

The Role of Cystatin C and Albumin in the Differential Diagnosis of Primary Chronic Glomerulonephritis and Hypertension-Induced Renal Injury

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Abstract—Background: It was difficult to differentiate diseases in patients of Primary chronic glomerulonephritis (PCG) and Hypertension-induced renal injury (HRI). This study aimed to investigate the role of serum Cystatin C and Albumin in differential diagnosis of PCG and HRI. **Materials and Methods:** A total of 150 patients (79 PCG and 71 HRI) considering for our clinic between October 2017 and August 2018 were included. Patients were included in the study newly diagnosed by biopsy. Urinary samples and blood samples were collected and measured. The diagnostic roles were investigated using the receiver operator curve curves. **Results:** There was an increased serum levels of Albumin in patients with PCG compared with that in patients with HRI. Patients with HRI had a significantly increased serum levels of Cystatin C than those with PCG. An Albumin level of 2.295 g/L had an 80.3% sensitivity and a 73.4% specificity, Cystatin C level of 36.200 mg/L had a 79.7% sensitivity and a 93% specificity for differential diagnosis of PCG and HRI. **Conclusions:** Serum Cystatin C and Albumin level might be used as tool to differentiate PCG from HRI. Further studies are needed to confirm the definite the role of those markers.

Keywords: *primary chronic glomerulonephritis, Hypertension-induced renal injury, Cystatin C, Albumin*

I. INTRODUCTION

Primary chronic glomerulonephritis (PCG) is one of major causes of end-stage renal disease (ESRD) among chronic renal disease patients in China [1-3]. Hypertension-induced renal injury (HRI) generally occurs during the middle and late stages of hypertension and has become an important public health problem [4-5]. Both of them are typically characterized by proteinuria, hematuria, hypertension, renal inflammation and so on, which makes it difficult to clinically diagnose PCG and

HRI. In addition, because hypertension can be both a cause and a consequence of renal diseases, it is difficult to determine newly PCG and HRI by consulting history of hypertension.

Kidney biopsy is considered the gold standard for diagnosing kidney disease. Because of partly harm to kidney, however, many patients may not choose kidney biopsy[6]. So patients with PCG and HRI were diagnosed by clinical features (e.g. proteinuria, hematuria, renal inflammation) and history of hypertension, currently. On the other hand, because of the subtle differences treatment, if there is a misdiagnosis, there will be a waste of medical resources. Therefore, simple and accurate markers are urgently used to diagnose PCG and HRI.

To clarify these issues, we collected the clinical and laboratory parameters of patients to explore possible markers. From data analysis, we find that cystatin-c and albumin maybe the role of the markers in the differential diagnosis of PCG and HRI, which will be perhaps widely used in clinical practice.

II. MATERIAL AND METHODS

A. Participants

The study population included patients who had been diagnosed with PCG and HRI. Patients were included in the study newly diagnosed by biopsy. All specimens of renal tissue were obtained by percutaneous renal biopsy, immunofluorescence was performed by an experienced morphologist, and standard examination of the cortical tissue by light microscopy. In hypertensive patients, renal biopsy was performed after blood pressure control. Patients were excluded if they had any of the following: any concomitant disease and the symptoms or sign of acute and chronic inflammation other than glomerulonephritis, pregnancy and lactation.

A total of 150 patients, among them 79 and 71 patients had been respectively diagnosed with PCG and HRI, were considered for our clinic between October 2017 and August 2018. This study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University. All subjects or their family members provided informed consent.

B. Clinical Characterization

Laboratory data included proteinuria 24h, Urine volume 24h, complement C3 and C4, total iron binding capacity (TIBC), low density lipoprotein cholesterol (LDL), non-high-density lipoprotein cholesterol (non-HDL-C), triglyceride (TG), high density lipoprotein cholesterol (HDL), very low density lipoprotein cholesterol (VLDL), albumin, Cystatin C. Urinary samples were measured from a fresh morning spot urine sample and blood samples was collected after an overnight fast of at least 10 h.

C. Statistical Analysis

The normality of distribution was assessed with the Kolmogorov-Smirnov test. Continuous variables are presented as the mean ± standard deviation (SD) or median ± interquartile range. Differences in categorical variables among the groups were examined using the χ^2 test. Comparisons of continuous variables were analysed with Mann-Whitney U test or t-test. Factors were tested in univariate analysis, and factors with values of P < 0.1 were tested in binary logistic regression analysis (Forward: LR). Receiver operating characteristic (ROC) curves was constructed to establish the predictive factors for the differentiation of PCG from HRI. Correlations were analysed with Pearson's correlation. All analyses were performed using Statistical Package for Social Sciences software (SPSS, version 17.0; SPSS Inc., Chicago, IL, USA). Tests were two-tailed, and values of P < 0.05 were considered to be statistically significant.

