Original Article

Title: Fibroblast growth factor 21 (FGF21) in children and adolescents with chronic kidney disease.

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Short title: FGF21 in chronic kidney disease in children

Abstract

Fibroblast growth factor 21 (FGF21) is one of the members of endocrine arm of FGF family. Its actions as a glucose and lipids metabolism regulator are widely known. Although the mechanism of FGF21 action in kidneys is still under investigation, FGF21 was considered as a marker of early kidney function decline. While many researchers focused on adult subjects in this matter, there are no data regarding children. Therefore, we have investigated the relationship between plasma or urine FGF21 levels and kidney function in a group of 42 pediatric patients with chronic kidney disease (CKD). Anthropometrical parameters and blood pressure were taken, routine biochemical tests were performed. The concentration of FGF21 in serum and urine was determined by enzyme immunoassay. The results revealed significantly higher serum FGF21 concentration among children from CKD group. However, serum FGF21 level was not related to gender, proteinuria, eGFR or renal replacement therapy. Urine FGF21 concentration correlated negatively with albuminuria and positively with eGFR. Documented negative correlation of FGF21 fractional excretion and eGFR is not enough to support the role of FGF21 as a biomarker for predicting kidney disease progression in children and adolescents. Other mechanisms including local kidney FGF21 production or enhanced excretion due to higher extrarenal production may result in higher urine FGF21 concentrations.

Key-words: children, chronic kidney disease, FGF superfamily, FGF21, renal replacement therapy

Introduction

Chronic kidney disease (CKD) is a condition of irreversible kidney damage, which usually proceeds with a progressive decrease in glomerular filtration (KIDGO 2012). The degree of renal impairment in children with CKD is assessed by calculating the estimated glomerular filtration rate (eGFR) according to the Schwartz equation (Mian and Schwartz 2017), based on the measurement of serum creatinine. The epidemiology of CKD in children originates mostly from data available of stage 5 CKD patients on renal replacement therapy (RRT). The median incidence of RRT in 0-19 years was 9 per 1 million of age-related population (Harambat *et al.* 2012; Warady and Chadha 2007). In younger patients the dominant causes are congenital kidney and urinary tract defects (CAKUT), when in older children acquired diseases are usually recognized - mostly glomerulonephritis. Reports draw attention to the growing problem of obesity and diabetes in the pediatric population, predicting an increase in CKD prevalence (Czarniak *et al.* 2010). This problem also concerns children born prematurely or born with low body weight (Crump*et al.* 2019).

Human fibroblast growth factor (FGF) superfamily is a group with a wide range of biological function such as development, repair and metabolism (Itoh 2010).For the first time FGF was isolated from bovine pituitary in 1975 (Gospodarowicz 1975). Since then the studies on FGF family gradually expanded, showing that the group consists of 22 proteins, numbered as FGF1 to 23 (FGF19 is a human ortholog of rodent FGF15), divided into three subgroups: intracellular, paracrine and endocrine, according to their mechanisms of action (Itoh 2010; Krejci *et al.* 2016). The “classic” FGFs are heparin-binding proteins that interact with cell-surface localized FGF receptors (FGFRs) for signal transduction. Members of the endocrine group, comprising FGF19, FGF21 and FGF23, are targeting cells from the bloodstream and have a unique receptor activation mechanism. They require α-Klotho or β-Klotho proteins as a cofactor for FGFR (Angelin *et al.* 2012; Itoh *et al.* 2015; Szymczyk *et al.* 2013).

Human fibroblast growth factor 21 (FGF21) is a 209 amino acid protein acting as a multifaced metabolic regulator. The FGF21 gene is localized in chromosome 19 and the mRNA expression takes place primarily in the liver, but also in the white adipose tissue (WAT), skeletal muscle, thymus and pancreas (Anuwatmatee *et al.* 2019). As it was mentioned before, the FGF21 activity depends on its binding to FGFR or β-Klotho/FGFR complex (depending on the targeting tissue) (Adams *et al.* 2012; Szymczyk *et al.* 2013). The FGF21 expression is regulated by different transcriptional factors such as peroxisome proliferator-activated receptor α (PPARα) in the liver or PPARγ in adipocytes (Szymczyk *et al.* 2013). The biology of FGF21 was studied over the past years emphasizing its role in carbohydrate, lipid and energy metabolism, especially during fasting.

The effects of FGF21 on different tissues are presented in Figure 1. The major actions of FGF21 are stimulation of fatty acid oxidation, gluconeogenesis and ketogenesis in the liver, increasing glucose uptake, lipolysis and thermogenesis in WAT, WAT conversion to brown adipose tissue (BAT), improvement of insulin sensitivity in cells and preservation of β-cell function in pancreas (Anuwatmatee *et al.* 2019; Itoh 2010; Kharitonenkov and DiMarchi 2015; Zhang *et al.* 2018). The cardiovascular protective role of FGF21 is expressed through the anti-atherosclerotic properties in vessels and reduction of oxidative stress in the heart (Kokkinos *et al.* 2016). It has been proposed that some of metabolic effects may correlate with the activation of central nervous system, where meanwhile FGF21 follows circadian behavior (Andersen *et al.*2011; Liang *et al.* 2014). Many of these relationships were recognized in studies on animal models (FGF21/FGFR/β-Klotho-knockout mouse) rising questions about the possible use of FGF21 to ameliorate diseases in humans.

