FRACTIONAL EXCRETION OF SURVIVIN, EMMPRIN AND MMP-7 IN CHILDREN WITH CHRONIC KIDNEY DISEASE

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INTRODUCTION

Epithelial-mesenchymal transition (EMT) is defined as a transformation of tubular epithelial cells into the mesenchymal ones, their migration through the extracellular matrix and change into active myofibroblasts, responsible for the matrix excessive deposition. The consequences of such changes are tubular dysfunction and fibrosis of the renal parenchyma, characteristic for chronic kidney disease (CKD).

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. Moreover, the ***extracellular matrix metalloproteinase inducer*** (EMMPRIN) is also interacting with the fibrotic signaling pathways.

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 except for our preliminary results concerning children

Analysis of the ***fractional excretion*** of various parameters, as a substitute of tubular dysfunction, has not been widely used in the CKD patients and it mainly has concerned the assessment of the phosphate metabolism so far.

TAB. 1

The patient characteristics

Parameter	*Median values (lower – upper quartile) 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) *

TAB. 2

The concentrations of analyzed parameters in serum (s) and urine (u) of CKD children and controls

Serum and urine concentrations of analyzed parameters	Median values (lower – upper quartile)	
	Control group (n = 23)	**CKD (n = 41)**
sSurvivin [ng/ml]	44.40 (40.42 – 47.97) *	98.51 (88.19 – 107.13)
sE-cadherin [ng/ml]	31.45 (30.45 – 32.68) *	98.50 96.34 – 103.58)
sEMMPRIN [pg/ml]	871.93 (854.86 – 906.07) *	1175.03 (1150.55 – 1211.54)
sMMP-7 [ng/ml]	2.23 (2.17 – 2.91) *	2.97 (2.27 – 3.05)
sTGFbeta1 [ng/ml]	1221.99 (1195.0–1242.9) *	1738.88 (1717.61–1760.18)
sCreatinine [mg/dl]	0.69 (0.64 – 0.76) *	2.55 (1.3 – 3.7)
uSurvivin [ng/ml]	41.49 (38.21 – 45.42) *	86.50 (80.85 – 93.84)
uE-cadherin[ng/ml]	3.34 (3.17 – 3.42) *	6.54 (6.30 – 6.67)
uEMMPRIN [pg/ml]	394.39 (375.02 – 413.56) *	800.42 (767.25 – 849.63)
uMMP-7 [ng/ml]	1.05 (1.02 – 1.11) *	2.36 (2.29 – 2.38)
uTGFbeta1 [ng/ml]	48.37 (45.96 – 49.98) *	195.10 (192.39 – 199.62)
uCreatinine [mg/dl]	114.00 (100.00 – 126.00) *	74.97 (60.0 – 82.0)

TAB. 3

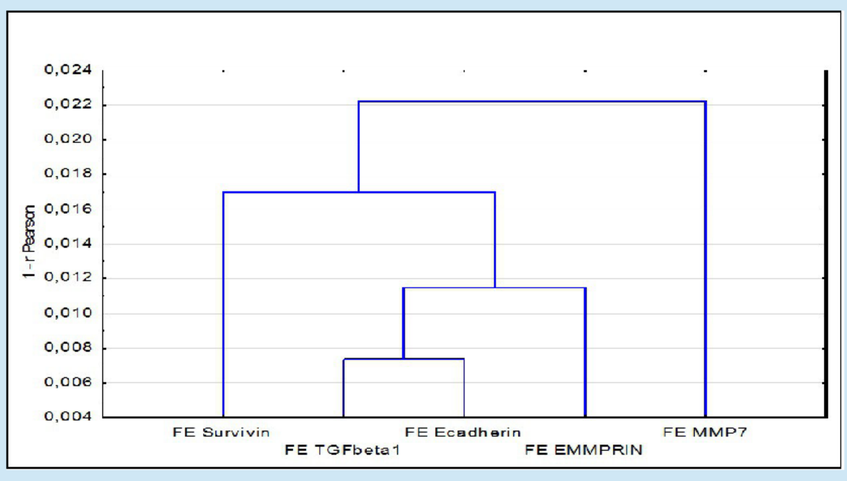
Fractional excretion of examined parameters in CKD children and controls.

