

## LOW MOLECULAR WEIGHT PROTEINURIA IN CHILDREN WITH DISTAL RENAL TUBULAR ACIDOSIS

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### ABSTRACT

Distal renal tubular acidosis (dRTA) (MIM #267300, #602722 and #179800) is a rare inherited tubulopathy characterized by the inability of the distal tubule to acidify the urine with consecutive systemic acidosis. The clinical features include polyuria, polydipsia, poor appetite, failure to thrive, short stature and rickets. Prominent biochemical features are hypokalemia, hypercalciuria and hypocitraturia. There are reports on patients who presented with unusual biochemical features such as low molecular proteinuria, hypophosphatemia, hypouricemia, generalized hyperaminoaciduria, hyperoxaluria and other making diagnostic confusion to the clinicians. In this work, we report on a series of 8 children with clinically, biochemically and genetically proven dRTA who present with low molecular proteinuria at the disease onset. With metabolic compensation of the disease, there was complete resolution of the low molecular weight proteinuria and other proximal tubular abnormalities in all children. Late recognition of the disease with long standing hypokalemia and acidosis may result in abnormal expression and function of the transporters in the proximal tubules. Sodium dodecyl sulphate polyacrylamide gel electrophoresis is an accurate method for detection and follow up of patients with low molecular weight proteinuria.

**Keywords:** distal renal tubular acidosis, low molecular weight proteinuria, SDS-PAGE

### INTRODUCTION

Distal renal tubular acidosis (MIM #267300, #602722 and #179800) is a rare inherited tubulopathy characterized by the inability of the distal tubule to acidify the urine with consecutive systemic acidosis. The clinical features include polyuria, polydipsia, poor appetite, failure to thrive, short stature and rickets. Prominent biochemical features are hypokalemia, hypercalciuria and hypocitraturia. Ultrasound investigation of the kidneys reveals presence of medullary nephrocalcinosis and stones. The disease may be associated with early or late sensorineural hearing loss depending on the

mutated H<sup>+</sup>ATPase genes. [1, 2, 3] There are reports on patients who presented with unusual biochemical features such as low molecular proteinuria, hypophosphatemia, hypouricemia, generalized hyperaminoaciduria, hyperoxaluria and other making diagnostic confusion to the clinicians. [4, 5, 6, 7] We have already reported two infants with atypical presentation of dRTA, who showed signs of proximal renal tubular dysfunction. In this paper, we present a small series of Macedonian children with dRTA with emphasis on the presence of low molecular proteinuria at diagnosis. [4]

## PATIENTS AND METHODS

The diagnosis of dRTA was established on the basis of systemic acidosis ( $\text{HCO}_3^- < 20 \text{ mmol/l}$ ) with simultaneous inappropriate high urine pH determined with an electrode ( $\text{pH} > 5.30$ ). All children underwent thorough physical examination with measurement of the weight and height and searching for signs of rickets. Audiometry was performed for assessment of the hearing status. In infants, brainstem auditory evoked potential study was performed. The kidneys were evaluated with ultrasound scanning for the presence of nephrocalcinosis and stones.

Standard biochemistry included complete blood count, serum electrolytes, uric acid, acid base status, alkaline phosphatase and parathormone. Twenty four hour urine samples were obtained for assessment of urine electrolyte excretion, uric acid, citrate, oxalate, amino acids and low molecular weight proteins. Hypercalciuria was defined as  $>4 \text{ mg/kg/d}$ , hyperoxaluria  $>0.5 \text{ mmol/1.73m}^2/\text{d}$ , hypocitraturia  $<220 \text{ mg/d}$ . In infants and non-toilet trained children, spot urine samples were obtained in which the ratio of calcium/creatinine, oxalate/creatinine and citrate/creatinine were determined and compared with referent values for the age. [8, 9, 10]

Low molecular weight proteinuria was assessed with SDS-PAG electrophoresis according to the method described by Görg et al. [11] In addition, single urinary markers such as beta-2 microglobulin, alfa-1 microglobulin were measured and compared to referent values. [12, 13, 14]

Genetic tests for  $\text{H}^+$  ATP-ase genes (ATP6V1B1 and ATP6V0A4) and anion exchanger 1 gene (SLC4A1) were performed in referent laboratories.

