Endocan Levels in Children with Polycystic Kidney Disease

Polikistik Böbrek Hastalığı Olan Çocukların Endocan Düzeyi

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ABSTRACT

Objective: Autosomal dominant polycystic kidney disease (ADPKD) is a common renal disorder that is characterized by hypertension and renal failure. Recently, it has been emphasized that endocan which is an endothelial dysfunction biomarker, could increase in many renal diseases. High endocan levels have also been reported in hypertensive ADPKD adult patients with renal failure. However, studies are limited on children. In this study, we investigated the serum endocan levels in normotensive ADPKD children with normal renal function.

Material and Methods: The study consisted of 20 ADPKD children without hypertension and renal failure as a patient group, and 20 healthy age- and sex-matched children as a control group. Serum endocan levels were determined by enzyme-linked immunosorbent assay techniques and compared between the two groups.

Results: The mean age of patients was 9.9±4.12 years, and the mean age of the control group was 10.2±3.83 years. There was no significant difference between the two groups in terms of gender, age, and BMI (p=0.751, p=0.813, p=0.781, respectively). The leukocyte (p=0.449), hemoglobin (p=0.337), platelets (p=0.134), serum uric acid (p=0.671) and serum creatinine (p=0.074) levels, eGFR (p=0.459) were not significantly differed between groups. The mean serum endocan level in the PKD group was 345.8±169.5 pg/ml, and in the control group was 448.61±258.2 pg/ml. Serum endocan levels did not change between groups (p=0.159).

Conclusion: Unlike the adult ADPKD patients, this study suggested that serum endocan level was normal in children with ADPKD without hypertension and renal failure.

Key Words: Child, Endocan, Polycystic kidney disease

ÖZ

Amaç: Otozomal dominant polikistik böbrek hastalığı (ODPKB), hipertansiyon ve böbrek yetmezliği ile karakterize yaygın görülen bir böbrek hastalığıdır. Son zamanlarda endotel disfonksiyonu biyobelirteçlerinden olan endocanın birçok böbrek hastalığında artabileceği vurgulanmıştır. Böbrek yetmezliği olan hipertansif ODPKB'li erişkin hastalarda da yüksek endocan seviyeleri bildirilmiştir. Ancak, çocuklar üzerinde yapılan çalışmalar sınırlıdır. Bu çalışmada, normal böbrek fonksiyonu olan normotansif ODPKB'li çocuklarda serum endocan düzeylerini araştırdık.

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Gereç ve Yöntemler: Çalışmaya, hasta grubu olarak hipertansiyonu ve böbrek yetmezliği olmayan 20 ODPBH'li çocuk ve kontrol grubu olarak yaş ve cinsiyet açısından benzer 20 sağlıklı çocuk dahil edildi. Serum endocan seviyeleri, enzime bağlı immünosorbent tahlil teknikleri ile belirlendi ve iki grup arasında karşılaştırıldı.

Bulgular: Hastaların yaş ortalaması 9.9±4.12 yıl, kontrol grubunun yaş ortalaması 10.2±3.83 yıldı. Cinsiyet, yaş ve VKİ açısından iki grup arasında anlamlı fark yoktu (sırasıyla p=0.751, p=0.813, p=0.781). Gruplar arasında Lökosit (p=0.449), hemoglobin (p=0.337), trombosit (p=0.134), serum ürik asit (p=0.671), serum kreatinin (p=0.074) seviyeleri, ve eGFR (p=0.459) düzeyleri açısından anlamlı fark bulunmadı. PKB grubunda ortalama serum endocan düzeyi 345.8±169.5 pg/ml, kontrol grubunda 448.61±258.2 pg/ml'di. Serum endocan seviyeleri gruplar arasında değişmedi (p=0.159).

Sonuç: Erişkin ODPKB hastalarından farklı olarak, bu çalışmada hipertansiyon ve böbrek yetmezliği olmayan ODPKB'li çocuklarda serum endocan düzeyinin normal olduğu saptandı.