Yet we still don’t know the exact effects of FGF21 on kidneys. Most of the FGF21 studies were carried out in adult patients and only few studies described the children population, mostly taking obesity under consideration. Although in the adult population

FGF21 has been considered as a prognostic factor for the progression of renal disease (Lee *et al.* 2015; Anuwatmatee *et al.* 2019), data on this topic among children with CKD have not been published.

The aim of our study was to evaluate the usefulness of FGF21 measurements in serum and urine in children diagnosed with CKD as a biomarker of early renal function decline.

Material and Methods

The study group (CKD) consisted of 42 patients (18 girls and 24 boys) aged 2 to 18 years with CKD. The causes of CKD were CAKUT - 19 children (45.2%), polycystic kidney disease - 8 patients (19%); tubulointerstitial nephritis - 6 (14.2%), glomerulonephritis- 5 (11.9%), nephronophthisis - 2(4.7%), renal toxicity - 1 (2.5%) and tubulopathy - 1 patient (2,5%). Twenty-six children (62%) were diagnosed with hypertension. Children from the study group were treated pharmacologically with antihypertensive agents, iron formulas, vitamin D, calcium and sodium bicarbonate. Mean time of CKD duration was 5.9±4.5 years. Ten children (23%) were treated with renal replacement therapy (RRT) using maintenance hemodialysis (3 patients) or peritoneal dialysis (7 patients). On admission, weight, height and blood pressure were taken and routine biochemical tests were performed. Body mass index (BMI) was calculated using the formula [BMI=body weight (kg)/height (m^2)]. Estimated glomerular filtration rate (eGFR) was calculated according to the Schwartz formula (mL/min/1.73 m^2) (Mian and Schwartz 2017). Additionally, albuminuria (mg/day) using 24 h urine collection was evaluated.

The control group consisted of 21 healthy children (11 girls and 10 boys) aged 1 to 18 years admitted for procedures of one-day surgery or patients with monosymptomatic nocturnal enuresis. All children presented no signs of kidney disease, were in good clinical condition and had no symptoms of acute infection. Anthropometric measurements and the age

of the study subjects and controls are presented in Table 1. The average age, weight, height and BMI in the study group and the control group did not differ significantly.

The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice (resolution No. KNW/0022/KB1/110/I/12 of 03.12.2013) and written consent from parents or legal guardians, and/or the patients was obtained.

Blood samples (3-5mL) for laboratory tests were drawn in Eppendorf tubes in the morning (8:00-9:00) during examination related to periodic control in the outpatient clinic. After centrifugation 1000x for 15 minutes at 4°C, the serum was stored at -20°C until assayed. Urine samples (50-100mL) were collected in children with CKD at the same time as the blood samples, and also kept at -20°C until evaluated. Determination of concentration of albumin and creatinine in urine samples as well as concentration of albumin in 24 hour urine collection was performed.

Determination of FGF21 levels

Determination of serum and urine concentrations of FGF21 was performed in the Chair and Department of Medical and Molecular Biology, Faculty of Medical Sciences in Zabrze, SUM in Katowice, using immunoenzymatic tests of the CLOUD-CLONE company (Cloud-Clone Corporation, USA, catalog number SEC 918 Hu). The kit was chosen because it was approved for serum, plasma, tissue homogenates, cell lysates, target culture supernates and other biological fluids. Before determining the concentration of FGF21 in urine, collected and frozen samples (after thawing) were centrifuged for 20 minutes at 1000x g. Harvested supernatants were then diluted 100-fold with cold PBS (resulting from preliminary laboratory tests). To determine the concentrations of the samples tested, a calibration curve was prepared using the parent standard. After reconstructing the parent standard which was included in the lyophilized form, a series of its dilutions was prepared - 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, 15.6pg/ml, 7.8pg/ml, 0pg/ml. The analytical procedure was carried out

according to the manufacturer's instructions attached to the kit. Absorbance readings were carried out using the Universal Microplate Spectrophotometer - μQUANT by BIO-TEK INC (Bio-Tek World Headquarters, California, USA) at a wave length of 450 nm, and the results were processed using the KCJunior computer program (Bio-Tek, USA) using a log-log curve (both results and absorbance were logarized). The sensitivity of the set was 2.7 pg/ml, 10% in-series error and 12% out-of-series error. Additionally fractional excretion of FGF21 (FE FGF21) has been calculated and compared between the groups of children with CKD.

Statistical analysis

A database was prepared in a Microsoft Excel spreadsheet. For statistical calculations STATISTICA software licensed v. 10.0 was used (StatSoft Inc, Tulsa, USA). Level of statistical significance was assumed at $p<0.05$. As the parameters of descriptive statistics, the arithmetic mean, median, minimum and maximum value, lower and upper quartile and standard deviation were chosen. For all parameters, the compatibility of their distributions with a normal distribution was checked with Shapiro-Wilk test. For variables with normal distribution, parametric test were used (t-test for independent variables in comparative analyses and Pearson's test for analyses of correlation). For other variables, nonparametric test were applied (Mann-Whitney U-test for comparisons and Spearman's rank correlation test for analyses of correlation).

