MARKERS OF EPITHELIAL-MESENCHYMAL TRANSITION IN CHILDREN WITH CHRONIC KIDNEY DISEASE

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TAB. 1

The patient characteristics

Parameter	Median values (interquartile ranges) of analyzed parameters	
	Control group (n = 23)	**CKD (n = 41)**
age [years]	10.5 (5.0-14.5)	11.0 (4.0-17.0)
gender	13 girls; 10 boys	17 girls; 24 boys
eGFR [ml/min]	105.0 (97.0 - 112.3)	26.0 (16.8 – 38.0) *
urea [mg/dl]	32.0 (25.5 - 37.0)	77.0 (55.0 – 94.5) *
albumin [g/dl]	N/A	4.3 (3.8 - 4.5)
hemoglobin [g/dl]	12.8 (11.7 - 13.9)	11.2 (10.5 - 12.2) *
parathormone [pg/ml]	N/A	125.0 (46.1 - 223.0)
hsCRP [mg/l]	0.5 (0.24 – 1.34)	0.6 (0.18 – 1.37)
proteinuria [g/l]	0.01 (0 – 0.1)	0.4 (0.03 – 0.6) *
urine creatinine [mg/dl]	114.0 (100.0 – 126.0)	90.0 (72.0 – 110.0) *

TAB. 2

The examined parameters in serum of CKD children and controls

Parameters in serum	Median values (lower – upper quartile)	
	Control group (n = 23)	**CKD (n = 41)**
MMP-2 [ng/ml]	94.53 (88.88–115.77)	159.45 (156.05 167.92) *
MMP-9 [ng/ml]	94.30 (91.00–100.8)	415.60 (397.90–427.6) *
TGFbeta1 [ng/ml]	1221.99 (1195.0–1242.9)	1738.88 (1717.61–1760.18) *
E-cadherin [ng/ml]	31.45 (30.45 – 32.68)	98.50 (96.34 – 103.58) *
Survivin [ng/ml]	44.40 (40.42 – 47.97)	98.51 (88.19 – 107.13) *
sFas [pg/ml]	3611.93 (3190.52-3721.49)	4943.56 (4716.0-5617.8) *
sFasL [pg/ml]	43.97 (42.66-44.95)	59.29 (57.02-67.72) *
Hsp27 [ng/ml]	35.54 (34.16-36.70)	53.12 (50.94-55.56) *
hsCRP [mg/l]	0.50 (0.24 – 1.34)	0.61 (0.18 – 1.37)

TAB. 3

The examined parameters in urine of CKD children and controls

Parameters in urine	Median values (lower – upper quartile)	
	Control group (n = 23)	**CKD (n = 41)**
MMP-2 [ng/mg creat]	1.19 (1.03–1.43)	2.64 (1.92–3.87) *
MMP-9 [ng/mg creat]	1.84 (1.63–2.12)	4.49 (3.95–5.25) *
TGFbeta1 [ng/mg creat]	42.55 (38.44–47.97)	246.26 (217.62–277.43) *
E-cadherin [ng/mg creat]	3.0 (2.67–3.17)	8.72 (7.88–11.20) *
Survivin [ng/mg creat]	36.43 (32.53–42.06)	119.56 (102.52–147.77) *

Mann-Whitney U test:
* – p < 0.001 CKD vs. control group
N/A – not assessed in the control group

TAB. 4

The correlations between serum and urine parameters in CKD children assessed by the Pearson's correlation coefficient r

Parameter	Urine MMP-2	Urine MMP-9	Urine TGFbeta 1	Urine E-cadherin	Urine survivin
Serum MMP-2	p < 0.001 r = - 0.59	p = 0.039 r = - 0.32	p = 0.32 r = - 0.16	p = 0.25 r = - 0.18	p = 0.70 r = - 0.06
Serum MMP-9	p < 0.001 r = 0.61	p = 0.048 r = 0.31	p = 0.29 r = 0.17	p = 0.24 r = 0.18	p = 0.88 r = 0.03
Serum TGF beta 1	p = 0.50 r = - 0.11	p = 0.76 r = - 0.05	p = 0.71 r = - 0.06	p = 0.75 r = - 0.05	p = 0.92 r = - 0.02
Serum E-cadherin	p = 0.41 r = 0.96	p = 0.12 r = 0.25	p = 0.01 r = 0.40	p = 0.04 r = 0.33	p = 0.12 r = 0.25
Serum survivin	p = 0.32 r = - 0.16	p = 0.38 r = 0.14	p = 0.09 r = 0.26	p = 0.16 r = 0.22	p = 0.20 r = 0.20
Serum sFas	p = 0.02 r = 0.38	p = 0.03 r = 0.34	p = 0.08 r = 0.27	p = 0.04 r = 0.33	p = 0.09 r = 0.26
Serum sFasL	p = 0.14 r = 0.24	p = 0.01 r = 0.39	p = 0.03 r = 0.34	p = 0.03 r = 0.34	p = 0.07 r = 0.29
Serum Hsp27	p = 0.002 r = - 0.46	p = 0.03 r = - 0.34	p = 0.02 r = - 0.37	p = 0.02 r = - 0.36	p = 0.03 r = - 0.35

