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SERUM FIBROBLAST GROWTH FACTOR 23 AND CALCIUM-PHOSPHORUS METABOLISM PARAMETERS IN CHILDREN WITH CHRONIC KIDNEY DISEASE – PRELIMINARY REPORT

CZYNNIK WZROSTU FIBROBLASTÓW 23 I PARAMETRY GOSPODARKI WAPNIOWO-FOSFORANOWEJ U DZIECI Z PRZEWLEKŁĄ CHOROBĄ NEREK – DONIESIENIE WSTĘPNE

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Abstract

Introduction: *In chronic kidney disease (CKD) the function of all factors regulating mineral metabolism is disturbed, leading inevitably to renal osteodystrophy and vascular calcification.*

The aim *of the study is to assess concentrations of fibroblast growth factor 23 (FGF 23), osteoprotegerin (OPG) and other parameters of calcium-phosphate metabolism in children with CKD.*

Material and methods: 37 children with CKD 3-5, aged 1.6-17 years were included in the study. In all children serum levels of calcium (sCa), phosphate (sP), creatinine, alkaline phosphatase (ALP), FGF 23, intact parathormone (PTH), OPG and receptor activator nuclear factor κB ligand (RANKL) were measured.

Results: *Total calcium concentration was within normal limits in all children included in this study. Hyperphosphatemia was found in 2 children from group CKD 3 (12%), 6 from CKD 4 (54%) and 1 from CKD 5 (11%). FGF 23 level increased consecutively in subsequent CKD stages achieving the highest values in CKD 5 group. In all children with CKD, serum levels of OPG were correlated with FGF 23. In children with CKD 3-4 negative correlation between FGF 23 and PTH (r=-0.45; p=0.02) and positive correlation between FGF 23 and RANKL (r=0,59; p=0.006) has been found. Positive correlation between OPG concentration and $HCO3^-$ and BE levels has been observed, as well as negative correlation between RANKL/OPG ratio and $HCO3^-$ and BE levels.*

Conclusion: *Despite maintaining serum calcium, phosphorus and PTH levels within recommended limits, elevated levels of FGF 23 and OPG were observed in children with chronic kidney disease, especially in it's end-stage.*

Key words: FGF 23, osteoprotegerin, chronic kidney disease, children, calcium-phosphorus metabolism

Streszczenie

Wstęp: *W przewlekłej chorobie nerek (CKD) wszystkie czynniki regulujące gospodarkę mineralną ulegają zaburzeniu, prowadząc do rozwoju nerkowej osteodystrofii i zwapnienia naczyń.*
Celem pracy *była ocena stężeń czynnika wzrostu fibroblastów 23 (FGF 23), osteoprotegeryny i innych parametrów gospodarki wapniowo-fosforanowej u dzieci z przewlekłą chorobą nerek.*
Materiał i metody: *Badaniami objęto 37 dzieci z CKD w stadium 3-5, w wieku 1,6-17 lat. U wszystkich w surowicy oceniano stężenia: wapnia, fosforu, kreatyniny, fosfatazy alkalicznej, FGF 23, parathormonu, osteoprotegeryny i liganda aktywatora receptora jądrowego czynnika κB (RANKL).*
Wyniki: *Stężenia wapnia całkowitego u wszystkich badanych dzieci mieściły się w zakresie normy wiekowej. Hiperfosforemię stwierdzono u 2 dzieci z grupy CKD 3 (12%), 6 z CKD 4 (54%) i 1 z CKD 5 (11%). Stężenie FGF 23 rosło w kolejnych stadiach przewlekłej choroby nerek, osiągając najwyższe wartości w stadium 5. U wszystkich dzieci z przewlekłą chorobą nerek stężenie osteoprotegeryny korelowało ze stężeniem FGF 23. U dzieci z przewlekłą chorobą nerek w stadium 3-4 stwierdzono ujemną korelację między FGF 23 i parathormonem (r=-0,45; p=0,02) i dodatnią korelację między FGF 23 i RANKL (r=0,59; p=0,006). Obserwowano korelację między stężeniem osteoprotegeryny a stężeniem wodorowęglanów i niedoboru zasad, jak również ujemną korelację między RANKL/OPG oraz stężeniem wodorowęglanów i niedoborem zasad.*
Wniosek: *U dzieci z przewlekłą chorobą nerek, szczególnie w stadium ich schyłkowej niewydolności, mimo utrzymywania stężeń wapnia, fosforanów i parathormonu w zalecanych granicach stwierdza się wysokie stężenia FGF 23 i osteoprotegeryny.*

