Research Article

Complement C3 and Nonalcoholic Fatty Liver Disease in Chronic Kidney Disease Patients: A Pilot Study

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Keywords

Nonalcoholic fatty liver disease · Chronic kidney disease · Complement C3

Abstract

Context: Evidences have suggested complement C3 is a biomarker for nonalcoholic fatty liver disease (NAFLD) in the general population. **Objective:** The present study was conducted to explore the predictive function of C3 for NAFLD in chronic kidney disease (CKD) patients. **Design, Setting, and Participants:** CKD patients were recruited for evaluation of their liver function, kidney function, serum lipids, glycated hemoglobin, blood, and immune function. The glomerular filtration rate was calculated using the CKD-EPI equation. NAFLD was diagnosed according to predefined ultrasonographic criteria. **Results:** A total of 648 consecutive CKD patients were included, with 216 (33.3%) patients diagnosed with NAFLD. The NAFLD group had significant higher levels of serum protein, serum albumin, triglycerides, glycated hemoglobin, complement C3, hemoglobin ($p = 0.001$), alanine aminotransferase ($p = 0.002$), estimated glomerular filtration rate ($p = 0.007$), and C4 ($p = 0.043$) and lower levels of cystatin C, β_2 -microglobulin, proteinuria ($p = 0.001$), and high-density lipoprotein cholesterol ($p = 0.008$). In a logistic regression model, only complement C3 (OR = 1.003; 95% CI 1.002–1.004, $p = 0.001$) was associated with a higher likelihood of being diagnosed with NAFLD. Finally, we constructed ROC curves for complement C3 for prediction of having NAFLD. The best cut-off for complement C3 was 993.5 mg/L and it yielded a sensitivity of 63.9% and a specificity of 70.1%. **Conclusion:** Our study revealed that complement C3 can be used as a surrogate biomarker of NAFLD in CKD patients.

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Introduction

Chronic kidney disease (CKD) is a common disorder all over the world, with a prevalence of more than 10% in China [1]. A numbers of diseases contribute to the development of CKD, including chronic nephritis, diabetes mellitus, hypertension, lupus, and thyroidal dysfunction [2]. Lipid accumulation, another common metabolic disorder, may also be involved in kidney injury. The injury is named lipid nephrotoxicity and was first outlined by Moorhead et al. [3], who proposed that dyslipidemia might contribute to the progression of renal disease in 1982. However, no data to date are available about the absolute correlation between dyslipidemia and CKD.

Meanwhile, dyslipidemia in the liver induces nonalcoholic fatty liver disease (NAFLD) which incorporates a spectrum of conditions ranging from simple steatosis to steatohepatitis, advanced fibrosis, and cirrhosis. NAFLD is emerging as the most common cause of chronic liver disease worldwide [4]. In addition, NAFLD has been proven to be a strong independent risk factor for cardiovascular events (HR = 2.03; 95% CI 1.33–3.13; $p < 0.01$), but it was not associated with all-cause mortality (HR = 0.79; 95% CI 0.58–1.08; $p = 0.14$) or CKD progression ($p = 0.09$ for rate of decline of the estimated glomerular filtration rate [eGFR] slope) [5]. However, a number of studies have revealed the predictive function of NAFLD for CKD [6–10].

Several proinflammatory and oxidative stress mechanisms have been postulated to explain the relationship between these 2 conditions [11, 12]. Insulin resistance has also been suggested to contribute to the progression of kidney disease by worsening renal hemodynamics through activation of the sympathetic nervous system, sodium retention, and down-regulation of the natriuretic peptide system, making IR a possible mechanistic link between NAFLD and CKD [10]. Activated complement has also been indicated to be involved in NAFLD [13, 14] and CKD [15, 16]. NAFLD patients showed hepatic deposition of activated C3 and C4d. C1q, as well as mannose-binding lectin accumulation, was found in most activated C3-positive patients. Strikingly, 50% of the activated C3-positive patients also displayed membrane attack complex-associated C9 deposition. Deposition of complement factors was predominantly seen around hepatocytes with macrovesicular steatosis [17].

In the light of this background, this study aimed to explore the possible role of complement C3 as a candidate biomarker for detection of NAFLD in CKD patients.

