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Title: Mitochondrial dysfunction in children with chronic kidney disease.

Short title: Mitochondrial dysfunction in children with chronic kidney disease.

Abstract

Purpose: We aimed to determine serum mitochondrial open reading frame 12S rRNA-c (MOTS-C) levels as an indicator of mitochondrial dysfunction in childhood chronic kidney disease patients and to investigate the relationship of this parameter, which is a metabolic regulatory factor, with renal anemia, hypertension metabolic acidosis and renal osteodystrophy.

Materials and methods: The study included 46 children with chronic kidney disease and 46 healthy children of similar age and gender. The patient group was divided into G1-G5 subgroups according to glomerular filtration rate, etiology, renal replacement therapies and the presence of anemia, hypertension, hyperparathyroidism and metabolic acidosis. Data were analyzed using SPSS 25.0 package program.

Results: The mean MOTS-C level was 60.47±11.1 ng/ml in patients with chronic kidney disease and 105.2±54.7 ng/ml in healthy children (p=0.001). The MOTS-C level was significantly lower in children with chronic kidney disease. In addition, there was no significant difference between patients who underwent renal transplantation and patients on chronic dialysis or predialysis. MOTS-C levels were significantly lower in patients with hyperparathyroidism and anemia compared to those without.

Conclusion: In our study, we demonstrated that mitochondrial damage in children with chronic kidney disease begins even in the early stages, renal osteodystrophy and anemia contribute to this condition, and mitochondrial inflammation persists even after kidney transplantation in these patients.

Keywords: Mitochondria, MOTS-C, children, chronic kidney disease.

Makale başlığı: Kronik böbrek hastalığı olan çocuklarda mitokondriyal disfonksiyon.

Kısa başlık: Kronik böbrek hastalığı olan çocuklarda mitokondriyal disfonksiyon.

Öz

Amaç: Çocukluk çağı kronik böbrek hastalarında mitokondriyal disfonksiyonun bir göstergesi olarak serum MOTS-C düzeylerini belirlemeyi ve metabolik düzenleyici bir faktör olan bu parametrenin renal anemi, hipertansiyon, metabolik asidoz ve renal osteodistrofi ile ilişkisini araştırmayı amaçladık.

Gereç ve yöntem: Çalışmaya kronik böbrek hastalığı olan 46 çocuk ve benzer yaş ve cinsiyette 46 sağlıklı çocuk dahil edildi. Hasta grubu glomerüler filtrasyon hızına göre G1-G5, etiyoloji, renal replasman tedavileri ve anemi, hipertansiyon, hiperparatiroidizm ve metabolik asidoz varlığına göre alt gruplarına ayrıldı. Veriler SPSS 25.0 paket programı kullanılarak analiz edildi.

Bulgular: Ortalama MOTS-C düzeyi kronik böbrek hastalığı olanlarda 60,47±11,1 ng/ml iken sağlıklı çocuklarda 105,2±54,7 ng/ml idi (p=0,001). MOTS-C düzeyi kronik böbrek hastalığı olan çocuklarda anlamlı derecede düşüktü. Ayrıca, böbrek nakli yapılan hastalar ile kronik diyaliz veya prediyaliz hastaları arasında anlamlı bir fark yoktu. MOTS-C düzeyleri hiperparatiroidizm ve anemisi olan hastalarda olmayanlara kıyasla anlamlı derecede düşüktü.

Sonuç: Çalışmamızda, kronik böbrek hastalığı olan çocuklarda mitokondriyal hasarın erken evrelerde bile başladığını, renal osteodistrofi ve aneminin bu duruma katkıda bulunduğunu ve mitokondriyal inflamasyonun bu hastalarda böbrek naklinden sonra bile devam ettiğini gösterdik.

Anahtar kelimeler: Mitokondri, MOTS-C, çocuklar, kronik böbrek hastalığı.