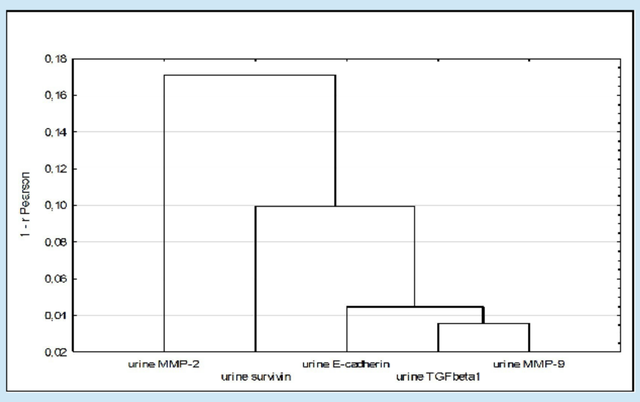
TAB. 5

The correlations between urine markers in CKD children assessed by the Pearson's correlation coefficient r

Parameter	Urine MMP-2	Urine MMP-9	Urine TGFbeta1	Urine E-cadherin	Urine survivin
Urine MMP-2	-	p < 0.001 r = 0.78	p < 0.001 r = 0.63	p < 0.001 r = 0.69	p < 0.001 r = 0.60
Urine MMP-9	p < 0.001 r = 0.78	-	p < 0.001 r = 0.94	p < 0.001 r = 0.96	p < 0.001 r = 0.86
Urine TGF beta1	p < 0.001 r = 0.63	p < 0.001 r = 0.94	-	p < 0.001 r = 0.95	p < 0.001 r = 0.86
Urine E-cadherin	p < 0.001 r = 0.69	p < 0.001 r = 0.96	p < 0.001 r = 0.95	-	p < 0.001 r = 0.89
Urine survivin	p < 0.001 r = 0.60	p < 0.001 r = 0.86	p < 0.001 r = 0.86	p < 0.001 r = 0.89	-

FIG. 1

The cluster analysis of urine concentrations of examined parameters in children with CKD.



INTRODUCTION

Renal interstitial fibrosis is the final common pathway in chronic kidney disease, irrespective of its origin. Epithelial-mesenchymal transition (EMT), in the course of which the tubular epithelial cells are transformed into the mesenchymal ones, is one of the main mechanisms responsible for the appearance of fibrotic changes. The newly formed mesenchymal cells migrate through the extracellular matrix and change into the active myofibroblasts, responsible for the matrix excessive deposition and the subsequent fibrosis progression.

Transforming growth factor *(TGF)* ***beta 1*** is the main EMT player and the master regulator of fibrosis, triggering early hypertrophy, apoptosis, the atrophy of tubular epithelial cells and their trans-differentiation in order to gain the phenotype characteristic for matrix-producing myofibroblasts.

The limits of ***matrix metalloproteinase*** *(MMP)* engagement in EMT and renal fibrosis have primarily been drawn as far as the regulation of extracellular matrix content and the tissue remodeling. However, recent studies have revealed that the MMP influence may be more significant as they can act pro-fibrotically through the EMT induction.

E-cadherin is an adhesion molecule released into the circulation as a consequence of the cell-cell detachment. Anoikis, a specific form of apoptosis, enables the elimination of those cells, thus preventing the reattachment in the inappropriate location and the metastasis formation. The loss of E-cadherin expression, resulting in the molecule accumulation in serum, is a hallmark of EMT, strictly connected with the resistance to anoikis. It has scarcely been analyzed in the patients with CKD.