Results

The results of routine laboratory tests are presented in Table 2. Children with CKD had elevated mean daily urinary albumin excretion and the albumin/creatinine ratio. Total serum proteins and albumin levels remained within the normal range. Mean eGFR in CKD group (with an average of 47.87±20ml/min/1.73m^2) and standardized levels of creatinine clearance were lowered without showing no gender differences. Analyzing the lipid profile, although the CKD group of girls presented increased mean total cholesterol, the average level of total

cholesterol, HDL and LDL cholesterol fractions were in the normal range and triglycerides concentration was elevated in whole CKD group. Among the entire CKD group, only two patients presented with obesity (BMI>97 p.c) and 9 with increased values of systolic blood pressure (SBP>97 pc). Mean MAP value was 79.1±10.0 mmHg.

Serum FGF21 concentrations were significantly higher in the CKD group (Figure 2). Further analysis showed that there were no significant differences concerning the age, method of therapy (RRT vs. conservative treatment) or the presence of proteinuria among the studied subjects (Figure 3-5). Mean FE FGF21 was 14.02±14.06% in the total CKD group, in girls and boys 16.7±19.1% and 12.13±9.2%, respectively. There was no significant gender difference. In RRT subgroup FE FGF21 was 41.0±17.24% and was higher as compared to predialysis group (9.38±6.21%;$p<0.0001$). In children with hypertension the value of FE FGF 21 was 16.12±17.47% and was comparable to value obtained in normotensive CKD children (11.37±7.74%).

There was no correlation between serum FGF21 level and any of measured anthropometric and laboratory parameters. eGFR correlated negatively with albumin/creatinine ratio ($r=-0.4603;p<0.01$) but no correlation with albuminuria was found.

Urine FGF21 concentration correlated negatively with urine albumin excretion ($r=-0.4965;p<0.01$) and positively with creatinine clearance ($r=0.4372;p<0.05$), eGFR ($r=0.5339;p<0.01$), age ($r=0.3414;p<0.05$), hemoglobin concentration ($r=0.3672;p<0.05$) and sodium level ($r=0.4377;p<0.01$). Detailed data are presented in Table 3.

FE FGF21 correlated positively with urine albumin excretion ($r=-0.4375$; $p<0.02$) and with albumin/creatinine ratio ($r=-0.7067;p<0.0001$).

Discussion

Many researchers had studied the relevance of FGF21 in various biological conditions. Some of them suggest that higher blood levels of FGF21 in patients with renal disease result

from impaired renal excretion (Hindricks *et al*. 2014). According to the literature type 2 diabetic patients with preserved renal function show significantly higher FGF21 serum level with correlation to albuminuria, therefore the decline in renal function with expected reduced FGF21 elimination cannot be the only cause of the increase of serum FGF21 (Crasto *et al.* 2012; Jian *et al.* 2012). On the other hand negative correlation of FGF21 serum level with eGFR was presented repeatedly (Anuwatmatee *et al.* 2019; Crasto *et al.* 2012; Lee *et al.* 2015). Researchers (Zhang *et al.* 2013) described also numerous positive effects of FGF21 including reduction of inflammation, fibrosis, lipid concentration and oxidative stress in kidneys. They discovered that FGF21 lowers the triglyceride level and lipid accumulation in renal tissue without plasma lipid suppression, suggesting its protective effect via local renal rather than systemic lipid reduction. It has been proven that serum FGF21 levels have been found to be even 15-fold higher in long term dialysis patients and were positively correlated with inflammatory markers such as interleukin-6, fibrinogen or C-reactive protein and negatively with residual renal function in peritoneal dialysis patients (Anuwatmatee *et al.* 2019). Furthermore, patients with end-stage kidney disease showing high FGF21 levels are expected to have high all-cause mortality (Kohara *et al.* 2017). Stage 5 CKD patients are considered to show signs of systemic and cellular metabolic stress due to uremic toxins, metabolic acidosis, anemia or hyperphosphatemia, contributing as well in FGF21 elevation. Zhang *at al*. 2013 manuscript presented the results of FGF21 administration to mice, preventing both free fatty acids (FFA) and diabetic renal damage, by reducing renal lipid accumulation and suppressing inflammation, oxidative stress and fibrosis. FGF21 shows as well hepatic, cardiac, vessel and lung protective actions (Suassuna *et al.* 2018). Analyzing the complexity of the processes in which FGF21 participates and the results of studies on both animals and humans, the question arises whether elevated level of FGF21 is an adaptation process supporting the survival of patients with CKD.