Materials and Methods

Patients

A total of 648 nondialysis CKD patients, including 428 hypertensive patients and 220 nonhypertensive patients, from August 2009 to October 2012 were registered via the case record system. We excluded subjects who were younger than 18 years, women who were pregnant, those who had a current or past history of significant alcohol abuse (≥ 30 g/day in men and ≥ 20 g/day in women), a previous diagnosis of HBV- or HCV-related liver disease, a primary or metastatic malignancy of the liver, Wilson's disease, an α_1 -antitrypsin deficiency, genetic hemochromatosis or other rare causes of liver dysfunction, autoimmune liver diseases, or parenteral nutrition, and those who were taking drugs potentially causing NAFLD, except for those used in treatment of CKD.

Laboratory Measures

Serum creatinine (Scr), cystatin C, uric acid, β_2 -microglobulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum protein, albumin, and proteinuria were

measured using an OLYMPUS AU5400 automatic biochemical analyzer (Olympus Corporation, Mishima, Japan). The calibrators for the enzymatic method were traceable to an isotope dilution mass spectrometric method for Scr using standard reference methods (NIST SRM 967) [18]. Hemoglobin (Hb) was detected by the Sysmex XT-1800i Automated Hematology System (Shanghai, China). The concentrations of serum C-reactive protein were measured using electrochemiluminescence by ELECSYS 2010 (Roche, Switzerland). Glycated Hb (HbA1c) was determined using a BioRad VARIANT II HPLC analyzer (Biorad, Hemel Hempstead, UK). The values of serum immunoglobulin and complement were measured by single immunodiffusion.

Definition of CKD

CKD stages were defined in accordance with KIDGO guidelines as follows: stage 1: $\text{eGFR} \geq 90 \text{ mL/min/1.73 m}^2$, stage 2: $60 \text{ mL/min/1.73 m}^2 \leq \text{eGFR} < 90 \text{ mL/min/1.73 m}^2$, stage 3: $30 \text{ mL/min/1.73 m}^2 \leq \text{eGFR} < 60 \text{ mL/min/1.73 m}^2$, stage 4: $15 \text{ mL/min/1.73 m}^2 \leq \text{eGFR} < 30 \text{ mL/min/1.73 m}^2$, and stage 5: $\text{eGFR} < 15 \text{ mL/min/1.73 m}^2$.

eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) 4-level race equation [19, 20] and the last Scr value before discharge was selected as the baseline level. The specific CKD-EPI 4-level race GFR estimation equation is shown in Table 1.

The expressions of the eGFR equation were as follows:

$$\begin{aligned} \text{eGFR} &= \text{EXP}(\text{LN}[51] - 0.328 \times \text{LN}[\text{Scr}/88.4/0.7] + \text{age} \times \text{LN}[0.993]) \text{ (if female and creatinine } < 0.7); \\ \text{eGFR} &= \text{EXP}(\text{LN}[151] - 1.210 \times \text{LN}[\text{Scr}/88.4/0.7] + \text{age} \times \text{LN}[0.993]) \text{ (if female and creatinine } \geq 0.7); \\ \text{eGFR} &= \text{EXP}(\text{LN}[149] - 0.412 \times \text{LN}[\text{Scr}/88.4/0.9] + \text{age} \times \text{LN}[0.993]) \text{ (if male and creatinine } < 0.9); \\ \text{eGFR} &= \text{EXP}(\text{LN}[149] - 1.210 \times \text{LN}[\text{Scr}/88.4/0.9] + \text{age} \times \text{LN}[0.993]) \text{ (if male and creatinine } \geq 0.9). \end{aligned}$$

Definition of NAFLD

NAFLD was diagnosed based on the presence of a characteristic ultrasound (US) pattern, including diffuse hyperechogenicity of the liver parenchyma compared to the kidney cortex, deep beam attenuation, and poor visualization of the intrahepatic vessels and the diaphragm border [21]. Liver ultrasonography was performed after overnight fasting by an experienced sonographer blinded to patients' clinical and laboratory data using a 3.5- to 5-MHz convex probe. The study image was then analyzed by another sonographer who was also blinded to the patients' clinical and laboratory data, and the final assignment to an US category (NAFLD yes/no) was done by agreement of the 2 operators.