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Introduction

Mitochondrial dysfunction is associated with increased oxidative stress (OS) and metabolic disorders and is known to contribute to the pathophysiology and progression of chronic kidney disease (CKD) [1-4]. Uremia in CKD leads to the release of proinflammatory cytokines (IL-1, IL-6, TNF). These cytokines are toxic to mitochondria, affect mitochondrial function and trigger cellular aging. OS, defined as disturbances in the pro-/antioxidant balance, is highly damaging to cells due to excessive formation of reactive oxygen (ROS) and nitrogen species [5]. Chronic inflammation and mitochondrial dysfunction are increasingly recognised as contributors to kidney fibrosis and end stage renal disease [4, 5]. Kidney is a mitochondria-rich organ and mitochondrial superoxide production leads to oxidative damage, which in turn damages mitochondrial DNA and electron transport chain. In patients with advanced stages of CKD, increased OS is associated with complications such as hypertension, atherosclerosis, inflammation, and anemia [5]. Persistence of oxidative stress and mitochondrial dysfunction leads to transition from acute kidney injury to chronic kidney disease [5].

Mitochondrial derived peptides (MDPs) humanin and mitochondrial open reading frame 12S rRNA-c (MOTS-c) are known to play a role in cell survival, apoptosis suppression and glucose control [4]. In response to an increase in oxidative stress, the MOTS-c protein translocates to the nucleus. MOTS-c has been demonstrated to interact with Nrf2 in the nucleus, thereby regulating the expression of antioxidant response element genes [4]. In the only study MDPs in patients with CKD, MOTS-C levels were found to be low in serum and muscle, while humanin levels were found to be low in muscle and normal in serum. The situation in childhood remains unclear. In our study, we aimed to determine serum MOTS-C levels as an indicator of mitochondrial dysfunction in childhood CKD patients and to investigate the relationship of this parameter, which is a metabolic regulatory factor, with renal anemia, hypertension, metabolic acidosis and renal osteodystrophy.

Materials and methods

A prospective cross sectional study involving children with CKD and healthy children of similar age and sex were conducted in at a tertiary care referral hospital. Weight (kg), height (cm) and manual blood pressure were measured. Clinical data including age, gender, CKD duration, etiology, and treatment were collected from patients' medical records.

Glomerular filtration rate (GFR) was calculated using the Schwartz formula (height (cm) x 0.413 /plasma creatinine (mg/dl)) [6]. CKD is defined as abnormalities of kidney structure or function, present for a minimum of 3 months, with implications for health [7]. CKD has classified based on GFR category (G1–G5) [8]. Patients with CKD were divided

into predialysis, hemodialysis, peritoneal dialysis and renal transplant groups according to their treatment. Additionally patients with CKD were divided into subgroups according to the presence of anemia, hypertension, renal osteodystrophy, and metabolic acidosis.

Anemia in children was defined using age-specific thresholds, namely for 0.5 to 4 years, a Hb <11 g/dl; for 5 to 11 years, a Hb <11.5 g/dl; and for 12 to 14 years, a Hb <12 g/dl [9]. Hypertension was defined as systolic and/or diastolic blood pressure above the 95th percentile for age, gender, and height [10]. The target range for PTH is 35-70 pg/mL in CKD stages 2-3 and 70-110 pg/mL in CKD stage 4 and <300 pg/mL in pediatric patients with CKD stage 5 [11]. Patients with a blood pH of less than 7.35 and a HCO3 of less than 22 mmol/l were defined as having metabolic acidosis [7].

Urea, creatinine, sodium, potassium, calcium, phosphorus, alkaline phosphatase, vitamin D and PTH were evaluated in routine laboratory tests in serum samples taken in the morning. Serum calcium, phosphorus and alkaline phosphatase levels were measured by photometric method and vitamin D and PTH levels were measured by electrochemiluminescence immunologic method. MOTS-C levels were analyzed from patient venous blood samples collected by enzyme-linked immunosorbent assay (ELISA) in the Medical Biochemistry research laboratory.

Data were analyzed using SPSS 25.0 (IBM SPSS Statistics 25 software) package program. Continuous variables were analyzed as mean ± standard deviation, median (IQR: Interquartile range) and categorical variables are given as numbers and percentages. When parametric test assumptions are met Independent samples t test was used in the comparison of independent group differences test; when parametric test assumptions are not met, independent group differences Mann Whitney U test was used for comparison. According to the reference study results [4], they had a strong effect size (d=1.43) for MOTSC results. Assuming we can achieve a lower effect size (d=0.7), when at least 90 participants (at least 45 participants per group) were included in the study, that would result in 80% power with %95 confidence level (%5 type 1 error rate).

Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study (date: 04.03.2025 and number: E-60116787-020-665941).

Results

The study encompassed 46 patients diagnosed with chronic kidney disease (20 female and 26 male) and an equivalent number of patients from the control group (21 female and 25 male). The mean age of patients with CKD was 13.7±5.6 years, while the

mean age of patients from the control group was 12.5±2.8 years. Age and gender of the patient and healthy control groups were similar.

The mean MOTS-C level was 60.47±11.1 ng/ml in patients with CKD and 105.2±54.7 ng/ml in healthy children (p=0.001). The MOTS-C level was significantly lower in children with CKD. Among the patients with CKD, 10 were in chronic peritoneal dialysis, 3 were in chronic hemodialysis program, 11 had received kidney transplantation, 22 were in pre-dialysis stage and were not yet on renal replacement therapy. In addition, there was no significant difference between patients who underwent renal transplantation and patients on chronic dialysis or predialysis (Table 1). Among the predialysis patients, 1 had stage 1, 3 had stage 2, 9 had stage 3, and 9 had stage 4 CKD. MOTS-C levels were found to be non-statistically lower in grade 4 compared to the other groups (Figure 2). When the etiologies of patients with CKD were analyzed, 42% had glomerular disease, 43% had tubular disease and 15% had unknown etiology, there was no significant difference between the groups in terms of MOTS-C levels (Table 2).

Anemia was present in 37% of patients with CKD, hyperphosphatemia in 43%, vitamin D deficiency in 57%, secondary hyperparathyroidism in 56%, metabolic acidosis in 43%, and hypertension in 58%. MOTS-C levels were significantly lower in patients with hyperparathyroidism and anemia compared to those without (Table 3). MOTS-C levels were similar in patients with metabolic acidosis, hyperphosphatemia or hypertension compared to those without.

Discussion

In the present study, it was demonstrated that mitochondrial dysfunction is present in patients with childhood CKD, independent of staging, etiology, and the type of renal replacement therapy received. Furthermore, we established that anemia and secondary hyperparathyroidism also increase mitochondrial dysfunction. While only one study in the literature showed that mitochondrial-derived peptides are decreased in CKD patients in adulthood, this study is the first to show that MOTS-C, one of the mitochondrial-derived peptides, is decreased in children with CKD.

The MDPs, humanin and MOTS-c are involved in cell survival, apoptosis suppression and glucose metabolism, there is only one study in the literature on its role and levels in CKD [4]. This study MOTS-C levels were found to be low in serum and muscle, while humanin levels were found to be low in muscle and normal in serum.

The results of the study suggest that MDP levels are associated with evidence of systemic inflammation and oxidative stress in muscles, two hallmarks of premature aging and uremia [4]. In another study, muscle biopsies were performed in adult patients with

CKD and it was found that the number of mitochondrial DNA decreased as the stage progressed [1]. In the present study, while MOTS-C levels were found to be significantly lower in paediatric patients with CKD compared to healthy children, no difference was observed between predialysis patients and patients receiving renal replacement therapy. Of particular interest was the observation that MOTS-C levels in patients with normal GFR post-renal transplantation, regarded as the optimal renal replacement therapy, were also significantly lower than those in the control group (p=0.002). This finding suggests that mitochondrial inflammation may persist in these patients, even in cases where GFR improves following renal transplantation. Regarding the etiology of CKD, MOTS-C levels were similar between the groups in terms of glomerular and tubular pathologies. In addition, the apparent decrease in MOTS-C as the grade 4-5 progressed in children with CKD was not statistically significant. These findings suggested the presence of mitochondrial inflammation in children with CKD from early stages and independent of etiology.

