

Autosomal Dominant Polycystic Disease is Associated with Depressed Levels of Soluble Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand

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Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by multiple, large renal cysts and impaired kidney function. Although the reason for the development of kidney cysts is unknown, ADPKD is associated with cell cycle arrest and abundant apoptosis of renal tubular epithelial cells.

Aims: We asked whether serum-soluble TNF-related apoptosis-inducing ligand (sTRAIL) might underlie ADPKD.

Study Design: Case-control study.

Methods: Serum sTRAIL levels were measured in 44 patients with ADPKD and 18 healthy volunteers.

The human soluble TRAIL/Apo2L ELISA kit was used for the in vitro quantitative determination of sTRAIL in serum samples.

Results: Mean serum sTRAIL levels were lower in patients with ADPKD as compared to the control group (446.9 ± 103.1 and 875.9 ± 349.6 pg/mL, $p < 0.001$). Serum sTRAIL levels did not differ among stages of renal failure in patients with ADPKD. There was no correlation between serum sTRAIL levels and estimated glomerular filtration rate in patients with ADPKD ($p > 0.05$).

Conclusion: Our results show that ADPKD patients have depressed sTRAIL levels, indicating apoptosis unrelated to the stage of chronic renal failure.

Keywords: Serum-soluble TNF-related apoptosis-inducing ligand, chronic kidney disease, autosomal dominant polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD) is a disorder characterized by multiple, large renal cysts and impaired kidney function. Although the mechanism involved in the pathogenesis of ADPKD is unknown, it is thought to involve cellular proliferation, ending in the development of multiple and big cysts, modified renal epithelial cell polarity, dislocation of Na⁺-K⁺ ATPase pump and remodeling of the matrix (1,2). The precise mechanism implicated in the patho-

genesis of ADPKD has not been identified. Apoptosis is a pathologic feature of ADPKD (3-5).

TNF-related apoptosis-inducing ligand (TRAIL) derives from the tumor necrosis factor superfamily and induces apoptosis in transformed cells (6,7). TRAIL can stimulate both pro-apoptotic and anti-apoptotic pathways, but the mechanism by which a particular pathway is triggered remains less well understood (8). TRAIL binds to TRAIL-R1, R2, R3, and R4 receptors

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and a soluble receptor that it is called osteoprotegerin (9-14). TRAIL-R1 and R2 are death-inducing receptors, and TRAIL-R3, -R4, and osteoprotegerin may act as trap receptors (15).

Although increased levels of apoptosis are observed in human ADPKD (3-5), data regarding sTRAIL levels in patients with chronic renal failure are limited (16). To our knowledge, no previous study evaluated circulating sTRAIL levels in patients with ADPKD. We therefore investigated the circulating sTRAIL in patients with ADPKD and the relationship between serum sTRAIL levels and severity of renal failure in these patients.

MATERIALS AND METHODS

The study was approved by the local ethics committee and was performed in appropriate with the ethical rules of the Declaration of Helsinki. Inform consent was taken from all patients. A total of 44 patients with ADPKD (22 males, 22 females, mean age 56.7 ± 12.1 years) who had been diagnosed by modified Ravine criteria and a positive family history of ADPKD were included in the study, with 18 healthy volunteers as a control group (9 male, 9 female, mean age: 57.8 ± 14.5 years) were included in this study. The healthy subjects with comparable gender, age and body mass index were enrolled to study as a control group. Subjects with diabetes mellitus, obesity, dyslipidemia, coronary artery disease, cerebrovascular disease, peripheral arterial disease, malignancy, smoking history were excluded from the study.

Glomerular filtration rate (GFR) values were measured by chronic kidney disease epidemiology collaboration (CKD-EPI) formulation (17). The patients were classified by chronic kidney disease (CKD) stages consequently to the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines (18).

The human soluble TRAIL/Apo2L ELISA kit (ReproTech; New Jersey, USA) was used for the in-vitro quantitative determination of sTRAIL in serum samples. All the clinical follow-up markers including blood urea nitrogen, serum albumin, creatinine, uric acid, calcium, chloride and C-reactive protein were also evaluated by clinical biochemistry diagnostic laboratory kits.

