

Incidental Detection of Dent-2 Disease in an Infant with Febrile Proteinuria

Shpetim Salihu^a Katerina Tosheska^b Svetlana Cekovska^b Velibor Tasic^c

^aDepartment of Neonatology, University Clinical Center, Prishtina, Kosovo; ^bInstitute of Medical and Experimental Biochemistry, Medical School Skopje, Skopje, Macedonia; ^cUniversity Children's Hospital, Medical School Skopje, Skopje, Macedonia

Significance of the Study

- Persistence of proteinuria after resolution of fever requires its quantification and SDS-PAGE is an accurate screening tool for low molecular weight proteinuria. The presence of low molecular weight proteinuria and hypercalciuria is a diagnostic clue for Dent disease.

Keywords

Functional proteinuria · SDS-PAGE · Dent-2 disease · Infants · OCRL mutation

Abstract

Objective: Febrile proteinuria is functional proteinuria and is seen as a transitory phenomenon during acute febrile illness, mainly viral infections. It is a benign phenomenon and clears promptly with resolution of the infection. **Clinical Presentation and Intervention:** In this report, we present a patient who was thought to have febrile proteinuria. Persistence of significant proteinuria after resolution of the infection prompted biochemical and genetic workup which led to the diagnosis of Dent-2 disease. **Conclusion:** We recommend the use of SDS-PAGE (sodium dodecyl sulfate electrophoresis) for the detection of low molecular weight proteinuria.

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Published by S. Karger AG, Basel

Introduction

Increased urinary excretion of proteins is an important marker for kidney damage. Analysis of the pattern of proteinuria gives clinicians insights into the pathophysiologic changes in the affected nephrons [1, 2]. The upper limit of excretion of daily protein excretion is 150 mg/24 h with albumin as the dominant fraction. Pathologic proteinuria is classified as glomerular, tubular, mixed, prerenal, or postrenal. Tubular proteinuria is characterized by increased urinary levels of low molecular proteins such as β_2 -microglobulin, α_1 -microglobulin, and retinol-binding protein [1]. Febrile proteinuria is functional proteinuria and is seen as a transitory phenomenon during acute febrile illness, mainly viral infections. It is a benign phenomenon and clears promptly with resolution of the infection. Here, we present a patient who was initially suspected to have febrile proteinuria. Persistence of significant proteinuria after resolution of the infection prompted us to characterize the proteinuria by biochemical methods. We wish to emphasize the role of SDS-PAGE (sodium dodecyl sulfate

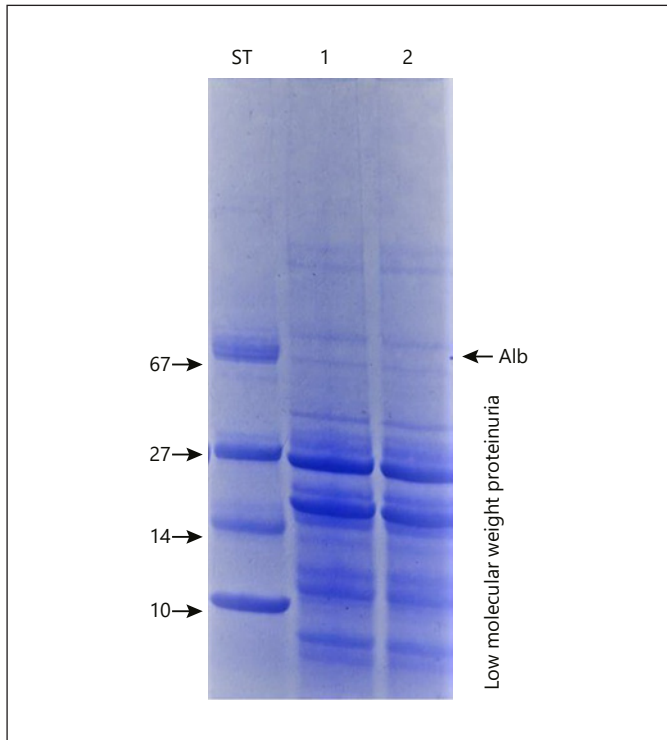


Fig. 1. The patient's electropherograms (lanes 1 and 2) reveal low molecular weight proteinuria. ST, standard (molecular weight in kilodaltons); Alb, albumin.

electrophoresis) for the detection of low molecular weight proteinuria. Further biochemical and genetic workup will help in the diagnosis of Dent-2 disease.