The mechanism by which high parathyroid hormone levels are a risk factor for cardiovascular diseases has been suggested to be that they cause oxidative damage by causing endothelial damage [12]. Two studies in the literature have demonstrated that oxidative stress markers are elevated in patients with hyperparathyroidism, and serum levels of these markers decrease following parathyroidectomy [13, 14]. In our study, we observed that MOTS-C levels were lower in CKD patients with hyperparathyroidism in comparison to those without hyperparathyroidism, thus indicating that the presence of hyperparathyroidism, in addition to CKD, is a contributing factor to mitochondrial dysfunction.

Hypertension is known to increase ROS production, leading to endothelial dysfunction and mitochondrial dysfunction. In hypertension, an excess of ROS generation cannot be counterbalanced by endogenous mitochondrial protective antioxidant mechanisms, leading to an increased state of mitochondrial oxidative stress [15]. While it is known that high blood pressure causes oxidative damage, our study did not show that high blood pressure also causes damage to the mitochondria in patients with CKD.

In a study investigating the relationship between anemia and oxidative damage, it was shown that both oxidative stress and DNA damage were increased in patients with iron deficiency anemia. It was interpreted that increased oxidative stress is an important factor causing DNA damage in patients with iron deficiency anemia [16]. In a study evaluating oxidative damage and renal function in children with iron deficiency anemia, markers of renal damage and oxidative damage such as urinary microalbumin were found to be high in children with iron deficiency anemia. At the end of the study, they suggested that oxidative damage contributed to the pathogenesis of renal function in these patients [17]. In the

present study, MOTS-C levels were found to be significantly lower in CKD patients with anaemia in comparison to children without anaemia. This finding indicates that the presence of anemia contributes to the exacerbation of mitochondrial dysfunction in patients with CKD.

One of the causes of chronic inflammation and oxidative damage in CKD is metabolic acidosis [18]. Metabolic acidosis was found in 43% of our patients and MOTS-C levels in these patients were similar to those in patients without acidosis.

It is known that the presence of mitochondrial dysfunction in CKD and concomitant pathologies such as uremia, metabolic acidosis, hypertension and anemia increase dysfunction and that mitochondrial dysfunction contributes to CKD progression. In our study, which is the first of its kind in childhood, we have shown that mitochondrial damage begins even in the early stages in children with CKD, renal osteodystrophy and anemia contribute to this condition, and mitochondrial inflammation continues in these patients even after kidney transplantation.

The single-centre, modest sample size and cross-sectional nature of our study are limitations.

MOTS-c has recently attracted attention as a potential prevention or therapeutic option for obesity and T2DM [19]. We believe that our small study on MOTS-c, a mitochondrial polypeptide, in childhood CKD patients will shed light on similar studies on a larger scale. In addition, our study may pave the way for studies in which mitochondrial peptides can be used in therapeutic treatment to prevent mitochondrial dysfunction, which plays a role in the progression from acute kidney injury to chronic kidney injury.

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Authors contributions: I.G. has constructed the main idea and hypothesis of the study. She developed the theory and edited the material and method section. E.A. has done the evaluation of the data in the Results section. Discussion section of the article Written by I.G. and E.A. and they reviewed, corrected and approved. In addition, all authors discussed the entire study and approved the final version.

Conflict of interest: No conflict of interest was declared by the authors.

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Table 1. MOTS-C levels in healthy and chronic kidney disease children

	MOTS-C (ng/ml)		MOTS-C (ng/ml)		
	Chronic Kidney Disease (n=46)	**Healthy group (n=46)**	**Pre-dialysis (n=22)**	**Kidney transplanted (n=11)**	**Dialysis (n=13)**
Mean±SD	60.4±11.1	105.2±54.7	62.1±10.7	61.2±13.2	56.5±9.8
Median	60.09	102.1	62.8	59.7	57.4
IQR	(53.1-66.8)	(49.9-61.4)	(55.7-67.3)	(52.6-71.5)	(49.9-61.4)
p	0.0001* (t=-5.15)		0.360[a] (t=0.92)	0.607[b] (t=-0.51)	0.72[c] (t=1.34)

SD: Standard Deviation; IQR: Interquartile Range (25^{th} – 75^{th} percentiles)
*$p<0.05$ statistically significant, Comparison of the CKD and healthy groups
[a] Comparison of the predialysis group with other groups in patients with CKD
[b] Comparison of the kidney transplanted group with other groups in patients with CKD
[c] Comparison of the dialysis group with other groups in patients with CKD
[a-b-c] Independent t test was used to compare groups