Statistical analysis

The results were evaluated statistically using the Statistical Package for the Social Sciences (SPSS Corp.; version 16.0, Chicago, IL, USA). Continuous variables are shown as mean \pm standard deviation; categorical variables are shown as frequency and percentage. Normal distribution was evaluated by Shapiro Wilk's test. According to the presence of normal

distribution of the parameters, for the analysis of continuous variables the Mann-Whitney U test and t-test (independent samples t-test) were used. One-way ANOVA and Kruskal Wallis tests were used for comparisons of age and sTRAIL by stage. Pearson and Spearman's rho tests were used for correlation analysis. With 95% confidence interval, a p value less than 0.05 was considered to be statistically significant.

RESULTS

Mean age was similar in patients with ADPKD and the control group (56.7 ± 12.1 and 57.8 ± 14.5 years, $p > 0.05$). Mean serum sTRAIL levels were lower in patients with ADPKD than control group (446.9 ± 103.1 and 875.9 ± 349.6 pg/mL, $p < 0.001$) (Table 1).

In patients with ADPKD, mean hemoglobin level was 12.7 ± 1.4 g/dL; serum creatinine level was 4.0 ± 3.7 mg/dL; serum albumin level was 4.2 ± 0.9 g/dL; C-reactive protein level was 1.8 ± 0.5 mg/L; GFR level was 36.1 ± 24.0 mL/min (Table 1).

There was negative correlation between serum sTRAIL levels and hemoglobin in patients with ADPKD ($r = -0.348$, $p = 0.02$). There was no correlation between serum sTRAIL levels and the other parameters (age, GFR, serum creatinine, serum albumin, C-reactive protein) (Table 2). Serum sTRAIL levels did not differ with stages of renal failure in patients with ADPKD (Table 3).

TABLE 1. Characteristics of patients with ADPKD and HV

| | ADPKD (n=44) | HV (n=18) | p |
|--------------------------------------|-------------------|-------------------|--------|
| Age (years) | 56.7 ± 12.1 | 57.8 ± 14.5 | 0.471 |
| Female, No (%) | 22 (50%) | 9 (50%) | 0.955 |
| Male, No (%) | 22 (50%) | 9 (50%) | 0.930 |
| Body Mass Index (kg/m ²) | 28.21 ± 5.34 | 27.37 ± 4.31 | 0.400 |
| Hemoglobin (g/dL) | 12.7 ± 1.4 | 13.9 ± 0.9 | 0.250 |
| Creatinine (mg/dL) | 4 ± 3.7 | 0.77 ± 0.2 | <0.001 |
| eGFR (ml/min/1.73m ²) | 36.1 ± 24 | 98.1 ± 10.7 | <0.001 |
| Calcium (mg/dL) | 8.9 ± 2.3 | 9.37 ± 0.39 | 0.095 |
| Phosphate (mg/dL) | 4.51 ± 1.7 | 4.0 ± 0.67 | 0.089 |
| Parathyroid hormone (pg/mL) | 162.3 ± 268.9 | 47.7 ± 12.3 | <0.001 |
| Albumin (g/dL) | 4.2 ± 0.9 | 4.6 ± 0.28 | 0.150 |
| Serum sTRAIL (pg/mL) | 446.9 ± 103.1 | 875.9 ± 349.6 | <0.001 |

HV: healthy volunteers; ADPKD: autosomal dominant polycystic kidney disease; eGFR: estimated Glomerular filtration rate; sTRAIL: soluble TNF-related apoptosis inducing ligand

TABLE 2. Correlation between serum sTRAIL levels, demographics and laboratory parameters in patients with ADPKD

| | Serum sTRAIL (pg/mL) | |
|--------------------------------------|----------------------|-------|
| | r | p |
| Age (years) | 0.175 | 0.445 |
| Body Mass Index (kg/m ²) | 0.150 | 0.550 |
| Hemoglobin (g/dL) | -0.348 | 0.02 |
| C-Reactive Protein (mg/L) | 0.059 | 0.940 |
| Creatinine (mg/dL) | 0.135 | 0.750 |
| eGFR(ml/min/1.73m ²) | 0.127 | 0.720 |
| Calcium (mg/dL) | 0.120 | 0.355 |
| Phosphate (mg/dL) | 0.025 | 0.625 |
| Parathyroid hormone (pg/mL) | 0.190 | 0.945 |
| Albumin (g/dL) | 0.155 | 0.150 |

ADPKD: autosomal dominant polycystic kidney disease; eGFR: estimated Glomerular filtration rate; sTRAIL: soluble TNF-related apoptosis inducing ligand