Statistical Analysis

Statistics analysis was performed using PASW 18.0 statistical software (SPSS Inc., Chicago, IL, USA). Data were expressed as means $\pm$ SD. The prevalence of NAFLD in different CKD stages was calculated using the χ^2 test. Student's *t* test was used to compare means for continuous variables. Fisher's exact test was used to compare prevalence dichotomic variables. Binary logistic regression was employed to explore risk factors for NAFLD. Multicollinearity between independent variables was evaluated by using the variance inflation factor before entering each value in the regression model. $p < 0.05$ was considered statistically significant. Receiver operating characteristic (ROC) curves were built to evaluate the predictive value of significant variables for the likelihood of having NAFLD. The best cut-off for ROC curves was calculated with the Youden index.

Table 1. Characteristics of the subjects in the CKD and CKD-NAFLD groups

	CKD (n = 432)	CKD-NAFLA (n = 216)	p value
Age, years	65.15±17.48	64.92±14.74	0.86
Male sex	212 (49.1)	94 (43.5)	0.211
ALT, UI/L	20.78±22.54	26.78±23.9	0.002 [#]
AST, UI/L	25.91±21.84	25.72±13.44	0.588
Serum protein, g/dL	59.88±9.63	64.92±8.28	0.000 [#]
Serum albumin, g/dL	34.64±6.31	37.98±5.33	0.000 [#]
Glutamyltransferase, g/dL	35.65±54.48	43.95±66.72	0.091
Cystatin C, mg/dL	2.18±1.37	1.65±0.8	0.000 [#]
β ₂ -microglobulin, mg/dL	5.81±4.28	4.04±3.8	0.000 [#]
Urine acid, μmol/L	403.26±157.95	399.45±133.21	0.747
eGFR, mL/min × 1.73 m ²	57.92±38.54	66.11±29.7	0.007 [#]
Total cholesterol, mmol/L	4.65±1.76	4.79±1.47	0.293
Triglycerides, mmol/l	1.49±0.99	2.12±1.47	0.000 [#]
HDL cholesterol, mmol/L	1.11±0.35	1.04±0.28	0.008 [#]
LDL cholesterol, mmol/L	2.86±1.29	2.93±1.12	0.481
Glycated hemoglobin	6.04±1.27	6.48±1.24	0.000 [#]
IgG, mg/L	11.41±5.85	11.96±3.47	0.2
IgM, mg/L	1.03±1.13	0.91±0.49	0.128
IgA, mg/L	2.40±1.45	2.51±1.1	0.324
C3, mg/L	890.5±239.92	1,085.3±270	0.000 [#]
C4, mg/L	218.65±69.9	230.39±68.98	0.043 [*]
CRP, mg/L	15.97±33.03	22.54±54.6	0.115
T2DM	135 (31.3)	93 (43.1)	0.003 [#]
Proteinuria, g/24 h	1.27±2.11	0.77±1.60	0.000 [#]
Hb, g/L	117.83±21.62	129.1±17.28	0.000 [#]
Hypertension, n (%)	270 (62.5)	158 (73.1)	0.007 [#]
Albuminuria (n = 456)	243/286	141/170	0.01 [*]

Values are presented as means ± SD or numbers (%). HDL, high-density lipoprotein; LDL, low-density lipoprotein; IgG, immunoglobulin G; IgM, immunoglobulin M; IgA, immunoglobulin A; CRP, C-reactive protein; T2DM, type 2 diabetes mellitus. * $p < 0.05$. [#] $p < 0.01$.

Results

Characteristics of Subjects in the CKD and CKD-NAFLD Groups

A total of 648 consecutive CKD patients were recruited and 216 (33.3%) patients were diagnosed with NAFLD. The causes of CKD were chronic glomerulonephritis, asymptomatic hematuria, diabetic nephropathy, polycystic kidney disease, chronic renal failure (of unknown or indeterminate etiology), hypertensive nephropathy, and obstructive nephropathy. The NAFLD group had significant higher levels of serum protein, serum albumin, triglycerides, HbA1c, complement C3, Hb ($p = 0.001$), ALT ($p = 0.002$), eGFR ($p = 0.007$), and C4 ($p = 0.043$) and a higher frequency of hypertension ($p = 0.007$). Additionally, markedly lower levels of cystatin C, β₂-microglobulin, proteinuria ($p = 0.001$), and high-density lipoprotein cholesterol ($p = 0.008$) and a lower frequency of albuminuria (only a total of 72 patients had albuminuria values within the normal range, while 384 patients had values exceeding the normal range) were also observed in the NAFLD group.