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Table 2. MOTS-C levels according to the etiology of childhood chronic kidney disease

Classification of Chronic Kidney Disease	Etiology of Chronic Kidney Disease	n (46)	MOTS-C (ng/ml) Mean±SD	*p*
Glomerular (n=19)	Focal Segmental Glomerulosclerosis	9	58.8±11.8	0.268* t=-1.12
	Hemolytic Uremic Syndrome	2		
	Crescentic Glomerulonephritis	1		
	IGA nephropathy	1		
	Congenital Nephrotic Syndrome	2		
	Lupus Nephritis	2		
	Chronic Tubulointerstitial Nephritis	2		
Tubular (n=20)	Cystic Kidney Disease	2	62.5±9.9	
	Neurogenic Bladder	6		
	Vur Nephropathy	5		
	Cystinosis	3		
	Other Urological Anomalies	4		
Unknown Cause (n=7)**	Chronic Kidney Disease of Unknown Cause	7	57.9±14.7	

SD: Standard Deviation; *=Comparison of glomerular and tubular CKD patients; **Unknown Cause group was not included in the comparison due to lack of numbers, t= independent samples t test

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Table 3. Comparison of MOTS-C levels with the presence of anemia, hyperparathyroidism, hyperphosphatemia in Chronic Kidney Disease group

MOTSC (ng/ml)	Anemia		Hyperparathyroidism		Hyperphosphatemia	
	Yes	**No**	**Yes**	**No**	**Yes**	**No**
Mean±SD	54.8±9.2	63.7±10.9	57.6±10.3	64.2±11.3	56.9±10.8	63.3±10.8
Median;	56.6	64.3	57.0	64.0	57.0	64.3
IQR	50.6-59.2	56.7-70.0	50.6-65	57.7-71.1	50.4-60.8	55.7-69.8
p	0.007* (t=2.8)		0.045* (t=2.05)		0.053 (t=1.9)	

SD: Standard Deviation; IQR: Interquartile Range (25^{th} – 75^{th} percentiles)
*$p<0.05$ statistically significant
t= independent samples t test

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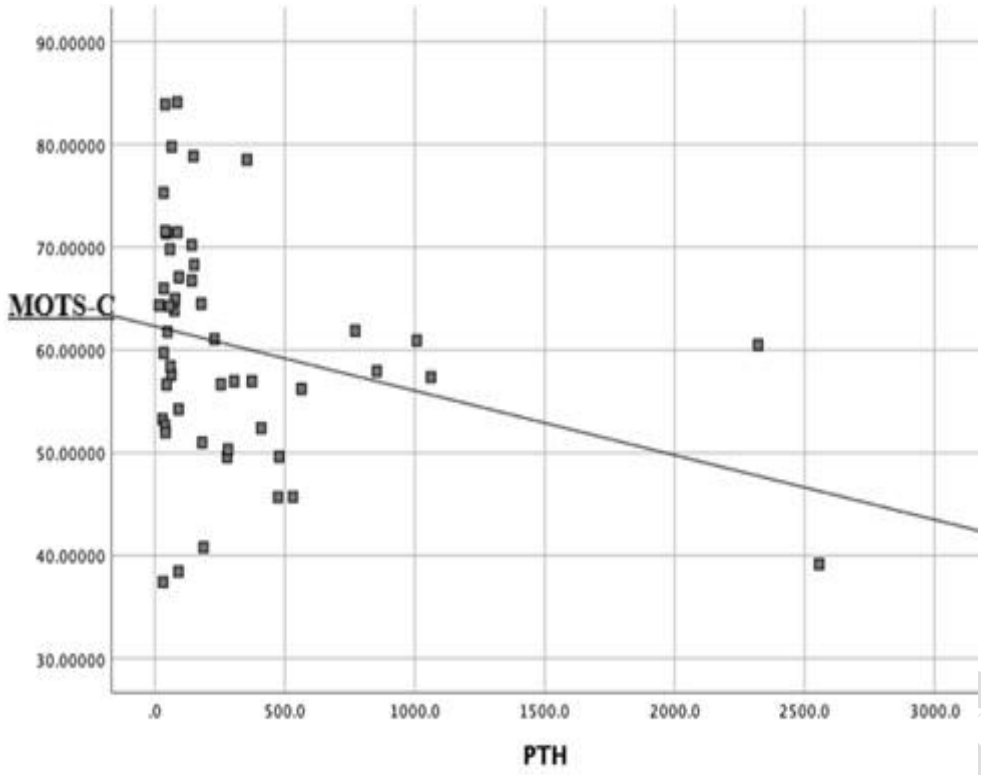