Słowa kluczowe: FGF 23, osteoprotegeryna, przewlekła choroba nerek, dzieci, gospodarka wapniowo-fosforanowa

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INTRODUCTION

Several new factors participating in mineral and bone metabolism have been found in the last years. Apart from calcium, phosphate, parathormone (PTH) and active form of vitamin D, other molecules of significance include osteoprotegerin – receptor activator of the nuclear factor κB ligand system (OPG/RANKL system) and fibroblast growth factor 23 (FGF 23). In chronic kidney disease (CKD) the function of all factors regulating mineral metabolism is disturbed, leading inevitably to renal osteodystrophy and vascular calcification.

OPG/RANKL system, discovered and described in 1997, consists of tightly cooperating proteins: receptor activator of the growth factor κB (RANK), its ligand (RANKL) and soluble RANKL binding receptor – osteoprotegerin (OPG). Activation of RANK by RANKL induces the process of osteoclastogenesis. OPG, secreted by osteoblasts, inhibits this process by binding to RANKL, thus constituting endogenic bone remodelling regulator. RANK, RANKL and OPG are expressed not only in bones, but also in vessels. Antiresorptive and vascuoloprotective role of OPG has been confirmed in experiments on animal model – OPG knockout mice develop severe osteoporosis and vascular calcification (1). Epidemiological studies have suggested that serum OPG levels are significantly elevated in adult patients with CKD and are correlated with cardiovascular mortality (2, 3). In dialysed children serum OPG level was found to be elevated as well (4, 5).

FGF 23 is one of the main regulators of phosphate metabolism. It belongs to a group of phosphatonins – phosphaturic factors described in 1994 (6, 7). FGF 23 is secreted by osteocytes and osteoblasts and its production is stimulated by phosphate load in a diet. The action of FGF 23, leading to lowering serum phosphate concentration, is mediated by binding to FGF receptor (FGFR) in presence of a coreceptor – transmembrane Klotho protein. Although FGFR is expressed in the majority of cells in the human body, Klotho protein expression is much more restricted and can be found in FGF 23 target organs: the kidneys, the parathyroid glands and the choroid plexuses (8). FGF 23 reduces expression of sodium-phosphate cotransportes (type IIa and IIc) in the proximal tubule, thus increasing kidney phosphate excretion. It also inhibits expression of 1(OH)-hydroxylase, the enzyme responsible for activation of 25(OH)-cholecalciferol to active vitamin D, which leads to decrease in phosphate and calcium absorption in the small intestine (9, 10).

Serum FGF 23 concentration is elevated in patients

with CKD and it increases proportionally to decrease in glomerular filtration rate (GFR) (11, 12). In adults with CKD stage 5 serum FGF 23 levels achieve 100 to 1000 fold the upper limit of normal (13). In children with CKD FGF 23 serum concentration is also elevated (4, 14).

This study was designed to assess concentrations of FGF 23 and other parameters of calcium-phosphate metabolism in children with varying grades of CKD.

MATERIAL AND METHODS

Study population

All children included in the study had CKD stages 3-5 and attended the CKD management clinic at the Department of Pediatrics and Nephrology of Medical University of Warsaw from 2008 to 2010. The inclusion criteria were: (I) estimated glomerular filtration rate <60 ml/min/1.73 m^2 for at least 3 months, (II) age 1,5-18 years. 37 children aged 16-17 years were included in the study. The baseline demographics and clinical characteristics of the children with CKD are reported in table I. In group CKD 5 all children were dialysed: 6 children with peritoneal dialysis (PD) with Physioneal (Baxter) as dialysis solution and 3 children with hemodialysis (HD). The study population is part of a larger cohort of children with CKD from Poland.