Case Report

A male 22-month-old infant was admitted to the Children's Hospital due to aphthous stomatitis. His body temperature was 40 °C, pulse rate 126 beats/min, and respiration 24 breaths/min. There were no pathological murmurs, and auscultation of the lung did not reveal any pathological sounds. His mucous membranes were dry and several aphthous changes were seen in the oral cavity. The child was given parenteral fluids and antipyretics. His blood analysis showed normal white blood cell count and C-reactive protein was 3 mg/L. The clinical features and normal inflammatory parameters suggested a viral infection. Urinary examination revealed proteinuria 1–2+ and this was considered as functional due to the high fever. After 4 days, the infant was discharged afebrile with improved oral intake of fluids. The last urinary examination showed protein 1+ and advice was given to his pediatrician to recheck urinalysis.

Ten days later the infant was referred to our outpatient clinic due to persistent proteinuria. The dipstick analysis showed pro-

teinuria 2+ (1.0 g/L), and negative results for blood, leukocyte esterase, nitrites, ketones, glucose, bilirubin, and urobilinogen. The specific gravity was 1.020 and pH 6. The urinary sediment showed 2–3 red blood cells per high-power field. Serum electrolytes were as follows: Na 142 mmol/L, K 4.3 mmol/L, Ca 2.44 mmol/L, Cl 102 mmol/L, P 1.67 mmol/L, Mg 0.88 mmol/L, acid-base status pH 7.39, HCO₃ 23.5 mmol/L, and base excess +1.2 mmol/L. The proteinogram revealed normal results (total protein 76 g/L, albumen 40 g/L). The degradation products were as follows: creatinine 36 μmol/L (estimated glomerular filtration rate according to the new Schwartz formula 121 mL/min/1.73 m²), urea 2.5 mmol/L, and uric acid 176 μmol/L. Protein creatinine ratio was increased at 98 mg/mol (normal <20). SDS-PAGE analysis of the urinary proteins showed dominant low molecular weight fractions from 14 to 67 kD (Fig. 1). His urinary β₂-microglobulin was increased at 33.5 mg/g creatinine (normal <0.13). This prompted further analysis for urinary calcium excretion which revealed hypercalciuria (2.5 mmol/mmol creatinine, normal <2.2). Kidney ultrasound examination showed two normal-sized kidneys. There was no evidence for kidney stones or nephrocalcinosis. Serum biochemistry revealed increased levels of LDH (656 U/L) and CPK (496 U/L), suggesting Dent-2 disease. The family history was negative for stones and chronic kidney diseases. Ultrasound screening of the first-grade relatives did not show evidence for nephrolithiasis or nephrocalcinosis. Urinary testing for blood and protein in the relatives did not show abnormal findings.

The diagnosis of Dent-2 disease was confirmed by mutational analysis of the OCRL gene, resulting in the detection of pathogenic mutation [3]. The detected mutation was c.del261_264del TGTT (p.C87X). This mutation is predicted to produce a truncated protein as a result of a 4-base deletion, which would produce a frameshift and premature termination of transcription. The mutation was inherited from the patient's mother. His growth and motoric and neurocognitive development were according to his chronological age. Ophthalmologic and neurologic examination did not show any abnormality, excluding the diagnosis of oculocerebrorenal syndrome.

Discussion

Dent disease is a rare X-linked inherited tubulopathy which is diagnosed on the basis of the presence of low molecular weight proteinuria and hypercalciuria. The disease has great clinical variability from isolated low molecular weight proteinuria in Japanese patients to severe kidney disease with nephrocalcinosis, stones, and progression to end-stage renal disease [4]. Approximately 60% of patients have Dent-1 disease (OMIM300009), whereas 15% have mutations in *OCRL1* (Dent-2 disease; OMIM300555). It appears that other unidentified genes are responsible for 25% of patients. Dent disease is often underdiagnosed, since characterization of proteinuria is neglected in nephrologic patients [5]. The phenotype often overlaps with other proteinuric chronic kidney diseases; the diagnosis is established late after failed im-

mune suppression treatment for presumed glomerular disease.

