The Assessment of the Usefulness of Selected Markers in the Diagnosis of Chronic Kidney Disease in Children

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ABSTRACT

INTRODUCTION: The kidney deterioration, which starts in childhood often leads to end-stage renal failure in the future. Therefore, searching for an early, sensitive, and specific biomarkers became a paramount for chronic kidney disease diagnosis. The aim of this study was the assessment of markers: KIM-1, FGF-23, NAG, NGAL, and uromodulin for diagnosis of preclinical phase of the disease in children.

PATIENTS AND METHODS: 59 children (15 boys, 44 girls from 6 months to 17 years old) with kidney disorders, which had clinical indications for renoscintigraphy, were included in the study. All patients were divided depending on the result of renoscintigraphy (renal scarring vs normal kidney picture) and depending on the level of estimated glomerular filtration rate (glomerular hyperfiltration vs normal filtration rate). The concentration of uromoduline, KIM-1, FGF-23, NAG, and NGAL in serum and of NGAL and uromoduline in urine were measured in all studied groups.

RESULTS: The children with glomerular hyperfiltration had a statistically significantly higher serum values of FGF-23 and NGAL than the children with normal filtration rate ($P < .05$). There were no statistically significant differences in serum concentrations of tested markers in children with renal scars in comparison to children with normal renal image. There was no statistically significant difference in the concentration of tested markers in urine.

CONCLUSIONS: The study confirmed the possible usefulness of FGF-23 and NGAL in detecting the preclinical-stage of renal disease associated with glomerular hyperfiltration in children. The study do not allow to indicate markers, which could be useful in the early diagnosis of kidney damage visible in the scintigraphic examination.

KEYWORDS: Uromoduline, KIM-1, NGAL, FGF-23, NAG

Introduction

Chronic kidney disease (CKD) is a condition of irreversible kidney damage resulting from abnormalities of the kidney structure or function, lasting over 3 months. It is usually accompanied by albuminuria, proteinuria, the presence of abnormalities in histopathological or imagining studies, and a decrease in the glomerular filtration rate (GFR) below 60 ml/min/1.73 m². [1]

The CKD is called, "the disease of the 21st century" because of the increasing number of diagnosed patients. It is estimated that it affects 600 million people worldwide. [1] In the group of children under 16 years of age, the incidence of CKD is between 1.5 and 3 per 1 000 000. [2] The disease, in many cases, is diagnosed at later stages when the only treatment option is renal replacement therapy.

It is known that the early stages of CKD and the predictors of CKD progression are the impaired uptake of the 99 -Tc-EC tracer in renal scintigraphy and the glomerular hyperfiltration. [3,4]

The uneven tracer uptake reported in renoscintigraphy most often corresponds to post-inflammatory scars due to complex urinary tract infections or congenital kidney defects in the form of dysplasia or hypodysplasia. [5] These pathologies, relatively frequently coexisting with each other in the pediatric population, may lead to a gradual reduction of active kidney tissue after years of disease, and consequently to the development of chronic kidney disease. [3] Recently, the importance of glomerular hyperfiltration has also been recognized as a predictor of the development of chronic kidney disease. [4] Increased filtration may initiate glomerular damage secondary to increased intra-glomerular pressure and cause permanent interstitial remodeling. [6]

One of the difficulties in diagnosing early-stages of CKD results from the fact that it may be asymptomatic and because commonly used diagnostic markers of CKD, such as creatinine, urea, GFR, albuminuria, and proteinuria, are not sensitive enough. [7,8] There is also a lack of markers for patients in early

CKD or those possessing the risk factors of CKD development in the future.[7-10] The ability to identify these patients would create an opportunity to implement appropriate therapeutic interventions, which could slow down or even stop the progression of the disease.