16 children of hospital employees aged 1.9-16 years, mean 10.2 (±4.9) years, served as controls. Control group children were otherwise healthy and had normal kidney function.

The study protocol was approved by the Medical University of Warsaw Ethics Committee.

Table I. Baseline demographic and clinical characteristics of examined cohort.

Tabela I. Podstawowe dane demograficzne i charakterystyka kliniczna badanych pacjentów.

	All ***Razem***		**CKD 3 (n=17)**		**CKD 4 (n=11)**		**CKD 5 (n=9)**	
	N	**(%)**	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**
Gender ***Płeć***								
Female *Kobiety*	12	32	6	35	3	27	3	33
Male *Mężczyźni*	25	68	11	65	8	73	6	67
Causes of chronic kidney disease ***Przyczyny przewlekłej choroby nerek***								
Congenital anomalies of kidney and urinary tract *Wrodzone wady nerek i dróg moczowych*	20	54	9	53	7	64	4	44
Congenital nephropathies *Nefropatie wrodzone*	3	8	1	6	1	9	1	12
Glomerulonephritis *Kłębuszkowe zapalenie nerek*	5	14	1	6	(-)	(-)	4	44
Hemolytic uremic syndrome *Zespół hemolityczno-mocznicowy*	1	2,5	(-)	(-)	1	9	(-)	(-)
Cystic renal disease *Torbielowatość nerek*	1	2,5	1	6	(-)	(-)	(-)	(-)
Other *Inne*	7	19	5	29	2	18	(-)	(-)
Age Mean (SD) years *Wiek Średnia (SD) lata*	10.9 (4.3)		10.2 (4.5)		10.3 (4.5)		13.2 (3)	
Medications ***Leki***								
Calcium carbonate *węglan wapnia*	16	43	3	18	7	64	6	67
Sevelamer *sewelamer*	2	5	(-)	(-)	(-)	(-)	2	22
Sevelamer + $CaCO_3$ *sewelamer + $CaCO_3$*	2	5	(-)	(-)	1	9	1	11
Vitamin D *witamina D*	5	14	4	24	1	9	(-)	(-)
1-hydroxyvitamin D *1-hydroksywitamina D*	20	54	4	24	8	73	8	89

Renal function was determined by eGFR using the new revised Schwartz formula (15) and CKD stage defined according to The National Kidney Foundation Kidney Disease Outcome Quality Initiative (NKF K/DOQI) Guidelines (16), namely, stage 3 (GFR 30-59 ml/min/1.73 m^2), stage 4 (GFR 15-29 ml/min/1.73 m^2) and stage 5 (GFR<15 ml/min/1.73 m^2). All children included in the study with CKD 5 were dialysed.

Conservative treatment was given to the CKD patients when indicated: oral sodium bicarbonate, calcium carbonate or sevelamer as a phosphate binder, active vitamin D supplement (alphacalcidol) and antihypertensive drugs. The doses of phosphate binders and alpha-calcidol were adjusted in order to maintain target serum levels of calcium, phosphate and PTH according to the guidelines of the European Pediatric Dialysis Working Group by stage of CKD (17).

Laboratory methods

Blood samples were collected from antecubital vein, in hemodialysed children before dialysis. After centrifugation patients serum for FGF 23, OPG and RANKL determination was frozen in -20°C until assayed. FGF23 was measured with FGF-23 (Intact) EIA ALPCO Immunoassays cat nr 31-IFGHU-E01 (ALPCO Diagnostics, USA). OPG was measured with enzyme immunoassay for quantitative determination in serum cat nr BI-20402 (Biomedica, Wien, Austria). RANKL was measured with enzyme immunoassay for quantitative determination in serum cat nr BI-20452 (Biomedica, Wien, Austria). Serum levels of calcium (sCa), phosphate (sP), creatinine, alkaline phosphatase (ALP) and blood gases were determined on the day of blood collection with routine laboratory methods: Vitros 5600 analyzer (Ortho clinical Diagnostics, Johnson and Johnson Company) for biochemical parametrers, Premier GEM 4000 analyzer (Instrumentation Laboratory) for blood gases.