III. RESULTS

79 adult patients with PCG and 71 patients with HRI were admitted to our study. The characteristics of participants were shown in Table I. Urine Volume 24h, TIBC, Albumin in PCG were significantly higher than those in HRI. Mean age, LDL Proteinuria 24h, and Cystatin C were significantly higher in HRI than those in PCG (Table I).

Binary logistic regression analyses were used to investigate significant factors differentiating between PCG and HRI and healthy group participants. Results indicated that Albumin in PCG was significantly higher than those in HRI and Cystatin C of the patients in HRI was significantly higher than that of PCG (Table II).

Our ROC curve analyses indicated that in differentiating PCG from HRI, an Albumin level of 2.295 g/L had an 80.3% sensitivity and a 73.4% specificity (Fig. 1 A) and Cystatin C level of 36.200 mg/L had a 79.7% sensitivity and a 93% specificity (Fig. 1 B). We also determined using Pearson's correlation when all participating patients were analysed together, significant inversely correlations were found between Cystatin C and Albumin concentrations (Table III).

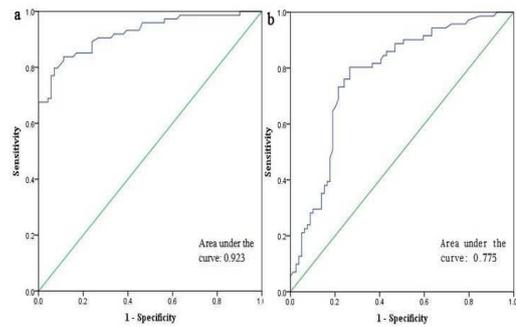


Fig. 1. ROC curve analysis of Cystatin C and Albumin for the prediction of primary chronic glomerulonephritis and hypertension-induced renal injury.

TABLE I. THE CHARACTERISTICS OF PARTICIPANTS FOR PRIMARY CHRONIC GLOMERULONEPHRITIS AND HYPERTENSION-INDUCED RENAL INJURY

| Parameters | Primary chronic glomerulonephritis | Hypertension-induced renal injury | P |
|-----------------------|------------------------------------|-----------------------------------|--------|
| Male/Female | 42/37 | 35/36 | 0.64 |
| Age (years) a | 42.73±13.26 | 48.80±12.60 | 0.005 |
| Urine Volume 24h (L)a | 1.79±0.58 | 1.33±0.70 | <0.001 |
| TIBC (umol/l) a | 45.10±9.21 | 40.13±7.94 | 0.001 |
| Complement C3 (g/l) a | 1.14±0.24 | 1.07±0.25 | 0.081 |
| Complement C4 (g/l) a | 0.34±0.14 | 0.38±0.13 | 0.126 |
| LDL (mmol/l) a | 2.69±0.99 | 3.04±1.12 | 0.047 |
| Non-HDL-C (mmol/l) a | 3.33±1.08 | 3.62±1.21 | 0.129 |
| HDL (mmol/l) a | 1.05±0.28 | 1.10±0.27 | 0.225 |
| VLDL (mmol/l) a | 0.61±0.34 | 0.58±0.32 | 0.688 |
| Proteinuria (g/24H) b | 1.01±1.29 | 1.34±3.43 | 0.007 |
| Albumin (g/l) b | 56.15±23.20 | 29.70±6.70 | <0.001 |
| TG (mmol/l) b | 1.41±1.05 | 1.34±0.81 | 0.626 |
| Cystatin C (mg/l) b | 0.80±2.65 | 3.93±2.70 | <0.001 |

a Data are presented as mean±SD; b Data are presented as median±IQR. TIBC: total iron binding capacity; LDL: low density lipoprotein cholesterol; non-HDL-C: non-high-density lipoprotein cholesterol; HDL: high density lipoprotein

VLDL: very low density lipoprotein cholesterol; TG: triglyceride

TABLE II. BINARY LOGISTIC REGRESSION ANALYSIS FOR PRIMARY CHRONIC GLOMERULONEPHRITIS AND HYPERTENSION-INDUCED RENAL INJURY

| Parameters | B | S.E. | Wald | P | Odds ratio (95% CI) |
|------------|--------|-------|--------|--------|---------------------|
| Albumin | -0.240 | 0.057 | 17.800 | <0.001 | 0.787 (0.704-0.879) |
| Cystatin C | 0.326 | 0.164 | 3.979 | 0.046 | 1.386 (1.006-1.910) |
| Constant | 8.272 | 1.889 | 19.172 | <0.001 | 3913.249 |

TABLE III. PEARSON'S CORRELATION RESULTS BETWEEN ALBUMIN AND CYSTATIN C LEVELS IN ALL PARTICIPATING PATIENTS

| Parameter | Albumin | |
|------------|---------|--------|
| | r | P |
| Cystatin C | -0.343 | <0.001 |

IV. DISCUSSION

To our knowledge, this is the first study to explore possible indicator in the differential diagnosis of PCG and HRI. We found increased serum levels of Albumin in patients with PCG compared with that in patients with HRI. Cystatin C was shown to be a significant factor differentiating PCG from HRI, patients with HRI had a significantly increased serum levels of Cystatin C than those with PCG.

