol* 2012;78:64–75.
- [30] Ahmed MS, Wong CF, Shawki H, et al. Rapidly deteriorating renal function with membranoproliferative glomerulonephritis Type 1 associated with hepatitis C treated successfully with steroids and antiviral therapy: a case report and review of literature. *Clin Nephrol* 2008;69:298–301.
- [31] Pipili C, Cholongitas E, Papatheodoridis G. Review article: nucleos(t)ide analogues in patients with chronic hepatitis B virus infection and chronic kidney disease. *Aliment Pharmacol Ther* 2014;39:35–46.