Statistical analyses

Data were expressed as the mean standard deviation (SD) for normally distributed parameters and median (interquartile range, IQR) for not normally distributed parameters. Normality was tested using Shapiro-Wilk normality test. Normally distributed variables were analysed using parametric tests, otherwise non-parametric methods were applied. The relationships between variables were determined using correlation analysis, univariate and multivariate regression analysis. Because FGF 23, sCa, sP, PTH, OPG and RANKL levels were not normally distributed, they were log-transformed prior to analysis. All statistical analysis was performed using the statistical software STATISTICA 10. For all analyses, a p value <0.05 was considered statistically significant.

RESULTS

Children in analysed groups did not differ according to age. Among all 37 CKD children 53% received phosphate binders and 68% were given vitamin D metabolites. In group CKD 5 all children took phosphate binders and only one patient did not receive vitamin D treatment at the time of the study.

Data concerning acid-base balance and calcium-phosphate metabolism in examined groups are presented in table II.

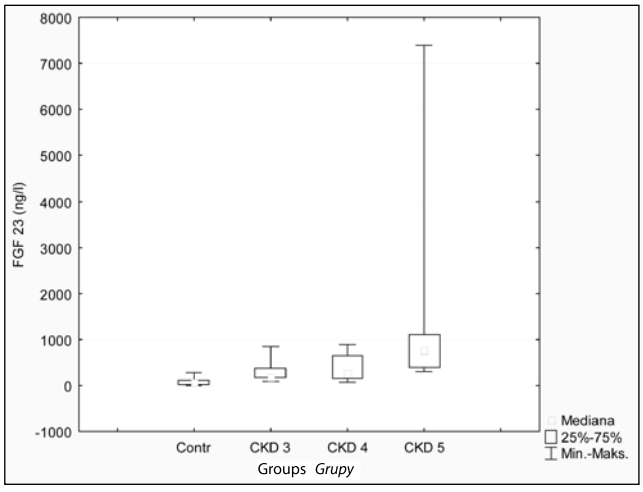
Total calcium concentration was within normal limits in all children included in this study. Hyperphosphatemia was found in 2 children from group CKD 3 (12%), 6 from CKD 4 (54%) and 1 from CKD 5 (11%).

Patients from CKD 5 group presented with best compensation of metabolic acidosis. Both HCO_3^- and BE (base excess) levels were highest in this group. Phosphate concentration differed significantly only between groups CKD 3 and CKD 4. OPG/RANKL ratio was highest in CKD 5 group.

FGF 23 concentrations in analysed groups are presented in figure 1. FGF 23 level increased consecutively in subsequent groups achieving the highest values in CKD 5 group.

In the univariate and multivariate analysis, in all children with CKD, serum levels of OPG were correlated with FGF 23 concentrations (tab. III). Such a correlation was not found in the control group. Correlations of examined variables were analysed in groups CKD 3 and CKD 4 under the assumption that aggressive treatment in children from CKD 5 group may have affected results. In children with CKD 3-4 negative correlation between FGF 23 and PTH (r=-0,45; p=0,02) and positive correlation between FGF 23 and RANKL (r=0,59; p=0,006) has been found (tab. IV).

In all children with CKD and in children with CKD 3-4 negative correlation between PTH and RANKL (r= -0.44; p=0.022 and, respectively r=-0.42; p=0.037) has been observed, as well as a tendency towards negative correlation between RANKL and OPG (r=-0.35; p=0,066 and, respectively r=-0,35; p=0,08).



C vs CKD 3 vs CKD 4 vs CKD 5 p< 0.0003

Fig. 1. Values of median and quartiles (25-75%) for FGF 23 in particular groups.

Ryc. 1. Wartości mediany i kwartyli (25-75%) FGF 23 w badanych grupach chorych.

Table II. Acid – base and calcium – phosphate metabolism parameters in examined children.