Human serum albumin is composed of 17 disulfides and one free cysteine, which is the most abundant intravascular protein and is widely used for routine clinical examination [7,8]. Moreover, serum albumin is the major intravascular antioxidant, is associated with the incidence of end-stage renal disease [7,9] and is a strong prognostic indicator for many disease processes [10,11]. In the study, we found using univariate analysis that Albumin in PCG was significantly higher than those in HRI. Additionally, binary logistic regression analysis also indicated the same result.

Lower serum albumin concentration may reflect renal tubular dysfunction impairing protein reabsorption from the filtrate, and thus may reflect tubular damage predisposing to development and progression of clinically apparent renal disease [12,13]. There are two possible explanations for Albumin in PCG was significantly higher than those in HRI. First, duration of hypertension impair the function of the renal, continually. Studies had shown that glomerular filtration rate (GFR) depends on the duration of hypertension [14], decreased GFR is associated with hypertension [15,16]. The function of GFR in patients with HRI reduced more than this in PCG. Further studies are needed to evaluate the mechanism of this association. Second, data suggested that the mean age was significantly higher in HRI than it in PCG. So, the physical condition of PCG might better than that those of HRI.

Serum cystatin C, is an endogenous cysteine proteinase inhibitor produced by nucleated cells, is freely filtered by the glomerulus and subsequently reabsorbed and catabolized by the healthy tubular epithelium [17], is almost independent of age and sex [18-19], is an alternative serum marker of renal function [20], especially now that a certified reference material is available [21]. Increased serum cystatin C predict the risk of renal injury [22]. In this paper, serum cystatin C concentration was increased in HRI and was inversely correlated with serum albumin concentration, which had been found by other studies [12, 20]. This might be further confirmed that The function of GFR in patients with HRI reduced more than this in PCG.

As we all known that hypertension is association with age. In the present study, our data showed a significant difference in the mean age of patients with HRI and with PCG. But, as far as possible to match the patients of HRI, the juvenile patients of PCG were consciously excluded from the study. Univariate analysis observed significant differences in Urine Volume 24h, TIBC, LDL Proteinuria 24h between patients of HRI and PCG However, binary logistic regression indicated no significant differences in those between the two groups. This results might be attributable to multi-colinearity between all of the data. In order to eliminate the problem of multi-colinearity, binary logistic regression analysis was used by the method of Forward:

LR. The results demonstrated that Albumin and Cystatin C were the biomarkers to differentiate HRI from PCG disease.

V. PROSPECT

Our study had some limitations. First, this is a small sample size, retrospective design and single-center study. Second, without acquiring the data regarding a family history of hypertension, alcohol consumption, serum creatinine, uric acid to adjust for all relevant confounding variables might have affected our results. Therefore, further studies with a larger sample of participants, more well-designed multicenter studies are needed to further confirm our study.

The results of the study highlight the need for future research to use a more representative sample, for example, to develop a model for predicting Cystatin C in individual patients with CKD, or built a validation of a score to predict HRI. Furthermore, we can recruit a development cohort from one hospital while a validation cohort from other hospitals in the same level. For both cohorts, CKD patients were eligible for inclusion if they were first visit. Otherwise, a Clinical Scoring System could be used to predict PCG or HRI in CKD.

In conclusion, our results indicated that Albumin and Cystatin C might be the biomarkers to differentiate the patients of HRI from PCG disease. Which will be perhaps widely used in clinical practice.