Anahtar Sözcükler: Çocuk, Endocan, Polikistik böbrek hastalığı

INTRODUCTION

Polycystic kidney disease (PKD) is an inherited kidney disease that can cause morbidity and mortality in childhood. (1). It is characterized by the increasing formation and expansion of multiple fluid-filled cysts in the parenchyma of the kidneys (2). The progression of cyst enlargement compresses the surrounding kidney areas and leads to fibrosis in renal parenchyma. The growth rate of the renal cysts and decreasing GFR also causes hypertension. Therefore, early diagnosis and optimal treatment of hypertension are essential to retard cyst growth and improvement of cystic disease (3). Many pathologic mechanisms that explain hypertension in PKD include activation of the renin-angiotensin-aldosterone system (RAAS), sympathetic nervous system, vascular vasopressin V1 receptors, macrophages, and reduction of nitric oxide production, and also an alteration of intracellular calcium levels (3-5). In addition, endothelial dysfunction which could lead to unbalanced vasoconstriction and ischemia of renal parenchyma is going to propose as an increasingly alternative pathway for renal damage in polycystic kidney disease (6).

Many biomarkers including kidney injury molecule–1 (KIM-1), N-acetyl-β-d-glucosaminidase (NAG), heart-type fatty acid-binding protein (HFABP), macrophage migration inhibitory factor (MIF), neutrophil gelatinase-associated lipocalin (NGAL), monocyte chemotactic protein–1 (MCP-1) have been investigated for understanding PKD progression (7). A new biomarker called endocan has also been investigated in PKD (6).

Endocan is a soluble that is secreted by vascular endothelial cells in various organs, as well as kidneys (8). It plays a key role in cell proliferation, migration, inflammation, neovascularization, and endothelial function (8). Its synthesis from the kidneys and its involvement in endothelial dysfunction suggests that endocan may be a potential early marker in PKD patients (6).

Raptis et al.(6) have reported that serum endocan levels are higher in adult hypertensive polycystic kidney diseases with renal failure compared to the healthy group. However, to our knowledge, there is no study about the endocan levels in PKD children with or without hypertension and renal failure. In this study, we want to investigate the serum endocan level in children with PKD without hypertension and renal failure.

MATERIAL and METHODS

Twenty children with ADPKD and 20 age-sex matched healthy children, ages between 5-17 years, were prospectively analyzed between February 2019 and January 2020 in our Pediatric Nephrology Department. Patients were included in the study if they had normal renal blood pressure and renal function with a documented diagnosis of ADPKD, according to the 'International consensus statement on the diagnosis and management of autosomal dominant polycystic kidney disease in children and young people' as PKD group (9). The child with a positive family history of PKD and one or more kidney cysts was accepted as PKD. Age-sex matched healthy children without any disease were also accepted as the control group.

Children with an acute or chronic inflammatory disease, history of recurrent urinary tract infection, asthma or chronic obstructive lung disease, cardiac disease, anemia, diabetes mellitus, insulin resistance, impaired glucose tolerance, hematologic or solid organ malignancies smoking habit, obesity, use of any medications, hepatic dysfunction, hypertension, and renal failure (estimated filtration rate below 90 mL/min/1.73m^2) were excluded from the study.

Normotension was determined from The American Academy of Pediatrics 'Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents' (10). Hypertension was defined as high in repeated office measurements (auscultatory confirmed blood pressure readings ≥95th percentile at 3 different visits) or ambulatory blood pressure measurements (mean systolic blood pressure and diastolic blood pressure > 95th percentile and systolic blood pressure and DBP diastolic blood pressure >25%) (10).

We used hemogram, CRP, urine analysis and urine culture for the parameters of inflammation status. Demographic data and medical history were collected from the files. Laboratory investigations of hemogram, urea, serum creatinine, uric acid, urine analysis, estimated glomerular filtration rate (eGFR), and also serum endocan level were all investigated. Body mass index (BMI, the ratio of height and weight, expressed as kg/m^2) was used to define obesity. The eGFR (ml/min per 1.73 m^2) was calculated using the original Schwartz equation.