Fractional excretion (FE) of analyzed parameters	Median values (lower – upper quartile)	
	Control group (n = 23)	**CKD (n = 41)**
FE survivin [%]	0.73 (0.58 – 0.75) *	1.99 (1.46 – 3.30)
FE E-cadherin[%]	0.07 (0.06 – 0.07) *	0.16 (0.10 – 0.28)
FE EMMPRIN [%]	0.31 (0.28 – 0.31) *	1.56 (1.07 – 2.86)
FE MMP-7 [%]	0.25 (0.23 – 0.29) *	1.89 (1.24 – 4.16)
FE TGFbeta1 [%]	0.02 (0.02 – 0.03) *	0.29 (0.17 – 0.55)

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

FIG. 1

The cluster analysis of fractional excretion (FE) values of examined parameters in children with CKD



AIM OF STUDY:

1) analysis of both known and new players engaged in epithelial-mesenchymal transition by assessing the concentrations of MMP-7, EMMPRIN, TGF beta 1, E-cadherin and survivin in the serum and urine of children with CKD stages 3 to 5
2) assessment of the fractional excretion of MMP-7, EMMPRIN, TGF beta 1, E-cadherin and survivin in children with CKD stages 3 to 5 and in the control group
3) analysis of the potential relations between those parameters
4) assessment of their applicability as markers of the CKD-related phenomena, like tubular damage and fibrosis

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 room temperature for 10 minutes, and then serum was stored at -20 deg until assayed. Urine was collected aseptically from the first morning sample, centrifuged at room temperature for 10 minutes and then stored at -20 deg until assayed.

The serum and urine concentrations of survivin, E-cadherin, EMMPRIN, MMP-7 and TGFβ1 were evaluated by commercially available ELISA kits (survivin - R&D Systems, reagent kit DSV00; E-cadherin - R&D Systems, reagent kit DCADE0; EMMPRIN - R&D Systems, reagent kit DEMP00; MMP-7 - R&D Systems, reagent kit DMP700; TGFβ1 - R&D Systems, reagent kit DB100B). Measurements were performed according to the manufacturer's instructions, results were calculated by reference to standard curves.

The fractional parameter excretion was calculated according to the formula: ([parameter urine concentration]x[serum creatinine concentration])/([parameter serum concentration]x[urine creatinine concentration])x100%.

The 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

Fractional urinary excretion of survivin, E-cadherin, EMMPRIN, MMP-7 and TGFbeta1

The median serum and urine concentrations of all examined parameters were elevated in the CKD patients vs. controls (table 2). The values of fractional urinary excretion in all cases were significantly elevated in the CKD children when compared to the control group (table 3). In particular, the FE values of E-cadherin and TGFbeta1 were below 1% both in controls and in the CKD group, whereas FE survivin, FE EMMPRIN and FE MMP-7 have raised from below 1% in the control group to the values exceeding 1% in the CKD children (table 3).

Correlations and cluster analysis

All FE values correlated significantly with each other ($r > 0.96$; $p < 0.000001$). Additionally, cluster analysis has revealed the similarity of features pictured by EMMPRIN, E-cadherin and TGFbeta1, suggesting that their efficiency as markers is comparable and choosing one out of 3 would be enough to get the required information (figure1).

None of the analyzed parameters correlated with analyzed biochemical markers or proteinuria.

CONCLUSIONS

1. *Fractional excretion of the examined markers has turned out a useful tool in assessment of tubular dysfunction in the course of chronic kidney disease.*
2. *The FE survivin, EMMPRIN and MMP-7 may be considered new independent markers of the kidney-specific EMT.*

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