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REFERENCES

- [1] Abbas F, El Kossi M, Jin JK, Sharma A, Halawa A, "Recurrence of primary glomerulonephritis: Review of the current evidence," *World J Transplant*, vol. 7, no. 6, pp. 301-316, 2017.
- [2] Qiu H, Fan W, Fu P, et al, "General acteoside of *Rehmanniae* leaves in the treatment of primary chronic glomerulonephritis: a randomized controlled trial," *Afr J Tradit Complement Altern Med*, vol. 10, no. 4, pp. 109-115, 2013.
- [3] Zhang L, Wang F, Wang L, et al, "Prevalence of chronic kidney disease in China: a cross-sectional survey," *Lancet*, vol. 379, no. 9818, pp. 815-822, 2012.
- [4] Gan Z, Huang D, Jiang J, et al., "Captopril alleviates hypertension-induced renal damage, inflammation, and NF- κ B activation," *Braz J Med Biol Res*, vol. 51, no. 11, pp. e7338, 2018.
- [5] Kannel WB, "Review of recent Framingham study hypertension research," *Curr Hypertens Rep*, vol. 2, pp. 239-240, 2000.
- [6] Orofino L, Quereda C, Lamas S, et al, "Hypertension in primary chronic glomerulonephritis: analysis of 288 biopsied patients," *Nephron*, vol. 45, no. 1, pp. 22-26, 1987.
- [7] Kawai Y, Masutani K, Torisu K, et al, "Association between serum albumin level and incidence of end-stage renal disease in patients with Immunoglobulin A nephropathy: A possible role of albumin as an antioxidant agent," *PLoS One*, vol. 13, no. 5, pp. e0196655, 2018.
- [8] Pupim LB, Ikizler TA, "Assessment and monitoring of uremic malnutrition," *J Ren Nutr*, vol. 14, pp. 6-19, 2004.
- [9] Goto M, Wakai K, Kawamura T, et al, "A scoring system to predict renal outcome in IgA nephropathy: a nationwide 10-year prospective cohort study," *Nephrol Dial Transplant*, vol. 24, pp. 3068-3074, 2009.
- [10] Snipelisky D, Jentzer J, Batal O, et al, "Serum albumin concentration as an independent prognostic indicator in patients with pulmonary arterial hypertension," *Clin Cardiol*, vol. 41, no. 6, pp. 782-787, 2018.

- [11] Heys SD, Walker LG, Deehan DJ, et al, "Serum albumin: a prognostic indicator in patients with colorectal cancer," *J R Coll Surg Edinb*, vol. 43, no. 3, pp. 163-168, 1998.
- [12] Walther CP, Gutiérrez OM, Cushman M, et al, "Serum albumin concentration and risk of end-stage renal disease: the REGARDS study," *Nephrol Dial Transplant*, vol. 33, no. 10, pp. 1770-1777, 2018.
- [13] Otaki Y, Watanabe T, Takahashi H et al, "Comorbid renal tubular damage and hypoalbuminemia exacerbate cardiac prognosis in patients with chronic heart failure," *Clin Res Cardiol*, vol. 105, pp. 162-171, 2016.
- [14] Rynkowska-Kidawa M, Kidawa M, Kapusta J, et al, "Evaluation of renal function in the course of hypertension in the elderly," *Pol Merkur Lekarski*, vol. 45, no. 265, pp. 11-16, 2018.
- [15] Imamura M, Hayashi C, Kim WJ, et al, "Renal scarring on DMSA scan is associated with hypertension and decreased estimated glomerular filtration rate in spina bifida patients in the age of transition to adulthood," *J Pediatr Urol*, vol. 14, no. 4, pp. 317.e1-317.e5 30417-0, 2018.
- [16] Paris G, Gorla SR, Arenas-Morales AJ, et al, "Comparison of echocardiographic changes in children with primary hypertension and hypertension due to mild to moderate chronic kidney disease," *Pediatr Nephrol*, vol. 34, no. 3, pp. 487-494.
- [17] Onopiuk A, Tokarzewicz A, Gorodkiewicz E, "Cystatin C: a kidney function biomarker," *Adv Clin Chem.*, vol. 68, pp. 57-69, 2015.
- [18] A. Bokenkamp, M. Domanetzi, R. Zinck, et al, "Cystatin C-a new marker of glomerular filtration rate in children independent of age and height. *Pediatrics*," vol. 101, pp. 875-881, 1998.
- [19] G.J. Schwartz, M.F. Schneider, P.S. Maier, et al, "Improved equations estimating GFR in children with chronic kidney disease using an immunonephelometric determination of cystatin C," *Kidney Int*, vol. 82, pp. 445-453, 2012.
- [20] Shlipak MG, Katz R, Kestenbaum B, et al, "Rate of kidney function decline in older adults: a comparison using creatinine and cystatin C," *Am J Nephrol*, vol. 30, no. 3, pp. 171-178, 2009.
- [21] A. Grubb, S. Blirup-Jensen, V. Lindström, et al, "First certified reference material for cystatin C in human serum ERM-DA471/IFCC," *Clin. Chem. Lab. Med*, vol. 48, pp. 1619-1621, 2010.
- [22] El-Gammacy TM, Shinkar DM, Mohamed NR, et al, "Serum cystatin C as an early predictor of acute kidney injury in preterm neonates with respiratory distress syndrome," *Scand J Clin Lab Invest*, vol. 22, pp. 1-6, 2018.