Therefore, there have been many attempts to discover early, sensitive, and specific markers of kidney damage. The introduction of such markers into everyday clinical practice would enable professionals to better identify the disease at its onset, before irreversible kidney damage. The KIM-1, FGF-23, uromodulin, NGAL, and NAG proteins seem to be very promising as the non-traditional markers for the diagnosis of chronic kidney disease.[7-10]

KIM-1 (kidney injury molecule-1, T-cell immunoglobulin, mucin-containing molecule) is a type 1 transmembrane protein located in the apical membrane of the nephron proximal tubule cells.[10,11] In response to hypoxia, ischemia or toxic damage of renal tubules, the ectodomain of KIM-1 protein is cleaved by metalloproteinases. KIM-1 is also involved in the process of phagocytosis[12] and the structural and functional renewal of the nephron tubular epithelium.[13] KIM-1 enables epithelial cells in the proximal part of the renal tubule to clear tubules from cellular debris. Therefore, this protein also reduces the immune response and mediates the structural and functional renewal of the nephron tubular epithelium.[12,14] In connection with these processes, KIM-1 has been found to be useful not only as a marker of renal function in AKI, but also in the diagnosis of CKD.[12,14]

The FGF-23 (fibroblast growth factor 23) is a hormone produced by osteoblasts responsible for the regulation of mineral and bone metabolism.[15] Factors regulating FGF-23 secretion are high levels of calcium, phosphates, *parathyroid hormone* and 1,25- dihydroxycholecalciferol in blood serum.[15] Recently, the role of FGF-23 as a marker of chronic kidney disease has also been underlined. One of the first metabolic disorders seen in the early stages of chronic kidney disease is phosphate retention. In response to this process, the secretion of FGF-23 and PTH increases.[16,17] The high concentration of FGF-23 in CKD correlates with the adaptation of kidneys to the decreased urinary phosphate excretion.[18] However, the concentration of FGF-23 also increases in oxidative stress, inflammation, escalated activation of renin-angiotensin-aldosterone system (RAAS) and iron deficiency.[19,20]

The uromodulin (UMOD), also known as Tamm – Horsfall protein, is a 95 kDa glycoprotein, synthesized by the epithelial cells of the distal tubules and the ascending arm of the Henle loop. A small amount of uromodulin is also secreted into the interstitial tissue, subsequently entering the bloodstream. Physiologically, it is the most abundant protein in urine.[21,22] Uromodulin is one of the factors responsible for protecting the urinary tract against infections and stones formation. It also contributes to the maintenance of fluid and electrolyte homeostasis in Henle loop.[22] The reduced number of nephrons, caused, among others, by tubules atrophy or fibrosis of kidney tissue in CKD, correlates with the uromodulin concentration in blood

serum and urine. Therefore, over the past few years, uromodulin has been accepted as a marker of kidney damage.[23-25]

NGAL – (neutrophil gelatinase-associated lipocalin, siderocalin) is a 25 kDa protein synthesized and released mostly by leukocytes, epithelial cells of the Henle loop and collecting ducts.[26,27] NGAL is also secreted by the cells of other organs.[26,27] This protein plays also a role in the embryogenesis when it transforms the nephron mesenchymal cells into epithelial cells.[26,27] Moreover, NGAL mediates, through the epithelial growth factor receptor (EGFR), the mitotic division of epithelial cells. In response to hypoxia, the EGFR stimulates nephron cell proliferation causing the gradual loss of nephron function and CKD progression.[26,27] The increased release of NGAL by renal epithelial cells is observed also in response to their acute kidney injury.[26,27]

NAG – (N-acetyl-β-D-glucosaminidase) is a lysosomal enzyme, which is secreted by microvilli of the epithelial cells of the proximal nephron.[28] The increased excretion of NAG correlates with nephron tubules injury and is a useful marker for the diagnosis of kidney damage[29] or nephrotoxicity.[30] Increased concentration of NAG is already present in the early stages of chronic kidney disease, even before excretory dysfunction appears.[28] Increased value of NAG in urine was also noted in the course of other nephrological diseases associated with damage to the nephron, for example, idiopathic nephrotic syndrome,[31] vesicoureteral refluxes and hydronephrosis,[32] and urinary tract infections.[33] NAG has also been shown to be useful in assessing the risk of diabetic nephropathy in patients with type I diabetes.[34]

According to the literature, there is a lack of reports of the usefulness of the non-traditional markers in monitoring patients in the early stages of kidney damage or those with risk factors of CKD development in the future. Moreover, there is a lack of research on paediatric population. The potencial, early markers may prove more useful in the paediatric population than among adult patients because of the general lack of comorbidities affecting the results of the study.

The aim of the study was the assessment of the usefulness of uromoduline, KIM-1, FGF-23, NAG, and NGAL for the early detection of CKD in children with indications for renal scintigraphy.

