Role of Urinary Neutrophil Gelatinase–Associated Lipocalin for Predicting the Severity of Renal Functions in Patients With Autosomal-Dominant Polycystic Kidney Disease

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ABSTRACT

Background. Autosomal-dominant polycystic kidney disease (ADPKD) has a feature of disruption of tubular integrity with increased cellular proliferation and apoptosis. There are several known tubular membrane proteins in the pathogenesis of ADPKD, and one of these proteins is the neutrophil gelatinase-associated lipocalin (NGAL). NGAL is a protein expressed on renal tubular cells of which production is markedly increased in response to harmful stimuli such as ischemia or toxicity.

Objective. We aim to study whether urinary NGAL levels could be used as a marker to identify the severity of ADPKD in patients.

Methods. Urinary NGAL levels were measured in 30 patients with ADPKD compared with 30 control patients who were matched by age, gender, and glomerular filtration rate (GFR). All patients with ADPKD were diagnosed by using both phenotypic and genotypic criteria, which showed that all cases of ADPKD were caused by *PKD1* gene mutation. The urinary NGAL level was measured using The NGAL Test by Roche, with analytic range of 25–1000 ng/mL.

Results. In the ADPKD group, there was significant negative correlation between urinary NGAL and GFR (Pearson $r = -0.472$; $P = .008$) and significant positive correlation between urinary NGAL and serum creatinine (Pearson $r = 0.718$; $P < .01$). Elevated urinary NGAL was increased as GFR of ADPKD patients was decreased.

Conclusion. Urinary NGAL might play role in the pathway of renal tubular damage in patients with ADPKD and might be useful in the prediction of the possibility to progress to chronic kidney disease in patients with ADPKD.

AUTOSOMAL-DOMINANT polycystic kidney disease (ADPKD) is the most prevalent, potentially lethal, monogenetic disorder with the prevalence of 1:500–1:1000 all over the world [1]. The characteristic of ADPKD is the enlargement of the kidney from numerous cysts present on the renal tubules, which gradually grow, resulting in the decline of glomerular filtration rate (GFR) and eventually turning into end-stage renal disease (ESRD) [2,3]. Several hypotheses have been proposed to elucidate the mechanism underlying this disorder. There is the mutation of *PKD1* and/or *PKD2* gene in patients with ADPKD resulting in abnormal formation of polycystin-1 and polycystin-2 protein. It has been shown that both polycystin-1 and polycystin-2 were found in the primary cilium, which is a nonmotile microtubule-based structure that extends from the apical membrane of tubular cells into the lumen.

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Abnormal polycystin-1 and polycystin-2 are associated with an abnormal intracellular signaling process that leads to increased cell proliferation and increased cell apoptosis [4,5]. Currently, there is no specific treatment for ADPKD or for the prevention of ESRD [6–9]. Nevertheless, understanding of the patho-physiology of the disease may lead to the development of some medications to slow the decline of GFR [7–9]. Currently, the diagnosis of ADPKD in clinical practice has been done using kidney ultrasound in patients with a family history of ADPKD [10]. However, this diagnostic tool has limitations in young patients. In patients with ADPKD who are younger than 30 years old with normal ultrasound results, the exclusion of ADPKD still cannot be made [10]. Thus, markers indicating the severity of disease that are more accurate than serum creatinine would be beneficial for follow-up of the disease and for development of treatments for patients with ADPKD.

Neutrophil gelatinase–associated lipocalin (NGAL) is a small molecule protein that consists of 178 amino acids. It is a part of the lipocalin superfamily, which consists of 20 structurally related secreted proteins [11]. NGAL can be found at low levels in the tissue of kidneys, bronchus, lungs, stomach, and colon. During inflammation or damage of the epithelia, the NGAL level can increase to a very high quantity [12]. In the year 2003, there was the first discovery of using NGAL as a marker for acute kidney injury. Moreover, NGAL was also found to express in high levels in the proliferating proximal tubular cells [13]. During the decreasing of GFR, the NGAL level was found to be increased in both serum and urine. Apart from the kidney, expression of the messenger RNA (mRNA) that produces NGAL is found to be up-regulated in other tissues, especially the liver and lungs. Although NGAL may act like an acute-phase reactant protein and can be released from the neutrophil, macrophage, and other immune cells, in the context of decreased renal function, it results in decreased NGAL elimination causing the serum NGAL to increase [14]. There are a limited number of studies on the association of urine NGAL and ADPKD, all of which use the diagnostic criteria of ADPKD based on ultrasound criteria [15,16]. We aim to study whether urine NGAL could be used as a marker to determine the severity of disease in patients with ADPKD.

METHODS

Patient Profile

The study was conducted on 30 ambulatory patients with ADPKD who had not undergone renal replacement therapy. The diagnosis of ADPKD was made using the typical ultrasound or computed tomography (CT) findings of renal cysts and positive family history. None of the patients had acute kidney injury, or were affected by any neoplastic disease or concomitant infections, and gave their fully informed consent to take part in the study, which was approved by the local ethics committee, Siriraj Institutional Review Board, Faculty of Medicine, Siriraj Hospital, Mahidol University. Table 1 provides details of the baseline characteristics of the patients with ADPKD and the control group.

Control Group

The control group consisted of 30 healthy volunteers matched for age, gender, and GFR. All control subjects gave their fully informed approval to take part in the study.

Collection of Urine

Ten milliliters of fresh midstream urine were collected using normal aseptic technique on the day each patient came for an out-patient visit and then were immediately centrifuged at 2000 rpm for 5 minutes. The 3 mL of supernatants were collected and stored at −80°C until assayed. All of the urine specimens were used for the study within 2 months after collection.

NGAL Enzyme-Linked Immunosorbent Assay

NGAL was measured in the urine using The NGAL Test Reagent Kit (BioPorto diagnostics A/S, Hellerup, Denmark) according to the manufacturer's instructions on the analyzer model of Abbott Architect c8000. All measurements were made in triplicate and in a blinded manner. NGAL levels were expressed as ng/mL.

Statistical Analyses

The statistical analysis of data was performed by using SPSS version 17.0 (Chicago, Ill, United States). An unpaired two-tailed t test was used for comparing the two groups; we used chi-square or Fisher exact test for comparison and independent t test or Mann-Whitney U test for continuous data. Pearson correlation coefficient was used to test correlations between variables. $P < .05$ was considered statistically significant. All data were expressed as mean ± SD.

RESULTS

Baseline Characteristics: Patient Profile

There were 30 ambulatory patients with ADPKD (14 males, 16 females; mean age, 50 ± 15 years) who had not undergone renal replacement therapy included in this study. The mean serum creatinine level of the patients was 2.10 ± 2.02 mg/dL, and the mean GFR, which is the creatinine clearance assessed using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula, was 54.4 ± 31.4 mL/min.

Control Group

There were 30 healthy volunteers in the control group (14 males, 16 females; mean age, 55 ± 15 years) with mean serum creatinine levels of 2.08 ± 2.25 mg/dL and a GFR of 58.5 ± 37.9 mL/min. Table 1 provides details of the baseline characteristics of the patients with ADPKD and the control group.

Urinary NGAL Levels in Patients With ADPKD and the Control Group

Urinary NGAL (uNGAL) was higher in the ADPKD group than in the control group, but there was no significant difference (uNGAL 155.40 ± 162.19 vs 118.13 ± 220.64; $P = .088$). In the ADPKD group, the uNGAL level appeared to increase with the decrease of the GFR. Table 1 provides details of the uNGAL of the patients with patients and the control group. Figure 1A shows the correlation of uNGAL