Survivin is another protein acting in an anti-apoptotic way, enabling the rescue from anoikis through the activation of nuclear factor (NF)-κB. Recent interest has turned into the potential nephrological engagement of survivin, revealing the paramount importance of its expression in mice undergoing the renal proximal tubule recovery after acute kidney damage. However, the role of survivin has not been assessed in patients with CKD so far.

THE AIM OF STUDY WAS TO

1. analyze both known and new markers of EMT in renal fibrosis by assessing the concentrations of MMP-2, MMP-9, TGF beta 1, E-cadherin and survivin in the serum and urine of children with CKD stages 3 to 5
2. evaluate the potential relations between those parameters
3. search for their correlations with the established markers of the CKD-related phenomena, like inflammation (hsCRP) or apoptosis represented by the death receptors sFas and sFasL or heat shock protein (hsp) 27

MATERIAL

Sixty four patients enrolled in this study were divided into 2 groups. Basic demographic and clinical data are shown in **Tab. 1**.

METHODS

Blood samples were drawn from peripheral veins after an overnight fast. Samples were clotted for 30 minutes, centrifuged at 4°C, 1000g for 10 minutes, and then serum was stored at -20°C until assayed. Urine was collected aseptically from the first morning sample, centrifuged as mentioned above and then stored at -20°C until assayed.

Serum and urine concentration of MMP-2, MMP-9, TGFβ1, E-cadherin, survivin, sFas, sFasL and Hsp27 were evaluated by commercially available ELISA kits (R&D Systems, Enzo Life Sciences). Measurements were performed according to the manufacturer's instructions, results were calculated by reference to standard curves.

High sensitivity (hs)CRP was assessed by immunonephelometry with Siemens CardioPhase hsCRP reagent on the BN II System analyzer. The estimated glomerular filtration rate (eGFR) was calculated according to the Schwartz formula.

Results are expressed as median values and interquartile ranges. Since the null hypothesis of normality of distribution was rejected by Shapiro-Wilk test, comparisons in pairs were evaluated by using nonparametric tests (Mann-Whitney U). Relations between parameters were defined by Pearson's correlation coefficient r and additionally pictured by the cluster analysis. Statistical analysis was performed using the package Statistica ver. 10.0 (StatSoft). A p value < 0.05 was considered significant.

RESULTS

MMPs, TGFbeta1, E-cadherin and survivin

The median values of MMP-2, MMP-9, TGFbeta 1, E-cadherin and survivin in serum of CKD children were significantly higher vs. controls (**Tab. 2**).

The urine levels of examined parameters, normalized to urine creatinine, were also significantly increased when compared to the control group (**Tab. 3**).

Correlations and cluster analysis

The MMP-2, MMP-9 and E-cadherin levels in urine correlated significantly with the corresponding values in serum, whereas the survivin and TGFbeta 1 urine concentrations did not (**Tab. 4**). All parameters examined in urine were related to selected apoptotic markers in serum. In particular, urine MMP-9 and E-cadherin correlated with all of them (sFas, sFasL, Hsp27), whereas urine survivin was significantly related only to serum Hsp27 **(Tab. 4)**. The most significant correlations concerned urine concentrations of all analyzed parameters **(Tab. 5)**. Additionally, cluster analysis has revealed the similarity of features pictured by MMP-9, TGFbeta 1 and E-cadherin, suggesting that their efficiency as biomarkers is comparable and choosing one out of 3 would be enough to get the required information **(Fig. 1)**.

Serum E-cadherin was the only parameter correlating with eGFR (R = - 0.64; p = 0.0002). None of the analyzed parameters correlated with hsCRP or other biochemical markers.

CONCLUSIONS

1. The increased urine levels of survivin, E-cadherin, MMPs and TGFbeta 1 may indicate the EMT-related apoptosis and the tissue remodeling, leading to the renal interstitial fibrosis in CKD children.

2. The urine MMP-9, TGFbeta 1 and E-cadherin concentrations form the panel of markers describing similar features of the EMT-related processes.

3. The urine survivin may be considered a new independent biomarker of the kidney-specific EMT.

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903--SP
N) Paediatric Nephrology.
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DOI: 10.3252/pso.eu.52era.2015
ePosters supported by F. Hoffmann- La Roche Ltd.
52nd ERA-EDTA CONGRESS London2015 UNITED KINGDOM MAY 28th-31st
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