Patients and Methods

The study was conducted from June 2016 to December 2018 at the Department of Paediatrics, Paediatric Nephrology and Allergology of the Military Institute of Medicine, Warsaw, Poland. A total of 59 patients with diseases of the urinary system and indications for renal scintigraphy were included in a consecutive manner. Table 1 summarizes the detailed characteristics and incidence of observed symptoms among studied patients. The listed conditions often coexisted with each other.

Renal scintigraphy was performed on each patient using 99 -Tc-EC and Infinia® II Nuclear Gamma Camera. The exclusion criteria were as follows: current urinary tract infection, other infectious diseases with fever, acute kidney injury

Table 1. Paediatric patients qualified for the study.

AGE	6 MO TO 17 Y
Sex	15 boys and 44 girls
Range of eGFR*	76.7 174.8 ml/min/1.73 m²
Diagnose	Recurrent urinary tract infections – n 52
	Vesicoureteral reflux – n 27
	Voiding disfunction – n 14
	Congenital anomalies – n 8
	State after posterior urethral valves ablation – n 5
	Pyelocalyceal system dilatation – n 3
	Kidney cyst – n 2
	Hydronephrosis – n 1

Abbreviation: n, number of patients.
*eGFR, estimated glomerular filtration rate by Schwartz Equation
(eGFR = 0.413 × (height/serum creatinine concentration)).

(estimated creatinine clearance decreased by 25% or/and urine output <0.5 ml/kg/h per 8 hours) or previously diagnosed CKD (estimated glomerular filtration rate <60 ml/min/1.73 m³, albumin excretion rate ≥ 30 mg/24 hours, albumin to creatinine ratio ≥ 30 mg/g, urine sediment abnormalities, electrolyte, and other abnormalities due to tubular disorders, history of kidney transplantation, hypertension due to kidney disease – systemic blood pressure ≥ 95th percentile for age, sex, and height) were excluded from the study. Additionally, children with other comorbidities, which would affect the results of the study, such as prematurity, heart failure, diabetes, vasculitis, other chronic inflammatory diseases, were also excluded. Patients did not undergo the nephroprotective treatment by angiotensin converting enzyme inhibitors or angiotensin receptor antagonists at the time of the study.

In the study the predictors of CKD progression were assumed as: the impaired uptake of the 99 -Tc-EC tracer in renal scintigraphy and the glomerular hyperfiltration.

Based on the above parameters, all patients included in the study were divided into several groups. The first selection was based on the renal scintigraphy images. The study group included children with impaired tracer uptake and the control group consisted of patients with normal kidneys scintigraphy images. The second selection was based on the estimated glomerular filtration rate calculated by Schwarzt Equation (eGFR = 0.413 × (height/serum creatinine concentration).[35] The study group included children with glomerular hyperfiltration (GFR ≥ 130 ml/min) and the control group contained children with normal glomerular filtration (<130 ml/min).

The markers tested in fasting venal blood serum included creatinine (mg/dl), cystatin C (mg/dl), KIM-1 (ng/ml), FGF-23 (pg/ml), NAG (U/l), NGAL (ng/ml) and uromodulin (ng/ml). The levels of NGAL (ng/ml) and uromodulin (ng/ml) were also measured in a first morning void.

Furthermore, albumin excretion rate in a total of 35 patients who were able to performed 24-hour urine collection and the albumin-to-creatinine ratio from a first-morning void in other 24 children were calculated. Serum and urine collected for the determination of KIM-1, FGF-23, NAG, NGAL, and uromodulin were centrifuged and then frozen at –80°C. The concentration of tested proteins was determined by *ELISA using commercial tests*: HAVcr1 ELISA Kit Catalog No: E0785h from EIAab®, FGF23 ELISA Kit Catalog No: E0746h from EIAab®, NAG ELISA Kit Catalog No: E0069h from EIAab®, Human Lipocalin 2/NGAL Immunoassay Quantikine® ELISA Catalog Number DLCN20 from R&D Systems, Uromodulin Human NATIVE Catalog No: RD172163100 from Biovendor.

All of the the procedures were in accordance with the latest version of the Declaration of Helsinki given by World Medical Association After explaining the details of the study to the patients and their guardians, written consent was obtained from guardians and patients over 16 years of age before enrolment into the study. The Bioethics Committee of the Military Institute of Medicine in Warsaw, Poland approved the study (Resolution no. 28/WIM/2015). The study was carried out as a part of the statutory project No. 397.