Reports related to children show a gender difference in FGF21 serum levels in a group of 179 Danish healthy children (Bisgaard et al. 2014), but such differences were not found in our CKD group. The same authors point out the lack of correlation of serum FGF21 levels with the lipid profile (excluding positive correlation with triglycerides) and BMI, and we also confirmed these observations. In Korean children and adolescents (Baek *et al.*2017) serum FGF21 levels were measured as an early marker of metabolic syndrome and type 2 diabetes, but those aspects in our patients haven't been analyzed as only two children had BMI value characteristic for obesity and all the children presented no diabetes. Kosola *et al.* 2012, conducted their research among pediatric patients receiving liver transplantation. This manuscript describes once again no FGF21 correlation with serum lipids and BMI, but a positive one with cystatin C and negative correlation with eGFR. The authors pointed out that FGF21 instead of being simple metabolic regulator may also reflect ongoing adaptive and proliferating processes not limited solely to kidneys. FGF21 transgenic mice are reported to be smaller than wild-type animals (Inagaki et al. 2008). The authors point to the possible involvement of FGF21 in this process by exerting tissue resistance to growth hormone and lowering insulin-like growth factor 1, which may have its consequence in growth retardation in CKD patient. In our study we didn't notice differences in height between children from CKD and control group, but despite of no correlation with serum FGF21 levels, there was a positive correlation of urine FGF21 concentration and height in CKD group.

Manuscript of Liu *et al.* 2015 that is reviewing clinical perspective of FGF21 in diabetes and its complications, analyzes patients with chronic kidney disease showing increased FGF21 plasma level correlating positively with albuminuria despite the eGFR. In our research urine FGF21 correlated negatively with urine albumin concentration and positively with eGFR. However, calculated FE FGF21 showed negative correlation with eGFR, which could be explained by higher extrarenal production of FGF21 and its enhanced

excretion or increased local production in the kidney, which requires further studies. These differences between adults and children serum and urine FGF21 level may also exist due to the differences in the primary cause of the CKD. The most common one in children are CAKUT, polycystic kidneys and glomerulopathies, which are primary kidney diseases, where urine FGF21 elevation might be the sign of early started healing process in parenchyma.

More advanced observations point to serum FGF21 elevation more as a healing tool rather than an early CKD marker. Anuwatmatee at al. 2019 analyzed patients from multiethnic study of arteriosclerosis, where patients free of clinically apparent cardiovascular disease were seen in a 10 year follow up period. The study does not support FGF21 as a biomarker of predicting kidney function decline or albuminuria in this group, even though higher levels of FGF21 were seen in those with impaired renal function.

Our study has some limitations. Major one is the number of examined children with CKD, which is the effect of epidemiological conditions and could be solved by longer data collection or organizing a multicenter study. The other one is lack of evaluation of urine FGF21 in healthy patients. Our observations are opposite to most of adult cohorts results and might have occurred because of small group of investigated children, but as well as overlapping of adult co-morbidities which are not that common in younger subjects.

In conclusion, the results show that serum FGF21 levels in children with chronic kidney disease although being elevated comparing to the healthy children group, show no correlation to the standard parameters used for chronic kidney disease evaluation. The positive correlation between urine FGF21 concentration and eGFR or creatinine clearance with negative correlation of FE FGF21 and eGFR is not enough to support the role of FGF21 as a biomarker for predicting kidney function decline in children. Other mechanisms including local kidney FGF21 production or enhanced excretion due to higher extrarenal production may result in higher urine FGF21 concentrations. Further studies on larger groups

of children with CKD are needed to elucidate the detailed role of FGF21 in this age population.

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References

ADAMS AC, CHENG CC, COSKUN T, KHARITONENKOV A. FGF21 requires βklotho to act in vivo. PLoS One **7**:e49977,2012.

ANDERSEN B, BECK-NIELSEN H, HØJLUND K. Plasma FGF21 displays a circadian rhythm during a 72-h fast in healthy female volunteers. ClinEndocrinol (Oxf)**75**:514–9,2011.

ANGELIN B, LARSSON TE, RUDLING M. Circulating Fibroblast Growth Factors as Metabolic Regulators—A Critical Appraisal. Cell Metab **16**:693–705,2012.

ANUWATMATEE S, TANG S, WU B, RYE K-A, ONG K. Fibroblast growth factor 21 in chronic kidney disease. ClinChimActa **489**:196-202,2019.

ANUWATMATEE S, ALLISON M, SHLIPAK M, McCLELLAND R, KRAMER H, TANG S, HOU L, RYE K, ONG K. Relationship of fibroblast growth factor 21 with kidney function and albuminuria: multi-ethnic study of atherosclerosis. Nephrol Dial Transplant **34**:1009-1016,2019.

BAEK J, NAM HK, RHIE YJ LK. Serum FGF21 Levels in Obese Korean Children and Adolescents. J ObesMetabSyndr.**26**:204–9,2017.

BISGAARS A, SØRENSEN K, JOHANNSEN T, HELGE J, ANDERSSON AM, JUUL A. Significant gender difference in serum levels of fibroblast factor 21 in Danish children and adolescents. Int J Pediatr Endocrinol **2014**:7,2014.

CRASTO C, SEMBA R, SUN K, FERRUCCI L. Serum Fibroblast Growth Factor 21 Is Associated With Renal Function and Chronic Kidney Disease in Community-Dwelling Adults. J Am Geriatr Soc **60**:792-793,2012.