Tabela II. Parametry gospodarki kwasowo-zasadowej i wapniowo-fosforanowej u badanych dzieci.

Parameter; *parametr* Mean ± SD; Median (IQR) *Średnia ± SD; Mediana (IQR)*	**Control group *Grupa kontrolna* (n=16)**	**CKD 3 (n=17)**	**CKD 4 (n=11)**	**CKD 5 (n=9)**
HCO_3 (mmol/l)	(-)	23.7±2.7	23.6±3	27±3[1]
Base excess (BE) *Nadmiar (niedobór) zasad*	(-)	-0.9±2.9	-1.3±3.4	2.9±3.4[2]
Serum calcium (mmol/l) *Wapń w surowicy*	2.5 (2.45-2.65)	2.5 (2.45-2.65)	2.53 (2.4-2.63)	2.5 (2.45-2.55)
Serum phosphate (mmol/l) *Fosfor w surowicy*	1.56 (1.44-1.61)	1.67 (1.5-1.83)	1.94 (1.67-2.17)[3]	1.78 (1.67-1.83)
Serum calcium x serum phosphate ($mmol^2/L^2$) *Wapń w surowicy x fosfor w surowicy*	3.87 (3.54-4.2)	4.03 (3.96-4.58)	4.63 (4.33-5.32)	4.39 (4.09-4.63)
PTH (ng/l)	(-)	51 (33-129)	82 (56-98)	101 (67-210)
ALP (U/L)	181±91	205±75	224±82	219±132
OPG (pmol/l)	3.05 (2.6-3.85)	2.48 (1.7-3.1)	2.61 (2.2-3.02)	5.88 (2.85-6.5)
RANKL	0.29 (0.25-0.54)	0.35 (0.17-0.71)	0.4 (0.25-1.37)	0.24 (0.19-0.33)
RANKL/OPG	0.12 (0.076-0.17)	0.087 (0.04-0.44)	0.17 (0.08-0.6)	0.039 (0.029-0.085)
OPG/RANKL	8.6 (5.7-13.2)	6.5 (2.38-17.9)	5.7 (1.63-12.3)	26 (11.8-35)[4]
FGF 23 (ng/l)	55 (30-108)	179 (152-359)	222 (138-550)	750 (330-1106)[5]

[1]p<0.05 CKD 5 vs CKD 3 and CKD 4; [2]p<0.03 CKD 5 vs CKD 4 and CKD 3; [3]p<0.05 CKD 3 vs CKD 4; [4]p <0.05 CKD 5 vs CKD 4 and Control; p=0.07 CKD 5 vs CKD 3; [5]p<0.0003

SD – standard deviation/*odchylenie standardowe*; IQR – interquartile range/*rozstęp kwartylny (ćwiartkowy)*; PTH – parathormone/*parathormon*; ALP – alkaline phosphatase/*fosfataza alkaliczna*; OPG – osteoprotegerin/*osteoprotegeryna*; RANKL – receptor activator κB ligand/*ligand aktywatora receptora jądrowego czynnika κB*, FGF 23 – fibroblast growth factor 23/*czynnik wzrostu fibroblastów 23*; CKD – chronic kidney disease/*przewlekła choroba nerek*

Table III. Factors associated with FGF 23 in all children with CKD: univariate analysis and multivariate regression analysis using log FGF 23 (n=37) as the dependent variable.

Tabela III. Zależność FGF 23 i badanych parametrów gospodarki wapniowo-fosforanowej u wszystkich dzieci z przewlekłą chorobą nerek: jednoczynnikowa i wieloczynnikowa analiza regresji z użyciem FGF 23 jako zmiennej zależnej.