The obtained data were analyzed using the R software, version 3.5.1. Before starting analyses, the data were verified with the normal distribution graphs and normalized using the Shapiro–Wilks test. Student's *t*-test was used to evaluate variables with a normal distribution. The selected variables lacking compliance were statistically evaluated by the U- Mann–Whitney test. For the correlation analysis, the Pearson correlation coefficient was used for data with normal distribution, and the Spearman rank correlation coefficient was calculated for abnormally distributed data. The values with $P < .05$ were considered statistically significant.

Results

The analysis of the groups selected on the basis of the renal scintigraphy images:

The study group consisted of 34 children (26 girls, 8 boys) and the control group of 25 children (18 girls, 7 boys).

There was no statistically significant difference between the study group (impaired tracer uptake) and the control group (normal kidneys in scintigraphy images) in terms of sex and levels of the "traditional" markers of renal function, such as concentration of creatinine and cystatin C in blood serum and albuminuria (the albumin-to-creatinine ratio). However, the average age of patients in the study group was significantly higher than in the control group (9.32 ± 4.8 vs 6.24 ± 4.1) (Table 2).

No statistically significant differences were found between the study group and the control group in terms of levels of the "novel" markers of renal function: KIM-1, FGF-23, NAG, NGAL, and uromodulin in blood serum and NGAL and uromodulin in urine (Table 3).

The analysis in groups based on glomerular filtration rate:

The study group consisted of 31 children (23 girls, 8 boys) and the control group of 28 children (21 girls, 7 boys).

Table 2. Characteristics of the control group and the study group selected on the basis of renal scintigraphy images.

	STUDY GROUP	CONTROL GROUP	*P*
Age (y) (mean ± SD)	9.32 ± 4.8	6.24 ± 4.1	<.05
Sex (girls:boys)	26:8	18:7	ns
Creatinine $_s$ (mg/dl) (median, Q1–Q3)	0.5 (0.4-0.7)	0.4 (0.3-0.5)	ns
Cystatin C $_s$ (mg/dl) (median, Q1–Q3)	0.95 (0.855-1)	1.0 (0.8-1.1)	ns
Albuminuria (ml/24 h) (median, Q1–Q3)	6 (4-8)	5 (3.75-6.25)	ns
Albumin-to-creatinine ratio (mean ± SD)	0.0053 ± 0.0055	0.0028 ± 0.0062	ns

There were no statistically significant differences between the study group (children with glomerular hyperfiltration) and the control group (children with normal glomerular filtration) in terms of sex and levels of the "traditional" markers of renal function such as concentration of creatinine and cystatin C in blood serum, and albuminuria (the albumin-to-creatinine ratio). However, the average age of patients in the study group was significantly higher than in the control group (9.09 ± 4.37 vs 6.0 ± 4.78) (Table 4).

There was a statistically significant increase of NGAL and FGF-23 concentration in blood serum in the group of children with glomerular hyperfiltration in comparison to the group with normal glomerular filtration rate (Table 5). Moreover, KIM-1 serum levels, although not statistically significant, were noticeably higher in the study group than in the control group. The uromodulin concentration in urine was lower in the study group than in the control group. There was no significant difference in NAG and uromodulin serum concentrations, and NGAL urine concentrations (Table 5).

In all studied patients, there was a correlation between the uromodulin and NGAL serum concentrations, and the age of examined children (*P* = .02 and *P* = .001), and between uromodulin and NGAL serum concentrations and the creatinine levels (*P* = .048 and *P* = .001) (Table 6).

There was no statistically significant correlation between the concentrations of KIM-1, FGF-23, and NAG in serum, and NGAL and uromodulin in urine, and the age and serum creatinine levels (Table 6). There was no statistically significant correlation between any of the studied protein markers and albuminuria, cystatin C levels or GFR (Table 6).

Discussion

The study analyzed the usefulness of the non-traditional protein markers (uromoduline, KIM-1, FGF-23, NGAL, and NAG) for the monitoring of the function of kidneys in children with a risk of CKD development.