The prevalence of NAFLD in each CKD group is shown in Table 2, with a significantly decreasing trend from CKD stage 1 to CKD stage 5. Additionally, levels of serum protein and albumin revealed a declining trend with increasing CKD stages (Tables 1, 2).

Table 2. Prevalence of NAFLD in the different CKD stages

	Non-NAFLD (n = 432), n	NAFLD (n = 216), n (%)	Albumin, g/L	Protein, g/L
CKD1	112	73 (33.8)	37.12	62.64
CKD2	66	48 (22.2)	36.3	62.66
CKD3	63	42 (19.4)	36	61.64
CKD4	140	50 (23.1)	36.15	62.62
CKD5	51	3 (1.4)	32.44	57.7

Table 3. Logistic regression analysis using NAFLD as the dependent variable

	OR	95% CI	p value
Male sex	1.069	0.704–1.623	0.753
C3	1.003	1.002–1.004	0.0001 [#]
Age	1.011	0.995–1.028	0.182
CRP	1.001	0.996–1.005	0.691
Glycated Hb	1.164	0.993–1.365	0.061
eGFR	1.006	0.999–1.013	0.113

CRP, C-reactive protein. [#] $p < 0.01$.

Correlations of NAFLD and Biomarkers

To explore risk factors of NAFLD, we used a binary logistic regression model including sex, age, C-reactive protein, C3, eGFR, and HbA1c. The regression showed that only complement C3 (OR = 1.003, 95% CI 1.002–1.004, $p = 0.001$) was associated with a higher likelihood of being diagnosed with NAFLD (Table 3).

Predictive Ability of C3 for NAFLD

According to the above analysis, the ROC curve was employed to evaluate the prediction efficiency of complement C3 and HbA1c for the likelihood of being diagnosed with NAFLD. The area under the ROC was 0.722 (95% CI 0.681–0.764, $p < 0.001$) for C3. The analysis of ROC revealed that the optimal cut-off for complement C3 was 993.5 mg/L and provided a sensitivity of 63.9% and a specificity of 70.1% (Fig. 1).

Discussion

Our study discussed clinical characteristics of NAFLD in CKD patients. Due to lipid accumulation in liver, serum lipids, especially triglycerides, were significantly higher in NAFLD patients. Meanwhile, NAFLD represents a high grade of metabolic disorder with elevated HbA1c. Because of inflammation induced by NAFLD, the level of ALT was a little higher, with a mean value within the normal range. Unlike previous studies that have focused on the increasing prevalence of CKD induced by NAFLD [6–10], our study showed the frequency of NAFLD declined as the elevation in CKD stage. As we all know, NAFLD is induced by insulin resistance and metabolic syndrome, which maybe attenuated because of uremic syndrome and malnutrition in CKD patients, especially in CKD stage 5. Values of protein and albumin go down as the CKD stage increases. Therefore, as the GFR decreases, an abnormal appetite and nutrition status may decrease the prevalence of NAFLD in CKD stage 5. On top of that, the levels of cystatin C, β_2 -microglobulin, and proteinuria were lower in NAFLD, which may be attributed

Fig. 1. ROC curves for complement C3 for prediction of NAFLD. The area under the ROC was 0.722 (95% CI 0.681–0.764, $p < 0.001$) for C3. The analysis of ROC revealed that the optimal cut-off for complement C3 was 993.5 mg/L and provided a sensitivity of 63.9% and a specificity of 70.1%.

to more CKD stage 1 to CKD stage 3 patients in the NAFLD group (241/432 vs. 163/216). However, complement C3 and C4 were both elevated in NAFLD and the logistic regression showed that C3 was an independent risk factor for NAFLD, indicating that complement system may be involved in the development of NAFLD. Furthermore, C3 had a promising prediction efficiency for NAFLD, with a sensitivity of 63.9% and a specificity of 70.1%.