CRUMP C, SUNDQUIST J, WINKLEBY MA, SUNDQUIST K. Preterm birth and risk of chronic kidney disease from childhood into mid-adulthood: national cohort study. BMJ **365**:l1346,2019.

CZARNIAK P, KRÓL E, SZCZEŚNIAK P. Wybrane aspekty epidemiologiczne przewlekłej choroby nerek u dzieci i młodzieży. Forum Nefrologiczne **1**:45-50, 2010.

GOSPODAROWICZ D. Purification of a fibroblast growth factor from bovine pituitary. J Biol Chem **250**:2515–2520,1975.

HARAMBAT J, STRALEN KJ, KIM JJ, TIZARD EJ. Epidemiology of chronic kidney disease in children. PediatrNephrol **27**:363-373,2012.

HINDRICKS J, EBERT T, BACHMANN A, KRALISCH S, LÖSSNER U, KRATZSCH J, STOLZENBURG JU, DIETEL A, BEIGE J, ANDERS M, BAST I, BLÜHER M, STUMVOLL M, FASSHAUER M. Serum levels of fibroblast growth factor-21 are increased in chronic and acute renal dysfunction. Clin Endocrinol (Oxf) **80**:918–24,2014.

INAGAKI T. Research Perspectives on the Regulation and Physiological Functions of FGF21 and its Association with NAFLD. Front Endocrinol (Lausanne) **6**:147,2015.

INAGAKI T, LIN VY, GOETZ R, MOHAMMADI M, MANGELSDORF D, KLIEWER S. Inhibition of growth hormone signaling by the fasting-induced hormone FGF21.Cell Metab **8**:77-83,2008.

ITOH N. Hormone-like (endocrine) FGFs: their evolutionary history and roles in development, metabolism, and disease. Cell Tissue Res **342**:1-11,2010.

ITOH N, OHTA H, KONISHI M. Endocrine FGFs: evolution, physiology, pathophysiology, and pharmacotherapy. Front Endocrinol **6**:154,2015.

KHARITONENKOV A, DI MARCHI R. FGF21 Revolutions: Recent Advances Illuminating FGF21 Biology and Medicinal Properties. Trends Endocrinol Metab **26**:608–17,2015.

KIDGO 2012 Clinical Practice Guideline for Evaluation and Management of Chronic Kidney Disease. Kidney International Supplements **3**:19–62,2013.

KOHARA M, MASUDA T, SHIIZAKI K, AKIMOTO T, WATANABE Y, HONMA S, SEKIGUCHI C, MIYAZAWA Y, KUSANO E, KANDA Y, ASANO Y, KURO-O M, NAGATA D. Association between circulating fibroblast growth factor 21 and mortality in end-stage renal disease. PLoSOne **12**:e0178971,2017.

KOKKINOS J, TANG S, RYE KA, ONG KL. The role of fibroblast growth factor 21 in atherosclerosis. Atherosclerosis **257**:259–65,2017.

KOSOLA S, LAMPELA H, GYLLING H, JALANKO H, NISSINEN MJ, LAURONEN J, MÄKISALO H, VAARALAHTI K, MIETTINEN TA, RAIVIO T, PAKARINEN MP. Cholesterol Metabolism Altered and FGF21 Levels High After Pediatric Liver Transplantation Despite Normal Serum Lipids. Am J Transplant **12**:2815–24,2012.

KREJCI E, PESEVSKI Z, NANKA O, SEDMERA D. Physiological Role of FGF Signaling in Growth and Remodeling of Developing Cardiovascular System. Physiol Res **65**:425-435,2016

LEE CH, HUI EY, WOO YC, YEUNG CY, CHOW WS, YUEN MM, FONG CH, XU A, LAM KS. Circulating fibroblast factor 21 levels predict progressive kidney disease in subjects with type 2 diabetes and normoalbuminuria. J Clin Endocrinol Metabol **100**:1368-75,2015.

LIANG Q, ZHONG L, ZHANG J, WANG Y, BORNSTEIN SR, TRIGGLE CR, DING H, LAM KS, XU A. FGF21 maintains glucose homeostasis by mediating the cross talk between liver and brain during prolonged fasting. Diabetes **63**:4064–75,2014.

LIU JJ, FOO JP, LIU S, LIM SC. The role of fibroblast growth factor 21 in diabetes and its complications: A review from clinical perspective. Diabetes Res Clin Pract **108**:382–9,2015.

MIAN AN, SCHWARTZ GJ. Measurement and Estimation of Glomerular Filtration Rate in Children. Adv Chronic Kidney Dis **24**:348–356,2017.

ORNITZ DM, ITOH N. The Fibroblast Growth Factor signaling pathway. Wiley Interdiscip Rev Dev Biol **4**:215–66,2015.

SUASSUNA PG, DE PAULA R, SANDERS-PINHEIRO H, MOE O, HU M-C. Fibroblast growth factor 21 in chronic kidney disease. J Nephrol **32**:365-377,2018.

SZYMCZYK A, KRZYWAŃSKI R, FORMA E. Struktura i funkcja białak βKlotho. Folia Medica Lodziensia **40**:99-132,2013.