This study is a part of the PhD thesis (S.S) and was approved by the Ethical Committee of the Medical School Skopje. All participants signed the informed consent.

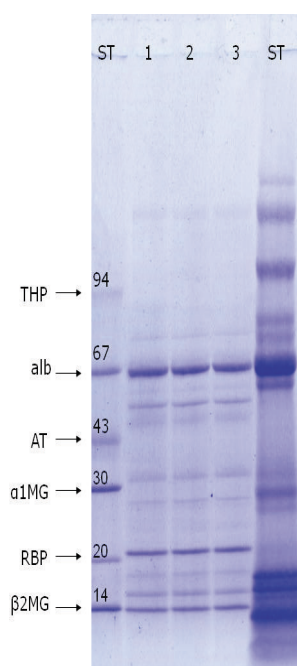
## RESULTS

Eight children were included in this study. Their basic demographic, clinical and biochemical data are presented in table 1. All fulfilled diagnostic criteria for distal RTA. Six children

had nephrocalcinosis, one child had single kidney stone and one child had normal kidney ultrasound scan. Molecular diagnosis was confirmed in 7 patients, only one patient was tested negative for the respective genes. Low molecular proteinuria was present in all six children. It was characterized with the presence of fractions with the size ranging from 10-67 kD (figure 1). It correlated with the presence of abnormal urinary levels of beta-2 microglobulin and alfa-1-microglobulin where tested. In seven children there were additional serum and urine abnormal parameters as a result of proximal tubular dysfunction. In one girl proximal tubular dysfunction was so severe so that initially she was considered to have Fanconi syndrome. Low molecular proteinuria resolved in all children after one to eight months, as well as other signs of proximal tubular dysfunction (hyperaminoaciduria, hypophosphatemia, hyperphosphaturia, hypouricemia, hyperuricosuria).

**Table 1.** Clinical biochemical and genetic features of 8 children with distal renal tubular acidosis

Patient	Sex	Age of diagnosis (years)	Kidney US	LMWP	B2M/ALMG	P (mmol/l)	UA (umol/l)	AA-uria	oxaluria	citraturia	Mutated gene
GV	M	5	NC	Y	?/?	1.47	154	?	?	?	ATP6V1B1
GA	M	3	NC	Y	?/?	1.07	145	?	?	?	ATP6V1B1
SM	F	2	NC	Y	?/ND	0.88	108	N	N	?	ATP6V1B1
GN	M	5	N	Y	?/ND	0.93	108	N	N	?	SLC4A1
HX	M	3	Stone	Y	ND/ND	0.7	111	?	?	?	SLC4A1
SB	M	0.8	NC	Y	ND/ND	1.55	73	?	ND	ND	ATP6V1B1
KF	F	5	N	Y	?/?	0.68	66	?	?	?	No mutation
BU	F	1.5	NC	Y	?/ND	0.98	73	ND	ND	ND	ATP6V1B1



**Figure 1.** Low molecular proteinuria in a patient with distal renal tubular acidosis.

Legend to figure 1. ST-standard; Lane 1,2,3 patient's elepherograms in triplicate; THP-Tamm Horsfall protein; alb-albumin; AT-antitrypsin; α1MG-alpha1 microglobulin, RBP-retinol binding protein. B2MG-beta2 microglobulin

## DISCUSSION

In this work we studied the presence of low molecular weight proteins in children with clinically, biochemically and genetically well defined distal renal tubular acidosis. All children had LMWP at diagnosis and this correlated with increased urinary levels of beta-2 microglobulin and alfa-1 microglobulin. It is of note that sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a relatively old method for differentiation of proteinurias but unfortunately neglected in the nephrological practice. [15] It was implemented in the clinical practice in the 80s and 90s of the previous century and was pretty popular in Germany. [16, 17, 18] This method enables differentiation between tubular and glomerular proteinuria and further analysis (selective, non-selective glomerular), tubular (complete, incomplete) or mixed glomerulo/tubular. We have successfully used it for differentiation of glomerular from non-glomerular hematurias and for analysis of functional proteinurias (orthostatic, exercise induced proteinuria). [19, 20, 21] Analysis of the elepherograms enables pan-