Figure 1. Negative correlation of MOTS-C with parathormone (PTH) levels in children with Chronic Kidney Disease (r:-0.311 *p*=0.036)

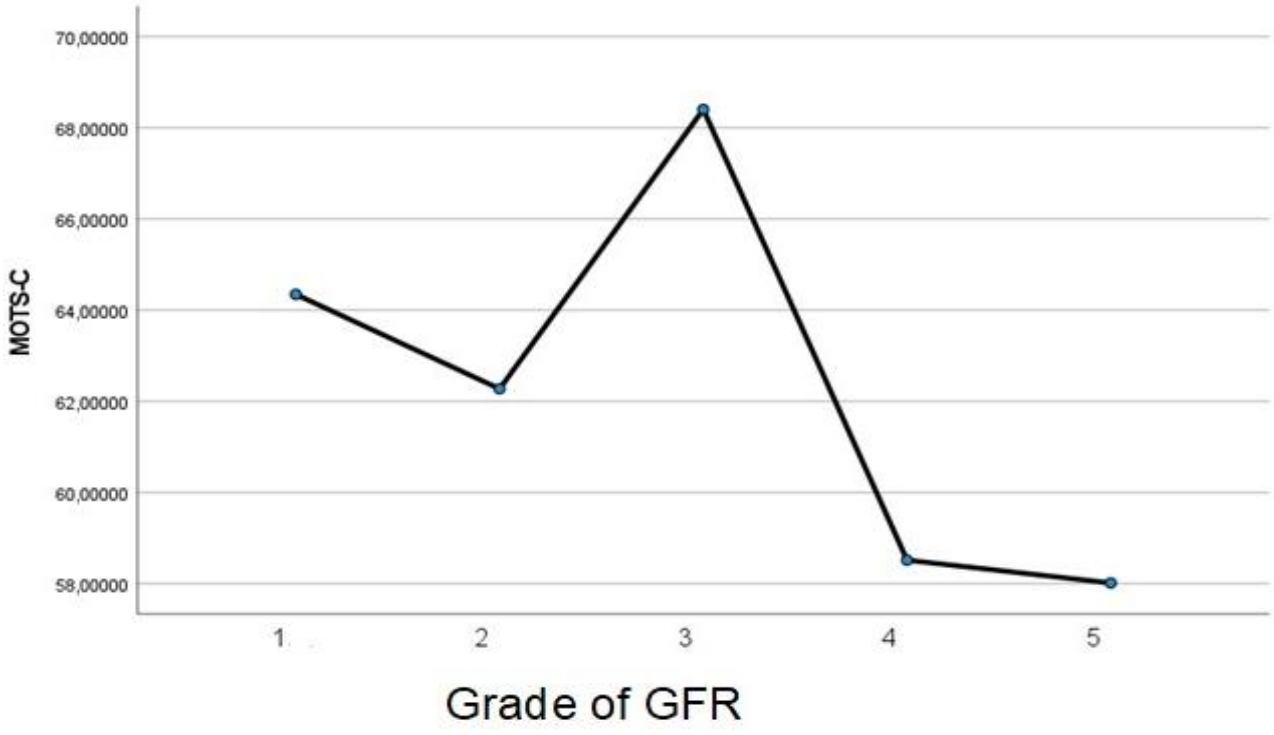

Figure 2. Serum MOTS-C levels according in stages of GFR in CKD

Girisgen I, Avci E. Mitochondrial dysfunction in children with chronic kidney disease. Pam Med J 2025;18:…-…

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