Emerging amounts of epidemiologic data have shown an association between the prevalence and severity of NAFLD and the incidence and stage of CKD. A cohort study showed that NAFLD proved to be a strong independent risk factor for cardiovascular events (HR = 2.03; 95% CI 1.33–3.13; $p < 0.01$), but it was not associated with all-cause mortality (HR = 0.79; 95% CI 0.58–1.08; $p = 0.14$) or CKD progression [5]. Nevertheless, a total of 9 observational studies with 96,595 adult individuals (34.1% with NAFLD) of predominantly Asian descent and 4,653 cases of incident CKD stage ≥ 3 (i.e., defined as the occurrence of an eGFR < 60 mL/min/1.73 m², with or without accompanying overt proteinuria) over a median period of 5.2 years showed that NAFLD (detected by biochemistry, the fatty liver index, or ultrasonography) is associated with a nearly 40% increase in the long-term risk of incident CKD [22]. Furthermore, a retrospective cohort study of 41,430 adult men and women (average age, 48.9 years) without CKD at baseline NAFLD was associated with an increased risk of CKD development. NAFLD may adversely affect renal function and patients may need to be carefully monitored for an increased risk of CKD [6].

When it comes to the pathogenesis of NAFLD, IR is the key pathophysiological factor for the development and progression of NAFLD. In addition, IR has been attributed to the development of CKD [23]. Other pathophysiological mechanisms, such as fetuin-A, adiponectin [24], and angiotensin II may be contributing factors for CKD development in patients with NAFLD [11]. The complement system may also be associated with the development of CKD and NAFLD. C3 is mainly synthesized in hepatocytes and undetectable in the liver of healthy subjects. However, 74% of the NAFLD patients showed hepatic deposition of activated C3 and

C4d [17]. C3 levels were positively associated with both NAFLD prevalence and severity regardless of whether metabolic syndromes exist or not [13]. Little data is available about whether C3 is directly a pathogen of NAFLD or not; however, C3 may induce the progression of NAFLD [17]. Moreover, C3 is an important immune regulatory factor in the pathogenesis of CKD [25–30]. Complement systems including C3 have been involved in most of cases of nephropathy, e.g., membrane nephropathy, lupus nephropathy, IgA nephropathy, and even diabetic nephropathy. In our study, C3 was an independent influencing factor for NAFLD, which is similar to the results of the previous study [13, 14]. We speculate that inflammation in CKD patients induces activation of the complement system which may result in deposition of activated C3 in the liver to assemble membrane attack complex (MAC) [17]. After deposition of C3 in the liver, activating C5 and formation of MAC emerge, which induces liver injury. However, to date, no data are available regarding the prediction efficiency of C3 for NAFLD in CKD. A study conducted in rheumatoid arthritis patients showed that the best cut-off of complement C3 for predicting NAFLD was 1.23 g/L and yielded a sensitivity of 76% and a specificity of 64% [14]. Our study also revealed sensitivity and specificity values comparable to the above result, suggesting that C3 can be used as a potential marker for predicting NAFLD in CKD.

There are 2 limitations to our study. Firstly, the BMI was not obtained. As we all know, the BMI is an important index to assess metabolic syndrome. It is associated with NAFLD, obesity, and diabetes. In our study, levels of serum lipids and HbA1c in CKD-NAFLD were both higher than those in CKD without NAFLD, suggesting that an abnormal metabolic status exists in these patients although the BMI was not recorded.

Secondly, histological confirmation of the diagnosis of NAFLD was deficient. However, patients in our cohort manifested without signs or symptoms of clinically relevant liver disease, including normal or near normal liver function indices, with only a small fraction of participants exhibiting an increase in ALT levels exceeding the upper limit of normal. Therefore, a liver biopsy may induce unbalanced ethical issues.

Taken together, C3 in CKD patients not only represent activity of kidney disease but it can also be used as a candidate biomarker for NAFLD.

Statement of Ethics

This study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board. Informed consent was obtained from all of the participants when they were admitted to the hospital.

Disclosure Statement

The authors have nothing to disclose.

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Author Contributions

The research was designed by B.P. and C.C. All of the authors helped to write this report and commented on it. B.P. and M.M. analyzed the data and advised on statistical issues at the time of the research write up. X.W. and C.C. were research administrators. M.M. was a research nurse responsible for recruitment and return of data.

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