All the tested, not routinely used proteins, except for FGF-23, are markers of renal tubular damage. Based on some previous studies available in the literature, it can be assumed that tubulointerstitial lesions may be more important in predicting renal disease progression than glomerular and vascular lesions in young patients with various renal diseases.[7-10] In the pediatric population, the most common causes of CKD are primary kidney diseases, including uropathies, polycystic disease, and kidney hypoplasia and dysplasia. In contrast, gromerulopathies are less common in this group of patients.[36] Therefore, the tubulointerstitial state may better reflect the total mass of nephrons and therefore could be helpful in more accurately identifying patients with high-risk chronic kidney disease. However, diagnostics based on such markers is not well established yet. The study result could contribute to further improvement of the early diagnosis of the disease and, consequently, contribute to the effective prevention, and monitoring of CKD progression

Unfortunately, the study did not confirm the usefulness of tested biomarkers in the prognosis of the development of chronic kidney disease in children with kidney damage visible in the scintigraphic examination. It might be due to the fact that the markers concentration in serum or urine is more correlated to functional injury of the nephron rather than to renal tissue damage itself.

There was also no statistically significant difference between studied groups in terms of the levels of the commonly used markers of renal function, such as the concentration of creatinine and cystatin C in blood serum and albuminuria/ the albumin-to-creatinine ratio. This confirms the limited use of these "traditional" markers for the diagnosis and monitoring early stages of renal damage in the pediatric population.

The study also assessed the usefulness of tested markers in diagnostics of children with glomerular hyperfiltration. The possible usefulness of sFGF-23 and sNGAL in detecting early stage of kidney disease associated with glomerular hyperfiltration in children was confirmed.

We found that children with glomerular hyperfiltration had a statistically significant increase of FGF-23 concentration in the blood serum, in comparison to the group with normal glomerular filtration rate. This result may correlate with the fact that this protein is not produced in the kidneys. Consequently its concentration closely depends on the glomerular filtration rate. Other authors also indicated the usefulness of FGF-23 in monitoring CKD. Lukaszyk et al[37] and Pavlik et al[38]

Table 3. Comparison of the levels of the novel markers of kidney damage in groups selected on the basis of the presence of post-inflammatory scars in renal parenchyma in renal scintigraphy images.

	MIN	MAX	MEAN	MEDIAN	SD	Q1	Q3	P
sKIM-1 ng/ml								
Study group	0.6	2.4	1.2	1.0	0.6	0.9	1.4	ns
Control group	0.5	3.1	1.3	1.0	0,7	0.9	1.7	
sFGF-23 pg/ml								
Study group	144.1	873.1	337.4	255.1	203.9	204.7	379.7	ns
Control group	134,1	867.8	337.4	238.5	231.8	155.9	471.8	
sUMOD ng/ml								
Study group	2.8	9.0	4.5	4.1	1.5	3.6	5.0	ns
Control group	2.9	7.8	5.1	5.4	1.7	3.4	5.9	
uUMOD ng/ml								
Study group	25.4	107.0	75.9	88.4	27.3	57.0	94.7	ns
Control group	15.7	138.9	77.7	93.9	33.3	46.0	97.5	
sNAG U/L								
Study group	72.1	867.0	329.5	192.1	280.2	84.7	541.5	ns
Control group	53.3	1351.1	422.4	312.4	384.6	99.3	642.2	
sNGAL ng/ml								
Study group	8.5	27.3	17.4	17.1	5.0	14.2	19.5	ns
Control group	5.8	27.2	16.9	16.7	4.5	13.9	19.7	
uNGAL ng/ml								
Study group	0.4	5.2	1.2	0.8	1.2	0.6	1.4	ns
Control group	0.2	4.8	1.6	1.2	1.2	0.6	2.0	

Abbreviations: sFGF-23, concentration of FGF-23 in blood serum; sKIM-1, concentration of KIM-1 in blood serum; sNAG, concentration of NAG in blood serum; sNGAL, concentration of NGAL in blood serum; sUMOD, concentration of uromodulin in blood serum; uNGAL, concentration of NGAL in urine; uUMOD, concentration of uromodulin in urine.

independently proved the usefulness of FGF-23 as an early marker of CKD in adults. Tranæus Lidblad et al[39] evaluated FGF-23 serum concentration in 74 pediatric patients with CKD. They reported the correlation between an increased level of FGF-23 and a decline in GFR, and thus CKD progression, in the pediatric patients with good controls of phosphate serum concentrations. Wesseing-Perry et al[40] presented similar conclusions in their study. They observed elevated FGF-23 levels in children with CKD stage 2-4. Cited authors did not conduct studies with patients with preclinical phase of CKD.