oramic investigation of various fractions according to their molecular weight and more subtle characterization of proteinuria (e.g. non selective glomerular with incomplete tubular proteinuria). The investigation of single urinary proteins may be helpful but offers limited information. The combination of 3 or more markers may be more sensitive for definition of the proteinuria type, but increases the costs and makes the analysis difficult for clinicians without experience in urinary protein biochemistry. Therefore, several expert (computerized) systems were developed to enhance the analysis of the data. Lun et al analyzed the values of different expert systems for characterization of proteinuria in children with various renal diseases. [22] They found that the UPES system has high accuracy for detection of LMWP proteinuria; in 19 children with clear clinically and pathologically defined tubulointerstitial diseases they detected tubular proteinuria in 16/19 and in additional 3/19 mixed glomerulo-tubular proteinuria.

We used SDS-PAGE to follow our children with dRTA. With metabolic compensation there was disappearance of LMWP in the period between 2 and 8 months. The presence of LMWP proteinuria may raise differential diagnostic dilemma with Dent's disease in which there is low molecular proteinuria, hypercalciuria and mild acidosis. [23] Dent disease affects males and LMWP proteinuria is persistent and not amenable to any treatment.

The majority of children in our series were diagnosed late. It seems that long lasting hypokalemia and acidosis affect the expression and function of various transporters in proximal tubules resulting in compromise of reabsorption of low molecular weight proteins, phosphate, uric acid and aminoacids. [24] In addition, we and others have evidenced hyperoxaluria at the disease onset, its mechanism has not yet been clarified. [4, 6]

## CONCLUSIONS

Herein we presented a series of 8 children with dRTA who present with low molecular proteinuria at the disease onset. It seems that late recognition of the disease with long standing hypokalemia and acidosis may result in abnormal expression and function of the transporters in the proximal tubules. With metabolic compensation

of the disease, there is complete resolution of the low molecular weight proteinuria and other proximal tubular abnormalities. SDS-PAGE electrophoresis is an accurate method for detection and follow up of patients with LMWP.

### Acknowledgment

We wish to thank Dr. Hae Il Cheong (Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Korea) for mutational analysis of the ATP6V1B1 and ATP6V0A4 genes and to Dr. John A Sayer (Institute of Genetic Medicine, Newcastle University, Newcastle, United Kingdom) for mutational analysis of the SLC4A1 gene.

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## Резиме

### НИСКО МОЛЕКУЛАРНА ПРОТЕИНУРИЈА КАЈ ДЕЦА СО ДИСТАЛНА РЕНАЛНА ТУБУЛСКА АЦИДОЗА

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Дистална ренална тубулска ацидоза (дРТА) (MIM #267300, #602722 и #179800) е ретка наследна тубулопатија, која се карактеризира со неспособност на дисталниот тубул да ја ацидифицира урината и консеквативна системска ацидоза. Клиничката слика вклучува полиурија, полидипсија, намален апетит, слабо напредување, низок раст и рахитис. Истакнати биохемиски црти се хипокалемија, хиперкалциурија и хипоцитратурија. Постојат презентирани описи на пациенти со невообичаени биохемиски знаци, какви што се нискомолекуларна протеинурија, хипофосфатемија, хипоурикемија, генерализирана хипераминоацидурија, хипероксалурија и други, кои водат до дијагностичка конфузија кај клиничарите. Во овој труд ние прикажуваме серија од осум деца со клинички, биохемиски и генетски докажана дРТА, кои се презентираа со нискомолекуларна протеинурија на почетокот на болеста. Со метаболичка компензација на болеста дојде до комплетна резолуција на нискомолекуларната протеинурија и на другите проксимални тубулски абнормалности кај сите деца. Доцното препознавање на болеста, долготрајната хипокалемија и ацидозата резултираа со абнормалната експресија и функција на транспортерите во проксималните тубули. Натриум додецил сулфат полиакриламид гел електрофорезата е сигурен метод за детекција и за следење на пациентите со нискомолекуларна протеинурија.

**Клучни зборови:** дистална ренална тубулска ацидоза, нискомолекуларна протеинурија, SDS-PAGE