Independent variables *Zmienne niezależne*	**Dependent variable Log FGF 23**			**Zmienna zależna**		
	Univariate analysis *Analiza jednoczynnikowa*			**Multivariate analysis *Analiza wieloczynnikowa***		
	B	Beta	p	B	Beta	p
Log Ca	-0.53	-0.03	0.86	-4.12	-0.24	0.27
Log P	0.98	0.14	0.44	-0.15	-0.02	0.93
Log PTH	-0.26	-0.24	0.17	-0.17	-0.16	0.53
Log OPG	**0.62**	**0.44**	**0.016**	**0.92**	**0.66**	**0.004**
Log RANKL	0.31	0.24	0.22	0.46	0.336	0.23

Ca-calcium, *wapń*; P-phosporus, *fosfor*; PTH-parathormone, *parathormon*; OPG-osteoprotegerin, *osteoprotegeryna*; RANKL-receptor activator of the nuclear factor κB ligand, *ligand aktywatora receptora jądrowego czynnika κB*

Table V presents relationships between acid-base homeostasis parameters with chosen variables of calcium-phosphate metabolism. The correlation between OPG concentration and HCO_3^- and BE levels has been observed, as well as negative correlation between RANKL/OPG ratio and HCO_3^- and BE levels.

Table IV. Factors associated with FGF 23 in children with CKD 3-4: univariate analysis and multivariate regression analysis using log FGF 23 (n=28) as the dependent variable.

Tabela IV. Zależność FGF 23 i badanych parametrów gospodarki wapniowo-fosforanowej u dzieci z przewlekłą chorobą nerek 3-4: jednoczynnikowa i wieloczynnikowa analiza regresji z użyciem FGF 23 jako zmiennej zależnej.

Independent variables ***Zmienne niezależne***	**Dependent variable** **Log FGF 23**			**Zmienna zależna**		
	Univariate analysis ***Analiza jednoczynnikowa***			**Multivariate analysis** ***Analiza wieloczynnikowa***		
	B	Beta	p	B	Beta	p
Log Ca	-2.4	-0.2	0.29	-3.32	-0.27	0.24
Log P	1.9	0.37	0.058	1.25	0.23	0.33
Log PTH	**-0.41**	**-0.45**	**0.02**	-0.21	-0.22	0.37
Log OPG	0.13	0.12	0.6	0.4	0.37	0.1
Log RANKL	**0.52**	**0.59**	**0.006**	0.37	0.42	0.16

Log – *logarytm*; Ca – calcium/*wapń*; P – phosphorus/*fosfor*; PTH – parathormone/*parathormon*; OPG – osteoprotegerin/*osteoprotegeryna*; RANKL – receptor activator of the nuclear factor κB ligand/*ligand aktywatora receptora jądrowego czynnika κB*

Table V. Influence of acid-base disturbances on examined bone metabolism parameters: univariate analysis and multivariate regression analysis using HCO3- and BE as the dependent variable.

Tabela V. Wpływ zaburzeń gospodarki kwasowo-zasadowej na badane parametry metabolizmu kostnego: jednoczynnikowa i wieloczynnikowa analiza regresji z użyciem HCO3- i BE jako zmiennej zależnej.

Independent variables ***Zmiennie niezależne***	**HCO_3**					
	Univariate analysis **Analiza jednoczynnikowa**			**Multivariate analysis** **Analiza wieloczynnikowa**		
	B	**Beta**	**p**	**B**	**Beta**	**p**
Log FGF 23	2.18	0.29	0.09	1.56	0.2	0.27
Log PTH	-0.12	-0.015	0.94	0.97	0.1	0.65
ALP	-2.8	-0.17	0.42	-0.007	-0.2	0.41
Log OPG	4.56	0.44	0.02	-1.14	-0.11	0.8
Log RANKL	-2.36	-0.23	0.25	2.1	0.17	0.6
Log RANKL/OPG	-5.2	-0.49	0.0096	-6.4	-0.59	0.2
Independent variables ***Zmiennie niezależne***	**BE**					
	Univariate analysis **Analiza jednoczynnikowa**			**Multivariate analysis** **Analiza wieloczynnikowa**		
	B	**Beta**	**p**	**B**	**Beta**	**p**
Log FGF 23	2.27	0.27	0.2	1.84	0.22	0.4
Log PTH	0.03	-0.004	0.9	0.76	0.08	0.76
ALP	-0.011	-0.26	0.21	-0.009	-0.2	0.4
Log OPG	5.26	0.46	0.02	-1.33	-0.11	0.8
Log RANKL	-3.31	- 0.24	0.25	1.69	0.12	0.7
Log RANKL/OPG	-6.25	- 0.52	0.01	-7.33	-0.6	0.2