WARADY BA, CHADHA V. Chronic kidney disease in children: the global perspective. Pediatr Nephrol **22**:1999-2009,2007.

ZHANG J, WU H, MA S, JING F, YU C, GAO L, ZHAOJ. Transcription Regulators and Hormones Involved in the Development of Brown Fat and White Fat Browning: Transcriptional and Hormonal Control of Brown/Beige Fat Development. Physiol Res **67**:347-362,2018.

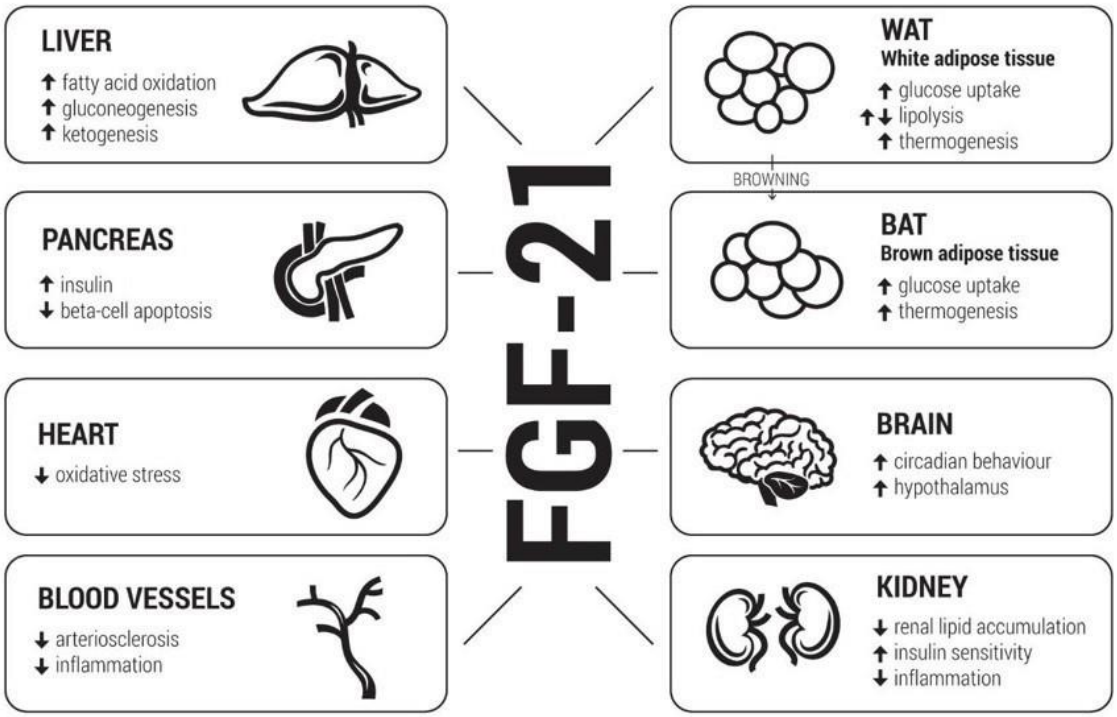

Figure 1. The effect od FGF21 on tissues.