Human Endocan Measurements

Blood samples were obtained after overnight fasting. Samples were allowed to clot for 30 min, centrifuged at 2000x g for 15 min for the separation of serum, and stored at -80°C until analysis.

The concentration of endocan was evaluated using an enzyme linked immunosorbent assay (ELISA) method. We used a commercially available human endocan ELISA kit (Uscn Life Science Inc, Wuhan, PR China). The ELISA process was applied according to the manufacturer's instructions. The absorbance was measured at 450 nm on a spectrophotometer. The intra-assay coefficient of variation was <10% while inter-assay coefficient of variation was <12%. The minimum detectable dose of this kit is typically less than 6.2 pg/ml. Linear measurement range of the assay was 15.6-1000 pg/ml.

The study was approved by the Clinical Research Ethics Committee of Ankara Pediatrics Hematology Oncology Training and Research Hospital (28.05.20219/2019-155).

Statistical analysis

SPSS v.20 was used for statistical analysis. Mann-Whitney U and t-tests were used for independent groups. The relationship between variables was examined by Chi-square analysis. p< 0.05 was considered statistically significant.

RESULTS

Twenty patients with PKD and 20 healthy controls were included in this study. The male/female ratio was 12/8 in the PKD group, and 10/10 in the control group. The mean age of patients with PKD was 9.9±4.12 years, the mean age of the control group was 10.2±3.83 years. The mean BMI of the PKD group was 18.7±2.47 kg/m^2, the mean BMI of the control group was 19.03±4.11 kg/m^2. There was no significant difference between the two groups in terms of gender, age, and BMI (p=0.751, p=0.813, p=0.781, respectively). The characteristics of the PKD and control groups are presented in Table I.

The mean follow-up period of the patients with PKD was 48.4±28.6 months.

The mean leukocyte level of patients with PKD and the control group were 7.6±2 µL, and 7.05±1.4 µL, respectively. The mea hemoglobin level of patients with PKD was 13.1±1.24g/dL, and the mean hemoglobin level of the control group was 13.5±1.23 g/dL. The mean platelets level of patients with PKD was 333.2±72.2 µL, the mean platelets level of the control group was 302.4±53.8 µL. There was no significant difference between groups for the levels of leukocyte (p=0.449), hemoglobin (p=0.337), and platelets (p=0.134).

Table I: The baseline characteristics of the PKD and control groups.

	PKD group (n=20)	Control group (n=20)	p
Age (year)	9.9±4.12	10.2±3.83	0.813
Gender (male/female)	12/8	10/10	0.751
Body mass index	18.7±2.47	19.03±4.11	0.781

Table II: The levels of complete blood count and biochemical parameters.

	PKD group (n=20)	Control group (n=20)	p
WBC (µL)	7.6±2	7.05±1.4	0.449
Hb (g/dL)	13.1±1.24	13.5±1.23	0.337
PLT (µL)	333.2±72.2	302.4±53.8	0.134
Urea (mg/dL)	28.1±7.5	22.7±6.4	0.031
Uric acid (mg/dL)	4.5±1.09	4.3±1.06	0.671
Creatinine (mg/dL)	0.57±0.17	0.63±0.13	0.074
eGFR (mL/min/1.73 m^2)	120.5±12.5	125±23.6	0.459
Blood endocan level (pg/ml)	345.8±169.5	448.6±258.2	0.159

The mean serum creatinine level was 0.57±0.17 mg/dL for patients with PKD, and 0.63±0.13 mg/dL for the control group. The mean serum uric acid level of patients with PKD was 4.5±1.09mg/dL, the mean serum uric acid level of the control group was 4.3±1.06 mg/dL. There was no significant difference between groups for the levels of serum creatinine (p=0.074), and uric acid (p=0.671).

The mean serum urea level for the PKD group was 28.1±7.5 mg/dL, the mean serum urea level for the control group was 22.7±6.4 mg/dL. The mean urea levels of the PKD group were significantly higher than the control group (p=0.031).