FGF 23 – fibroblast growth factor 23/*czynnik wzrostu fibroblastów 23*; PTH – parathormone/*parathormon*; ALP – alkaline phosphatase/*/fosfataza alkaliczna*; OPG – osteoprotegerin/*osteoprotegeryna*; RANKL – receptor activator of the nuclear factor κB ligand/*ligand aktywatora receptora jądrowego czynnika κB*

DISCUSSION

In accordance to other reports, the median of FGF 23 was higher in children with kidney failure than in healthy controls and it was highest in dialysed patients (4, 14, 18, 19, 20). However, no correlation between FGF 23 and eGFR in analysed children has been found, despite other authors observed such association (19, 20, 21). Those results could have been affected by relatively small number of CKD 3-4 patients included in the study. Irrespective of significant differences between FGF 23 medians in respective groups, in both CKD 3 and CKD 5 groups a broad spectrum of FGF 23 concentration values was observed. However, in group CKD 5 nobody had FGF 23 lower than 330 ng/ml.

Phosphate concentrations were significantly higher in CKD 4 than in CKD 3 group, but in CKD 5 in comparison to other groups no higher P levels were observed. It is probably a consequence of an effective phosphate-lowering therapy in those patients. In CKD 5 group all patients received phosphate binders, whereas in CKD 3 group only 18% and in CKD 4-73% of examined children. PTH concentrations in subsequent groups also indicate adequate treatment regimen. In CKD 5 group only one patient had high PTH level (>600 ng/l), whereas 4 children had PTH<100 ng/l. Relatively low concentrations of PTH in group CKD 5 suggest even too aggressive therapy with vitamin D metabolites (there was only 1 child in this group not receiving vitamin D) and phosphate binders. Results obtained in the study illustrate difficulties in providing PTH levels within narrow recommended limits, avoiding falls of PTH below recommended values (16, 22).

Results from the table II illustrate significantly higher HCO_3^- and BE values in dialysed patients. Surprisingly good acidosis compensation in this group results from many factors, including dialysis and treatment with calcium carbonate and $NaHCO_3$. Also the composition of the group affected those results, as 6 of 9 children were treated with peritoneal dialysis, which favours better acidosis compensation. Those findings may also be influenced by the fact that dialysed children attend medical control more frequently than patients on conservative treatment.

Among parameters presented in table II, higher OPG concentrations in CKD 5 group are remarkable, although those differences have not achieved statistical significance. On the other hand, OPG/RANKL ratio was significantly higher in CKD 5 group. Elevated OPG concentrations in dialysed patients have been observed by authors of this study (5) and by other investigators in the past (4). *Siomou* et al. (4) found increased OPG concentrations only in children not receiving vitamin D supplementation. In this study elevated OPG was observed in CKD 5 group irrespective of vitamin D treatment provided in almost all of those patients.

Relationships of FGF 23 with other variables are presented in tables III and IV. In all children with CKD the only correlation found was with OPG. However, in patients on conservative treatment, there was positive correlation with RANKL and negative correlation with PTH concentration. Relationship between OPG and FGF 23 may be an accidental phenomenon associating abnormalities in course of kidney failure, although experiments on animal model suggest that OPG plays a substantial role in FGF 23 production by osteoblasts in response to phosphate-rich diet (23). Other authors also noticed correlation between concentrations of FGF 23 and OPG (4).