Similar results were reported to NGAL. Our study showed significant increase of NGAL concentration in blood serum in the group of children with glomerular hyperfiltration in comparison to the group with the normal glomerular filtration rate. Additionally, there was the correlation between the sNGAL and creatinine serum levels. Mori and Nakao presented an interesting theory called the "Forest Fire Theory." This hypothesis assumed that the increase in NGAL concentration is not only a consequence of decreased renal clearance, but to a greater extent the result of increased production of this protein by damaged tubular epithelial cells. According to this theory, the concentration of NGAL is supposed to reflect the activity of current kidney damaging processes in the course of CKD. Meanwhile, the increase in serum creatinine concentration are the result of the loss of the number of functioning nephrons.[41]

There are multiple studies, which confirm the usefulness of NGAL in the monitoring of the CKD, including the early stages of CKD. Bolignano et al[42] assessed the usefulness of NGAL as a new marker of CKD progression. They found that sNGAL concentration was noticeably elevated in the patients with the CKD stage 2-4, in comparison to the control group with the CKD stage 0. In that study, NGAL serum and urine concentrations were negatively correlated with GFR. Mitsnefes et al[43] showed, in a small study in pediatric population, the

Table 4. Characteristics of the control group and the study group selected on the basis of the glomerular filtration rate.

	STUDY GROUP	CONTROL GROUP	*P*
Age (years) (mean ± SD)	9.09 ± 4.37	6.0 ± 4.78	<.05
Sex (girls:boys)	23:8	21:7	ns
Creatinine $_s$ (mg/dl) (median, Q1–Q3)	0.5 (0.5-0.6)	0.4 (0.3-0.4)	ns
Cystatin C $_s$ (mg/dl) (median, Q1–Q3)	1.0 (0.9-1.0)	1.05 (0.9-1.1)	ns
Albuminuria (ml/24 h) (median, Q1–Q3)	6.5 (5.5-8.0)	4 (3-6)	ns
Albumin-to-creatinine ratio (mean ± SD)	0.0052 ± 0.0052	0.0038 ± 0.0021	ns

Table 5. Comparison of the levels of the novel markers of kidney damage in groups selected on the basis of the glomerular filtration rate estimated from the fractional renal tracer uptake in renal scintigraphy.

	MIN	MAX	MEAN	MEDIAN	SD	Q1	Q3	*P*
sKIM-1 ng/ml								
Study group	0,5	2.4	1.5	1.6	0.7	1.0	2.1	ns
Control group	0.7	3.1	1.3	1.0	0,7	0.9	1.3	
sFGF-23 pg/ml								
Study group	174.3	867.8	409.8	359.4	225.7	241.8	552.5	.032
Control group	134,1	749.3	254.4	194.2	194.2	150.5	245.8	
sUMOD ng/ml								
Study group	3.3	9.0	5.0	4.0	2.0	3.5	5.8	ns
Control group	2.9	6.8	4.3	4.2	1.2	3.4	5.1	
uUMOD ng/ml								
Study group	25.0	138.9	70.4	75.1	37.1	38.7	94.7	ns
Control group	15.9	123.3	83.8	94.1	29.1	85.1	97.1	
sNAG U/L								
Study group	53,5	1060.7	335.1	90.2	377.5	73.3	608.5	ns
Control group	53.3	1351.1	535.5	536.2	373.8	209.5	706.1	
sNGAL ng/ml								
Study group	10.9	27.0	20.2	21.3	4.9	17.6	23.2	.048
Control group	8.5	27.7	16.5	16.1	4.9	12.9	18.7	
uNGAL ng/ml								
Study group	0.5	2.6	1.3	0.9	0.8	0.6	2.0	ns
Control group	0.2	5.2	1.4	0.8	1.3	0.5	1.6	

Abbreviations: sFGF-23, concentration of FGF-23 in blood serum; sKIM-1, concentration of KIM-1 in blood serum; sNAG, concentration of NAG in blood serum; sNGAL, concentration of NGAL in blood serum; sUMOD, concentration of uromodulin in blood serum; uNGAL, concentration of NGAL in urine; uUMOD, concentration of uromodulin in urine.

correlation between NGAL level and eGFR (estimated from the *Schwartz* equation). Thereby, they confirmed the accuracy of using NGAL to assess a renal function in pediatric patients with CKD.[43] Parmaksiz et al[44] proved the usefulness of NGAL in predicting presence of renal scars in children with the VUR, and Lee et al[45] showed that in the patients with urinary infection, the NGAL and KIM-1 serum levels are the predictors of the future development of renal scarring.