The mean eGFR for patients with PKD and the control group were 120.5±12.5 mL/min/1.73 m^2, 125±23.6 mL/min/1.73 m2, respectively. The eGFR (p=0.459) did not significantly differ between groups. The levels of complete blood count and biochemical parameters are presented in Table II.

None of the patients had hematuria or proteinuria in the PKD group.

The mean serum endocan level in the PKD group was 345.8±169.5 pg/ml and in control group was 448.61±258.2 pg/ml. Serum endocan levels did not change between groups (p=0.159). Serum endocan levels of groups are presented in Table II.

DISCUSSION

Polycystic kidney disease is an inherited, monogenetically, cilia-related disorder. The PKD affects an estimated greater than 10 million people around the world and eventually results in renal replacement therapy (RRT) and kidney transplantation (2). Many prognostic factors for disease progression in PKD have

been identified such as the early age of onset, hypertension, total kidney volume, and proteinuria. The identification of new molecular prognostic factors continues due to the growing knowledge of pathological cyst processes. To date, several cellular changes described with cyst formation and disease progression (1,3). Epithelial cell proliferation, fluid secretion, and extracellular matrix deposition are the major factors for cystogenesis. Increased inflammation is related to cyst formation. Many chemokines, cytokines, and growth factors, inflammatory cells contribute to cystogenesis. These molecular changes lead to alteration in the blood circulation of the regional kidney area (1,2).

Furthermore, increased cyst number and sizes induce local hypoxia which eventually results in fibrotic kidneys (2). The relationship between endothelial dysfunction and molecular alterations

suggests that new endothelial biomarkers could predict disease progression in PKD. Recently, a new endothelial biomarker called endocan was studied in various kidney diseases as well as PKD (6).

Endocan is a soluble proteoglycan that is secreted by vascular endothelial cells of many tissues such as lung, tumor endothelium, glandular tissues, germinal centers of lymph nodes, and also kidneys. It is an endothelial activation marker that is involved in inflammation, endothelial dysfunction, and angiogenesis (8).

Previous studies have demonstrated elevated plasma endocan levels in polycystic kidney disease, immunoglobulin A nephropathy, chronic kidney disease, and hypertension (11-14).

Yilmaz et al.(12) reported that serum endocan levels are inversely correlated with eGFR. Additionally, Raptis et al. (6) reported that serum endocan levels in ADPKD patients with impaired renal function were significantly higher compared to normal renal function. Also, they were reported high serum endocan levels in patients with ADPKD and normal renal function compared to healthy controls. From different studies, increased levels of serum endocan was explained by a marker of vascular inflammation which is an important process in the development of CKD (6,12). However, most of these studies includes adult patients, with and without hypertension and renal failure.

It is known that increasing endocan levels are correlated with inflammation and endothelial dysfunction which may play an important role in the pathophysiology of hypertension (13). Balta et al. (13) reported a significant increase in endocan levels in patients with newly diagnosed untreated HT compared with the normotensive group.

In this study, we evaluated the serum endocan levels in ADPKD children with normal renal function and normal blood pressure. We found no significant difference between the PKD and the control groups in terms of serum endocan levels.

Many studies reported that endocan is secreted from many cells as well as tubules and glomeruli (8). Li Recently et. al. (15) detected that endocan is mainly expressed from glomeruli in the kidneys. However, ADPKD is mainly a renal tubular disorder (1). Therefore, normal serum endocan levels of our patients who have normal renal function can be explained by the presence of mainly tubular alteration. In addition, none of our patients had hypertension. The high serum endocan levels in other studies with ADPKD adult patients may be related to the presence of hypertensive patients in their groups. It could be possible that serum endocan levels of our patients are going to be high when hypertension and renal dysfunction develop in their adulthood. However, we didn't include patients with hypertension and impaired renal function as a separated group. This was our limitation. On the other hand, to the best of our knowledge, this was the first study that evaluates serum endocan levels in children with ADPKD without hypertension and renal failure.