The probable role of FGF 23 and OPG in pathophysiology of cardiovascular complications in course of CKD is emphasised in literature recently. In 2009 *Gutierrez* et al. (13) observed that FGF 23 correlates independently with increased mortality in patients entering haemodialysis. Subsequent publications from 2009-2010 indicated correlation of serum FGF 23 concentration with cardiovascular disease (CVD) in both dialysed patients and those in pre-dialysis stages (24, 25). In adult patients with CKD a correlation between FGF 23 and vascular calcifications (26), carotid artery wall thickening (27) and left ventricular hypertrophy (LVH) has been found (24, 25). In the study analysing correlation of FGF 23 with LVH, Gutierrez et al. hypothesised that FGF 23 may be not only an important predictor of CVD in CKD, but may also participate in LVH pathogenesis by influencing cardiomyocytes directly (24). In 2011 *Faul* et al. confirmed that hypothesis in series of experiments on cardiomyocytes *in vitro* and on animal model. In their studies they found that FGF 23, present in high concentrations (as in CKD), induces cardiomyocytes hypertrophy by binding to FGFR, independently of Klotho protein coexistence (which is not expressed in the heart) (28).

OPG displays vasculoprotective effect in animal models (1), whereas in studies on humans an association of high OPG concentrations with frequent cardiovascular events has been found (29,30,31), but one human study has shown an inverse relationship between OPG and echogenicity of carotid plaques in adult patients (32). In face of proven vasculoprotective role of OPG in animals, elevated OPG concentration is rather a marker, not a trigger of vascular changes. OPG secreted by osteoblasts may be also produced by osteoblast-like cells in the vessels and thus its concentration may correlate with arteriosclerosis.

Studies concerning associations of FGF 23 with calcium-phosphate metabolism parameters are not unanimous. Expected correlation of phosphate concentration with FGF 23 has not been found in all children included in this study, although other authors described such an association (4, 19, 20). This is probably due to the fact, that in children with CKD 5 phosphate concentration was effectively decreased by phosphate-binders treatment. In groups CKD 3 and 4 there is a tendency towards a correlation between those two variables, although it should be remembered that in course of CKD increase in FGF 23 appears much earlier than increase in phosphate level (13, 33). A correlation between FGF 23 and phosphate concentration has not been found neither by *Wan* et al. (21) in 154 children with CKD or in the study of *Bacchetta* et al. (34).

Results of studies analysing correlations between FGF 23 and PTH vary as well. Some describe such correlations (4, 19), whereas others deny them (20, 21). Diversity of those findings may result from varying grade of hyperprathyroidism compensation in those

patients. Compensated levels of PTH and phosphate concentrations were also present, similarly as in this study, in a large group of children with CKD described by *Wan* et al. (21). Those authors have not observed a correlation between PTH and FGF 23 neither. Instead, they described a negative correlation between PTH and soluble Klotho protein, concluding that Klotho may directly regulate PTH synthesis.

The influence of metabolic acidosis on bone metabolism and children's growth is well known (34). Results of this study presented in table 5 confirm association between acidosis compensation degree and bone turnover parameters. In studies on animal models and *in vitro*, long lasting acidosis was found to stimulate bone resorption and inhibit bone formation by osteoblasts (36, 37). Metabolic acidosis compensation in dialysed patients resulted in decrease of PTH level and improved bone formation (38). Evident correlation of HCO_3^- and BE with OPG confirms those observations: OPG, which has protective impact on the bone, increases alongside with acidosis compensation. Negative correlation of bicarbonate and BE with RANKL/OPG ratio also indicates that acidosis compensation diminishes proresorptive action of RANKL/OPG system. Data about associations of acid-base parameters with RANKL/OPG system were not found in available literature.

Results of this study in children with kidney failure on CKD stage > 3 suggest, that despite outwardly appropriate treatment of calcium-phosphate disturbances, those patients present with abnormalities that may decrease their life expectancy. In most of children included in the study, especially in those undergoing dialysis therapy, acid-base balance and calcium-phosphate metabolism parameters were kept within normal limits. Despite that, their FGF 23 and OPG concentrations were elevated and a significant correlation between those parameters was found. Considering recent reports about impact of both those factors on cardiovascular risk in CKD patients, it may indicate that treatment regimen in those patients necessitates modification in future.

CONCLUSION

Despite maintaining serum calcium, phosphorus and PTH levels within recommended limits, elevated levels of FGF 23 and OPG were observed in children with chronic kidney disease, especially in it's end-stage.

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