TABLE 3. Age and sTRAIL in patients with ADPKD

| | Stage II N=19 | Stage III N=5 | Stage IV N=4 | Stage V N=16 | p |
|----------------|------------------|------------------|-----------------|-----------------|-------|
| Age (year) | 56.5±13.6 | 60.1±11.9 | 57.3±9.7 | 54.5±11.4 | 0.565 |
| sTRAIL (pg/mL) | 447.9±104.6 | 452.1±110.5 | 439.8±68.6 | 436.7±103.5 | 0.750 |

HV: healthy volunteers; ADPKD: autosomal dominant polycystic kidney disease; eGFR: estimated Glomerular filtration rate; sTRAIL: soluble TNF-related apoptosis inducing ligand

DISCUSSION

Autosomal dominant polycystic kidney disease is a genetic disease that presented by the development of many cysts in the kidney and other organs. The certain mechanism involved in the pathogenesis of ADPKD has not been determined. There have been major advances in the study of the pathophysiology of cyst formation and the progression of renal failure in ADPKD (2). One of those potential factors in cyst formation and loss of non-cystic tubules leading to renal failure in ADPKD is apoptosis. Increased levels of apoptosis are observed in human ADPKD (3-5) and experimental models of ADPKD (19-22). Furthermore, apoptosis has been reported in kidneys of humans with ADPKD regardless of renal function (5).

Cell death via apoptosis through the stimulation of specific intracellular pathways is a stimulation response of cells to affected microenvironments (22,23). Lethal cytokines, they are members of the TNF superfamily, stimulate decease receptors on the cell surface with consequent stimulation of caspases, central activators, and effectors of apoptosis (23-25). The apoptotic process is regulated by a host of checks and balances with a many of positive and negative factors (26). The

TNF-α is essential for induction of cytokine cascade which composing immune response. Also, TNF-α participates in immune response, inflammation and wound healing. The role and molecular function of the TRAIL and its receptors' system in the immune system has been extensively studied and various results were presented (8-14,27).

Vascular calcification and endothelial dysfunction are frequent in patients with chronic kidney disease. Accumulating experimental and clinical evidence suggests that TRAIL is associated with atherosclerosis. The circulating TRAIL level is positively associated with endothelial function, and TRAIL deficiency results in accelerated calcification in atherosclerosis (28-30).

Although increased levels of apoptosis are observed in human ADPKD, to our knowledge, no previous study has evaluated circulating sTRAIL levels in patients with ADPKD. In this study, we assessed the serum levels of sTRAIL as a marker of overall apoptotic activity in ADPKD. Serum sTRAIL levels were lower in patients with ADPKD than control group.

Data regarding sTRAIL levels in patients with CKD are limited (16). Liabeuf et al. (16) reported that patients undergoing hemodialysis had significantly lower serum sTRAIL levels when compared with patients with earlier stages of CKD, and lower serum STRAIL levels had been related to increased mortality in CKD patients. Furthermore, they found no correlation between serum sTRAIL and estimated GFR. Serum sTRAIL levels were not different between stages of CKD. This different result in patients with undergoing hemodialysis may be related etiological factor of chronic renal failure and blood sample collection time (predialysis or after dialysis period). Our study population was homogenous etiology (ADPKD) of CKD and sample for blood collection in pre-dialytic period. In addition, we did not find any correlation between STRAIL and the estimated GFR, similarly their study.

Clinical presentation of ADPKD differs from the other CKD causes with less frequent occurrence of anemia due to higher serum erythropoietin levels. Recent studies indicate that in ADPKD patients, proximal tubuli is the main site for erythropoietin production and erythropoietin is significantly enriched in cysts of proximal tubular origin (31,32). Both in vitro and in vivo studies have clearly documented that TRAIL shows regulatory functions in hematopoiesis under normal and pathological conditions (33-36). Choi reported that negative correlation between STRAIL and hemoglobin levels in healthy adults (34). We also found a negative correlation between sTRAIL and hemoglobin levels in patients with ADPKD.

Our study has several limitations. First, the sample size of our cohort (especially for each stage of CKD) was relatively small. Second, we relied on single measurement serum sTRAIL levels instead of serial monitoring. Third, we did

not analyze the cellular (for example cystic lesion) levels of sTRAIL. Therefore, new studies that have more sample sizes are necessary for this purpose. In conclusion, our results show that ADPKD patients have depressed sTRAIL levels as an indicator of apoptosis which was not related to stage of CKD.

Ethics Committee Approval: Ethics committee approval was received for his study from the ethics committee of Ministry of Health.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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