But the limitations of this study were that it has a small number of patients, and we did not use national reference for standard blood pressures in the pediatric age group.

CONCLUSION

Serum endocan levels did not change in ADPKD children without hypertension and renal failure. These results could suggest that endocan is mainly expressed from glomeruli, not from tubules, and the presence of hypertension and renal failure could elevate endothelial dysfunction and serum endocan levels in children with ADPKD. The study includes a low number of patients, and results require more prospective studies.

REFERENCES

1. Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. Lancet 2007; 369: 1287–301.
2. Harris PC, Torres VE. Polycystic kidney disease. Annu Rev Med 2009; 60: 321–37.
3. Rahbari-Oskoui F, Williams O, Chapman A. Mechanisms and management of hypertension in autosomal dominant polycystic kidney disease. Nephrol Dial Transplant 2014; 29: 2194–201.
4. Karihaloo A, Koraishy F, Huen SC, Lee Y,Merrick D, Caplan MJ et al. Macrophages promote cyst growth in polycystic kidney disease. J Am Soc Nephrol 2011; 22: 1809–14.
5. Hiesberger T, Gourley E, Erickson A, Koulen P, Ward CJ, Masyuk TV. et al. Proteolytic cleavage and nuclear translocation of fibrocystin is regulated by intracellular Ca2+ and activation of protein kinase C. J Biol Chem 2006; 281: 34357–64.
6. Raptis V, Bakogiannis C, Loutradis C, Boutou AK, Lampropoulou I, Intzevidou E et al. Levels of Endocan, Angiopoietin-2, and Hypoxia Inducible Factor-1a in patients with autosomal dominant polycystic

kidney disease and different levels of renal fuction. Am J Nephrol 2018; 47: 231–8.

7. Messchendorp AL, MEijer E, Boertien WE, Engels GE, Casteleijn NF, Spithoven EM. et al. Urinary biomarkers to identify autosomal dominant polycystic kidney disease patients with a high likelihood of disease progression. Kidney Int Rep 2018; 3: 291-301.
8. Lassalle P, Molet S, Janin A, Heyden JV, Tavernier J, Fiers W. et al. ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines. J Biol Chem 1996; 271: 20458–64.
9. Gimpel C, Bergmann C, Bockenhauer D, Breysem L, Cadnapaphornchai MA, Cetiner M et al. International consensus statement on the diagnosis and management of autosomal dominant polycystic kidney disease in children and young people. Nature Reviews Nephrology 2019;15, 713-26.
10. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Caroll AE, Daniels SR et al. Subcommittee on Screening and Management of High Blood Pressure in Children. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. Pediatrics 2017; 140: e20171904
11. Lee YH, Kim SJ, Kim SY, Kim YG, Moon JY, Jeong KH et al. Plasma endocan level and prognosis of immunoglobulin A nephropathy. Kidney Res Clin Pract 2016; 35: 152-9
12. Yilmaz MI, Siriopol D, Saglam M, Kurt YG, Unal HU, Eyileten T, et al. Plasma endocan levels associate with inflammation, vascular abnormalities, cardiovascular events, and survival in chronic kidney disease. Kidney Int 2014; 86: 1213-20.
13. Balta S, Mikhailidis DP, Demirkol S, Ozturk C, Kurtoglu E, Demir M et al. Endocan A novel inflammatory indicator in newly diagnosed patients with hypertension: A pilot study. Angiology 2014; 65: 773–7.
14. Ekinci I, Buyukbaba M, Cinar A, Tunc M, Cebeci E, Gursu M et al. Endothelial Dysfunction and Atherosclerosis in Patients With Autosomal Dominant Polycystic Kidney Disease. Cureus 2021; 13: e13561.
15. Li S, Wang L, Wang C, Wang Q, Yang H, Liang P et al. Detection on dynamic changes of endothelial cell specific molecule1 in acute rejection after renal transplantation. Urology 2012; 80: 738.e1–738.e8.