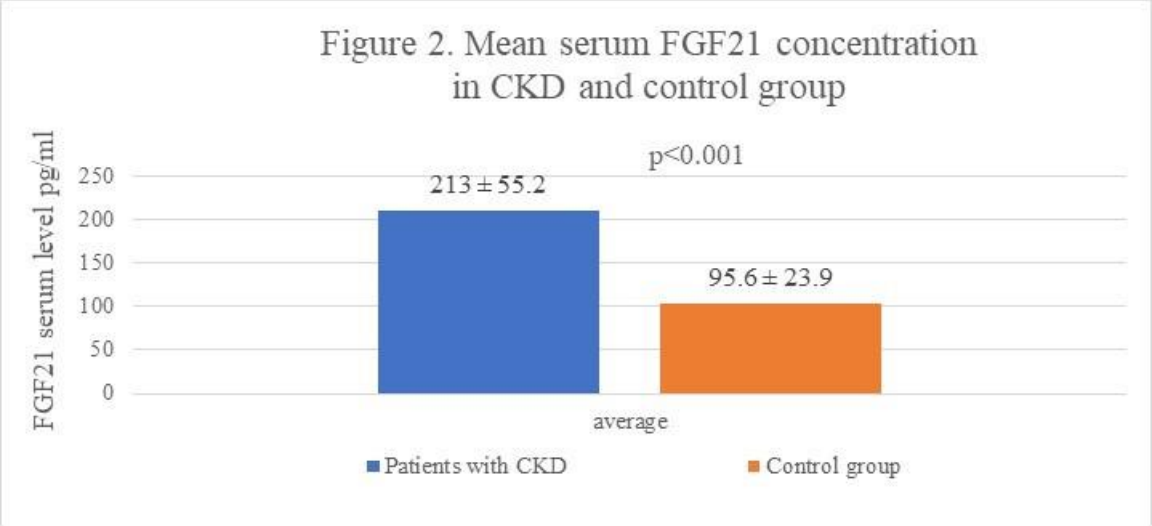

Figure 3. FGF21 level in children with CKD depending on gender

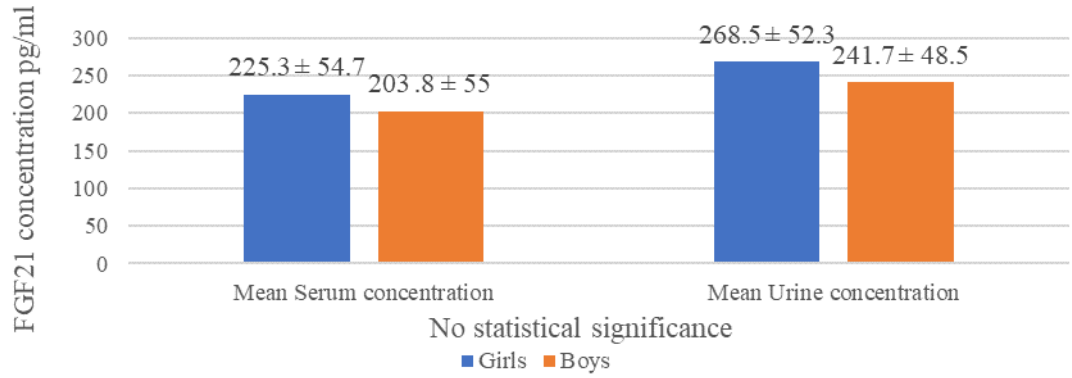

Figure 4. FGF21 level in children on renal replacement therapy (RRT) and conservative treatment

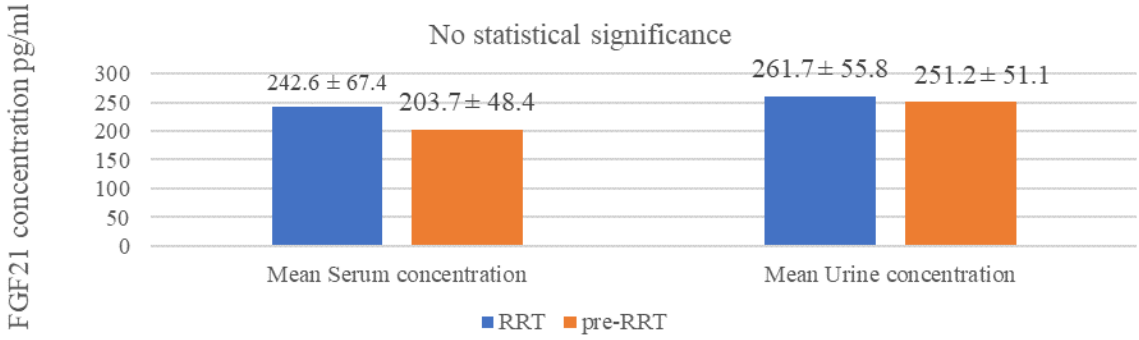

Figure 5. FGF21 level in children with CKD depending on proteinuria

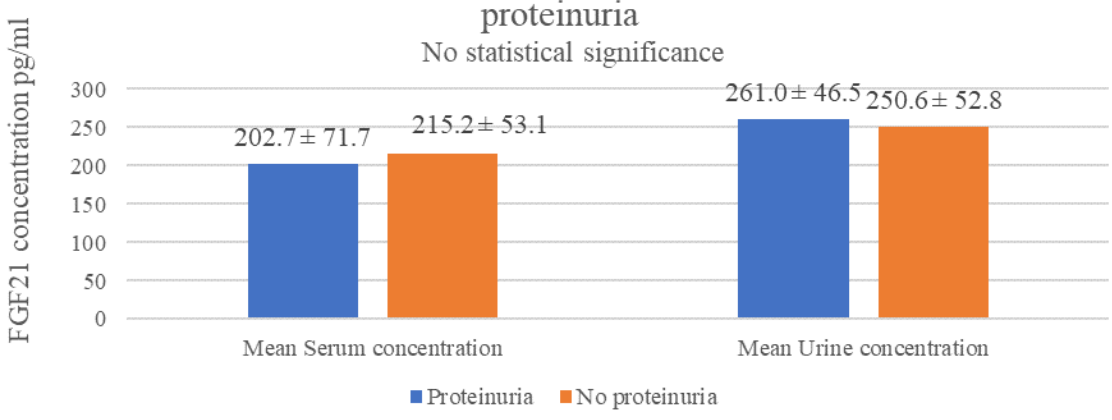

Table 1. aClinical characteristics of evaluated children (CKD and control group)

Parameter	CKD group (CKD)			Control Group (CG)			p-value
	Total group (n=42)	Girls (n=18)	Boys (n=24)	Total group (n=21)	Girls (n=11)	Boys (n=10)	CKD vs CG
Age (years)	10.7±4.6	10.6±4.6	10.7±4.7	8.4±4.1	6.7±3.6	10.3±3.8	NS
Height (cm)	134.3±26.5	132.1±25.3	135.9±27.8	128.5±24.5	116.1±21.8	142.2±20.2	NS
Height SDS	-1.6±1.43	-1.68±1.73	-1.54±1.19	0.106±0.96	-0.12±1.19	0.35±0.57	<0,01
Body weight (kg)	36.4±20.6	32.1±14.0	39.7±24.1	31.9±16.42	24.63±12.82	40.0±16.7	NS
Body weight SDS	-0.98±1.44	-1.22±1.3	-0.8±1.53	0.19±1.15	-0.08±1.36	0.49±0.84	<0,01
BMI (kg/m^2)	18.5±4.7	17.4±2.6	19.3±5.8	18.0±3.3	17.2±2.95	18.9±3.7	NS
BMI SDS	-0.19±1.11	-0.27±0.95	-0.002±1.23	0.296±1.05	0.2±1.16	0.397±1.02	NS

Data are presented as mean ± standard deviation.