Table 6. The correlation rate and probability values (*P*-values) as a determinant of the correlation between the novel and traditional markers of renal function, and between novel markers of renal function and the age of examined children.

	CREATININE (MG/DL)	CYSTATIN C (MG/DL)	ALBUMINURIA (MG/24 H)	GFR (ML/MIN)	AGE (Y)
sKIM-1	−0.028	0.239	0.147	0.218	0.072
	p = .875	*p* = .229	*p* = .572	*p* = .328	*p* = .692
sFGF-23	0.166	−0.021	0.227	0.323	0.205
	p = .345	*p* = .914	*p* = .335	*p* = .131	*p* = .243
sUMOD	0.340	0.139	−0.002	0.294	0.391
	p = .048	*P* = .479	*p* = .990	*p* = .161	*p* = .022
uUMOD	−0.010	0.054	−0.132	0.004	−0.009
	p = .948	*p* = .764	*p* = .547	*p* = .983	*p* = .953
sNAG	−0.043	0.175	0.135	−0.149	−0.071
	p = .796	*p* = .337	*p* = .546	*p* = .476	*p* = .667
sNGAL	0.518	0.168	0.190	0.311	0.524
	p = .001	*p* = .273	*p* = .303	*p* = .129	*p* = .001
uNGAL	0.029	−0.061	0.097	−0.202	0.066
	p = .837	*p* = .696	*p* = .629	*p* = .331	*p* = .642

Abbreviations: sFGF-23, concentration of FGF-23 in blood serum; sKIM-1, concentration of KIM-1 in blood serum; sNAG, concentration of NAG in blood serum; sNGAL, concentration of NGAL in blood serum; sUMOD, concentration of uromodulin in blood serum; uNGAL, concentration of NGAL in urine; uUMOD, concentration of uromodulin in urine.

We found that the level of KIM-1 in the serum, despite of the lack of statistical significance, was higher in the group of children with glomerular hyperfiltration than in the group with normal GFR values. Numerous studies are confirming the usefulness of KIM-1 in monitoring the advanced stages of CKD. Waikar et al[46] reported a strong correlation between albuminuria and increased KIM-1 serum levels measured in participants of 5 cohort studies conducted in the USA and Sweden. Lee et al[45] demonstrated that the levels of NGAL and KIM-1 in the serum of patients with urinary tract infections are the predictors of future development of renal scarring. Toker et al[47] reported increased KIM-1 serum concentration in the group of children with the post-inflammatory scars visible in the renal scintigraphy images of renal parenchyma and caused by the high-grade vesicoureteral reflux (VUR). Unfortunately, there was no information what was the stage of CKD in the studied population.[47] In contrast, Parmakisiz et al[44] and Novan et al[48] did not confirm the usefulness of KIM-1 urine concentration measurements in predicting the formation of post-inflammatory renal scars in children with VUR. However, as far as we know, no studies were assessing the level of KIM-1 in the group of patients only with glomerular hyperfiltration.

The study showed that the level of uromodulin in the serum and urine is not useful for the monitoring of kidney function either in patients with glomerular hyperfiltration or with abnormalities in renal scintigraphy. However, Steubl et al,[49] in the prospective study of the group of 426 adults showed the advantage of measuring uromodulin serum concentration (sUMOD) in monitoring function of the kidneys in CKD patients. They noticed the correlation between the gradual decline in sUMOD concentrations and the loss of kidney function. They also observed a statistically significant difference in the levels of sUmod between the CKD stage 0 and 1. In contrast, Fedak et al[50] study did not show a statistically relevant differences between patients with CKD stage 0 and stage 1. In our study, we showed a correlation between sUmod and creatinine serum concentrations. Also, Scherberich et al,[22] confirmed the correlation between declined uromodulin and cystatin C serum concentrations, creatinine urine levels, and GFR. Tan et al[51] reported the correlation between sUMOD and GFR and proved that patients with lower sUmod levels are more prone to the development of the ESRD in the future. Regardless of the usefulness of uromodulin for the monitoring of the CKD, proven in numerous clinical studies, there is not enough research on the uromodulin in the pediatric pre-CKD stages population.