Dent-2 patients have increased levels of CK and LDH in 100% of patients. This may be a clue for a diagnostic molecular strategy since a minority of Dent-1 patients have elevations of these enzymes [6–8].

Dent-2 disease should be differentiated from oculocerebrorenal syndrome of Lowe, which is a multisystem disorder characterized by congenital cataracts, hypotonia, severe neurologic deficits, intellectual disability, and renal Fanconi syndrome [9, 10]. Mild intellectual disability and peripheral cataracts may be seen in some patients with Dent-2 disease, therefore one should consider both entities within the spectrum of OCRL mutations.

The pathophysiology of proteinuria in Dent disease is very complex. In Dent-1 patients, ClC-5 (encoded by CLCN5) is expressed in proximal tubules and co-distributes with the vacuolar H⁺-ATPase (V-ATPase) in early endosomes which are responsible for the reabsorption and processing of the filtered albumin and low molecular weight proteins. These vesicles belong to the receptor-mediated endocytic pathway, which involves the multi-ligand receptors, megalin and cubilin. ClC-5 is important for the endosomal Cl conductance; defective ClC-5 would impair vesicular acidification, causing dysfunction of proximal tubular cells resulting in proteinuria.

In Dent-2 patients OCRL mutations lead to loss or dysfunction of the OCRL1 protein. OCRL1 is localized to lysosomes in renal proximal tubular cells and this localization is consistent with the role of OCRL1 in lysosomal enzyme trafficking. OCRL1 interacts with clathrin which enhances transport of the vesicles from the Golgi apparatus to the endosomes. OCRL1 also plays a role in the early endocytic pathway, by interacting with the Rab5 effector APPL1. This abnormality is similar to that observed in Dent-1 disease, and it seems that Dent disease, therefore, may be due to abnormalities in either endosomal acidification and sorting, or lysosomal trafficking.

We diagnosed our patient in early infancy due to the persistence of proteinuria. The initial step in the evaluation of a child with proteinuria is the identification of the type of proteinuria (e.g., glomerular, tubular, mixed, selective/nonselective). The panoramic presentation of the urinary protein fractions obtained with SDS-PAGE is according to their molecular weight, which enables clinically valuable classification of proteinuria. In this case we did not have any clue to the diagnosis of tubulopathy (negative family history, normal kidney ultrasound, no serum and urinary abnormalities which could indicate renal tubular dysfunction). The abnormal electrophero-

gram showing dominant low molecular protein fractions guided us to extend further investigations towards tubular disorders. Measurement of urinary β_2 -microglobulin level confirmed the SDS-PAGE finding.

β_2 -microglobulin is an established urinary marker for diagnosis and follow-up in children with tubulointerstitial disorders. Precaution should be taken for proper conservation of the urine. As a single marker urinary β_2 -microglobulin cannot classify the type of proteinuria. Some children with Dent disease may also have significant glomerular proteinuria and histologic lesion of focal segmental glomerulosclerosis [5]. In this respect SDS-PAGE of urinary proteins or a combination of urinary protein markers (e.g., Urinary Protein Expert System) can accurately classify the type of proteinuria. In addition, laser densitometry enables the quantification of glomerular and tubular fractions, which is very important for the follow-up of these patients.

Functional proteinuria is often encountered in pediatric practice, and clears with resolution of the fever and infection. Several theories have attempted to clarify the mechanism of febrile proteinuria. It is unlikely that transitory proteinuria is the response of the kidney to the infectious agent because increased excretion of protein is evident in patients with hyperthermia without obvious infection. In addition, injection of adrenal hormones may result in transitory proteinuria. The plausible explanation is that the stress induced by the fever would result in the release of adrenal hormones and subsequent proteinuria.

Conclusion

Persistence of proteinuria in infancy requires its quantification and characterization even if it is low grade proteinuria. SDS-PAGE is a reliable screening tool for low molecular weight proteinuria, as seen in our patient, and enabled the establishment of an early and correct diagnosis.

Statement of Ethics

This work was done as part of a PhD thesis (S.S) and was approved by the Ethics Committee of the Medical School Skopje. Informed consent was obtained from the parents.

Disclosure Statement

The authors declare no conflicts of interest.

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