CKD - chronic kidney disease; BMI - body mass index; SDS- standard deviation score.

Table 2. Biochemical parameters among the CKD group compared by gender.

Parameter	Total group n=42	Girls n=18	Boys n=24	p value
Serum albumin (g/l)	44.02±6.25	42.8±7.9	44.8±4.7	NS
Total proteins (g/l)	69.3±7.58	68.23±8.7	70.1±6.7	NS
Parathyroid hormone (pg/ml)	184.3±278	296.9±400	104.5±83.2	p<0.05
Total cholesterol (mmol/l)	4.8±2.2	5.49±3.18	4.29±0.72	NS
HDL Cholesterol (mmol/l)	1.3±0.39	1.165±0.38	1.40±0.38	NS
LDL Cholesterol (mmol/l)	2.74±1.98	3.43±3.02	2.31±0.69	NS
Triglycerides (mmol/l)	1.59±1.02	1.91±1.35	1.36±0.64	NS
Serum creatinine (mmol/l)	261.5±254	319.3±283.2	218.1±227.6	NS
Urea (mmol/l)	12.35±8.3	12.67±10.5	12.1±6.5	NS
HCO_3 (mmol/l)	21.9±2.3	22.7±2.05	21.3±2.32	p<0.05
Inorganic phosphate (mmol/l)	1.64±0.6	1.68±0.64	1.6±0.58	NS
Daily urine albumin excretion (mg/24 h)	269±628	97.5±243.0	392.9±785.5	NS
Albuminuria (mg/l)	59±50	30.92±48.3	76.5±43.8	p<0.01
Albumin/creatinine ratio (mg/g creatinine)	423.2±905	328.25±983	484.8±872	NS
Standardized creatinine clearance (ml/min/1.73m^2)	49.33±24.15	48.75±25.4	49.8±24.0	NS
eGFR (ml/min/1.73m^2)	47.87±20	50.56±22.6	46.26±20.17	NS
Fracrional excretion of FGF21	14.02±14.06	16.72±19.10	12.13±9.20	NS

Data are presented as mean ± standard deviation.

eGFR - estimated glomerular filtration rate. Significant boys vs. girls p<0.05.

Table 3. Correlation between FGF21 serum/urine concentration and the results of chosen anthropometrical measurements and biochemical parameters among CKD group.

Parameter	Serum FGF21		Urine FGF21	
Body weight	r=0.1846	p=.242	r=0.3182	p=.067
Height	r=0.2076	p=.187	r=0.3604	**p=.036**
BMI	r=0.1022	p=.520	r=0.1359	p=.443
Age	r=0.1507	p=.341	r=0.3414	**p=.048**
SBP	r=0.1712	p=.278	r=0.07	p=.694
DBP	r=0.1017	p=.521	r=0.002	p=.991
MAP	r=0.1364	p=.389	r=0.0263	p=.883
Serum albumin	r=-0.0971	p=.546	r=0.0177	p=.922
Total proteins	r=-0.0003	p=.999	r=0.2141	p=.224
Parathyroid hormone	r=0.0609	p=.705	r=-0.2153	p=.229
Total Cholesterol	r=0.0664	p=.676	r=0.0294	p=.869
HDL Cholesterol	r=-0.3297	p=.070	r=-0.2503	p=.228
LDL Cholesterol	r=0.0799	p=.669	r=0.3664	p=.072
Triglycerides	r=0.2805	p=.080	r=0.0464	p=.801
Serum creatinine	r=0.2165	p=.168	r=-0.1889	p=.285
Urea	r=0.0064	p=.968	r=-0.3071	p=.077
HCO_3	r=-0.1920	p=.022	r=0.223	p=.902
Inorganic phosphate	r=0.0449	p=.778	r=-0.313	p=,071
Daily urine albumin excretion	r=0.3222	p=.077	r=0.1469	p=.456
Albuminuria	r=-0.2147	p=.223	r=-0.4965	**p=.004**
Albumin/ creatinine ratio	r=0.2565	p=.150	r=0.0032	p=.987
Standardized creatinine clearance	r=0.1169	p=.553	r=0.4372	**p=.029**
eGFR	r=0.2158	p=.236	r=0.5339	**p=.003**

BMI - body mass index; SBP- systolic blood preasure; DBP- diastolic blood preasure; MAP- mean arterial preasure;eGFR - estimated glomerular filtration rate.
Significant correlation coefficients $p<0.05$.