Our study did not show the usefulness of NAG, either for monitoring patients with glomerular hyperfiltration or with abnormalities in renal scintigraphy. Furthermore, no correlation between NAG and "traditional" markers was observed. There are no reports in the literature on the usefulness of NAG in the monitoring of the early stages of the CKD. However, Jungbauer et al[52] studying 149 adult patients with heart failure, confirmed the usefulness of NAG as an independent marker of

the CKD progression. Furthermore, the Diabetes Control and Complications Trial (DCCT)[34] reported a positive correlation between NAG concentration and level of albuminuria in patients with type 1 diabetes. They also showed that NAG can be useful in assessing the risk of diabetic nephropathy development in those patients.[34]

There were several limitations to our study. One of them was a statistically significant difference in the age of studied groups. Both, in the group with the impaired tracer uptake in renal scintigraphy, and the group with the glomerular hyperfiltration, the average age was 3 years higher than in control groups. Assuming the progression of CKD over time, the disease can be more aggravated in older patients. In our study, we proved the positive correlation between the serum concentration of uromodulin and NGAL, and patient's age.

However, data from the literature are not equivocal. Van Donge et al. studied reference intervals for kidney markers in healthy infants, children and adolescents. It was noted that KIM-1 levels decreased with age in pediatric population. This study did not show any NGAL and UMOD age-dependency.[53] Scherberich et al[22] showed similarity of uromodulin serum levels in adults and children. In the group of 190 healthy adult patients, the mean sUMOD level was 207 ng/ml, whereas in pediatric population of 443 patients it was 193 ng/ml, and the difference was statistically insignificant. There was also no difference in sUMOD levels associated with sex. Bennett et al[54] conducted research to correlate selected markers with age and sex. When assessing the concentrations of selected markers, in 4 age groups, uNGAL and uKIM-1 levels were statistically significantly higher in older children. Similarly, we noted a positive correlation between NGAL concentrations and the age of children in our study. Cangemi et al[55] observed different reference ranges for uNGAL between neonates and older children. In the study, mean uNGAL levels were noticeably higher in neonates (44.2 ng/ml) than in older children (10.2 ng/ml).

In our study, there was no statistically significant difference between selected groups in terms of sex. However, levels of individual markers were not correlated with gender. Similarly, van Donge et al did not show significant differences in the serum values of UMOD, NGAL, and KIM-1 between healthy boys and girls.[53] In contrast, Bennett et al[54] reported statistically significant higher values of uNGAL in girls than boys. A similar relationship was not observed for KIM-1 level. Similar results were obtained by McWilliam et al[56] They also observed lower uKIM-1 levels in African – Americans, in comparison to Caucasians. In our studies, all children enrolled in the study were Caucasian.

The limitation of our study was also the lack of programmed correlation between the urine levels of studied markers and the concentration of excreted creatinine. However, we took into account the possibility of incorrect calculations of creatinine excretion in patients with hyperfiltration. Therefore it was decided to use this type of presentation.

The last limitation of our study was a small number of participants and heterogeneity in studied groups. Therefore, further research is needed, including a larger group of patients and all variables not explored in the current study that might affect the results.

Conclusions

The results of our study indicate the usefulness of FGF-23 and NGAL for the monitoring of the early stages of chronic kidney disease associated with glomerular hyperfiltration in children. The study did not confirm the usefulness of commonly used markers (creatinine, cystatin C, albuminuria) and not routinely used markers (KIM-1, uromodulin, and NAG) for diagnosis of the early stages of renal damage in the paediatric population.

Author Contributions

A.B. and K.J. are responsible for general conception and design. A.B and K.J. have participated in the conception, planning, collection, analysis, interpretation of data. A.B and A.B. have drafted the manuscript. B.K. and M.K. are responsible for critical revision of this manuscript. B.K. is also responsible for the final approval of the article. All authors of this paper have read and approved the submission of this version of the manuscript and take full responsibility for the manuscript.

Ethics Approval and Consent to Participate

After explaining the details of the study to the patients and their guardians, written consent was obtained from guardians and patients over 16 years of age before enrolment into the study. The Bioethics Committee of the Military Institute of Medicine in Warsaw, Poland approved the study (Resolution no. 28/WIM/2015).

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Data availability

The detailed statistical data used to support the findings of this study are available from the corresponding author upon request.

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