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Case Report

Acute kidney failure and discrepant values of urinary proteins: When the case is not “crystal clear”

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1. Case description

A 59-year-old woman was referred to the nephrology department of our Hospital from her general practitioner with acute renal function impairment. She had a history of well–controlled high blood pressure and seronegative arthropathy. There was no family history of any nephro-urological condition. The initial serum creatinine concentration was 185 μmol/L (2.10 mg/dL) with a normal serum uric acid. Chemical screening of the urine using reagent strips was negative for proteins and showed no abnormalities. In a couple of weeks, a sudden rise to 456 μmol/L (5.16 mg/dL) of serum creatinine was noted. A 24-h urine sample was collected and sent to the Laboratory for total proteins quantification in our automated analyzers. A non-nephrotic proteinuria was found (0.7 g/24 h) with negative urine albumin, while multiple reagent strips tests were negative for proteins. On urine immunofixation, a modest proteinuria with no monoclonal component (ratio of light chain kappa to lambda of 3.51) was the only noticeable finding. Serum complement factors were within the reference range. The patient was admitted to the Hospital for further studies. Renal ultrasonography showed both kidneys were normal in size and structure and identified a mild increase of their echogenicity at cortical level. There was no evidence of perinephric collections and/or dilation of the renal pelvis and/or calyces as a result of obstruction. The bladder was half-filled and had no abnormalities. Echography showed no free peritoneal liquid. Given the discrepancies in the two testing methods for urine proteins, a microscopic evaluation of the urine sediment was performed. It was hoped that this would clarify whether proteins were present or not. But the only significant discovery was the presence of rare, small and round crystals in abundance.

2. Discussion

Up to 150 mg of protein is excreted in the urine daily varying from 2 to 10 mg/dL depending on urine volume. A persistent excretion of 200 mg of protein a day could be considered a marker of kidney failure. Urine basically consists of two groups of proteins: One third is albumin, a plasma protein that passes through the glomerular basement but is usually reabsorbed by proximal tubular cells. In normal conditions, another third is uromodulin or Tamm - Horsfall glycoprotein (secreted by distal tubular cells and cells of the ascending loop of Henle). Apart from those, urine also contains proportions of globulins, low molecular weight proteins and light chains [1].

Given that protein reabsorption has a very low maximal tubular rate, the detection of an abnormal amount of proteinuria is an important indicator of kidney failure when increased filtration of proteins quickly saturates reabsorption mechanisms. Several screening methods and quantitative methods have been proposed for their analysis. It has been suggested that screening methods used to detect an abnormal protein excretion should not detect less than 8–10 mg/dL in a healthy adults with normal rate of urine flow.

Common screening tests include the semiquantitative colorimetric reagent strip test. Reagent strips (or dipstick) method takes advantage

Abbreviations: PRM, Pyrogallol Red Molybdate; AKF, Acute kidney failure; DHA, 2,8 – dihydroxyadenine; APRT, adenine phosphoribosyltransferase; AMP, adenine monophosphate

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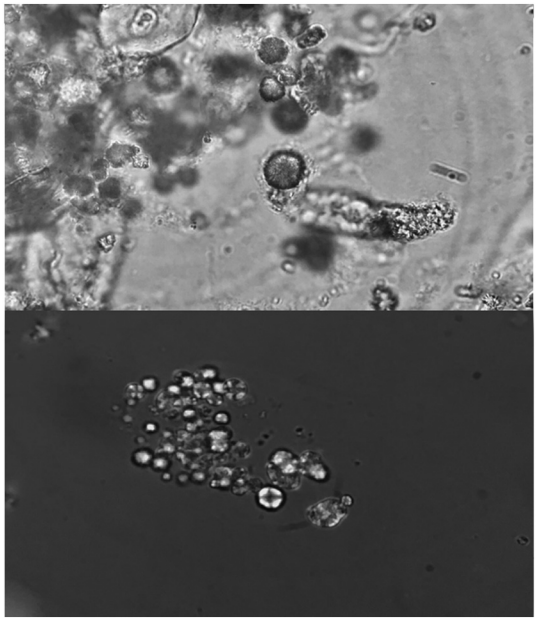

Fig. 1. Small, round, 2,8 – dihydroxyadenine (2,8 – DHA) crystals observed without and with polarized light microscopy (central Maltese cross pattern) in urine sediment.

of the protein error of pH indicators to produce a visible colorimetric reaction at constant pH. Depending on the manufacturer, the reagent strip is normally impregnated with pH–sensitive dyes like tetrabromophenol blue buffered to a pH 3 (our current method for dipsticks). In the absence of protein, the strip remains yellow, however, as the degree of proteinuria increases, the color progresses from green to blue. Results are normally reported in terms of semi-qualitative values based on color changes. Despite the fact that high salt levels will lower results, this method is unaffected by turbidity, radiographic media and most drugs or metabolites. Unfortunately, reagent strips appear to be more sensitive to albumin than to globulins [2]. Positively charged proteins like immunoglobulins may not be picked up even when their concentrations are high. Most dipstick methods have a lower limit of detection of 15 mg/dL for urinary proteins.

Due to this lack of sensitivity to globulins, acid proteins precipitation methods may be necessary for screening/quantification purposes. In this method, acid (e.g. Sulfosalicylic acid, Trichloroacetic acid) is added to the urine and proteinuria is quantified by measuring the turbidity caused by protein precipitation in cold. Based on the assay employed the sensitivity may be as low as 0.25 mg/dL although, highly buffered alkaline urines may cause false negative results.

Additionally, there are several dye-binding colorimetric methods used to quantify proteinuria such as Coomassie blue and Pyrogallol Red – Molybdate (PRM). This last one (the method that we perform in our lab for protein quantification), where amino groups of urinary proteins react to form a bluish-purple complex that absorbs at 600 nm, is commonly used in most clinical labs because of its sensitivity (up to 0.7 mg/dL in our lab), precision and practicability [3,4].

The patient presented herein, developed acute kidney failure (AKF) with non – nephrotic proteinuria that was not detected using screening methods (dipsticks). The discrepancy between dipstick and PRM methods may be explained by their differences of sensitivity to urinary proteins. Besides, it should be noted that reagent strips use random urine specimens that may have a falsely low protein value because of sample dilution. In fact, if an intermittent or transient proteinuria is suspected, repeat measurements may be needed.

In our patient, it seemed quite obvious that the modest amount of urinary protein measured was not due to albumin, ruling out a glomerular pattern as the main cause of proteinuria. The question therefore becomes: which protein (or compound) were we detecting using the PRM method?

The answer came from the examination of the urine sediment. Although all the dipstick results were negative for our patient, the presence of discrepant results for urinary proteins by the two methods and the lack of an explanation for her sudden AKF, were decisive factors in performing a microscopic evaluation of the urine. Small, round, brown crystals with a central Maltese cross pattern on polarized light microscopy were found in abundance (Fig. 1). These crystals were consistent with 2,8–dihydroxyadenine (2,8–DHA) crystals which is a pathognomonic feature of adenine phosphoribosyltransferase (APRT)

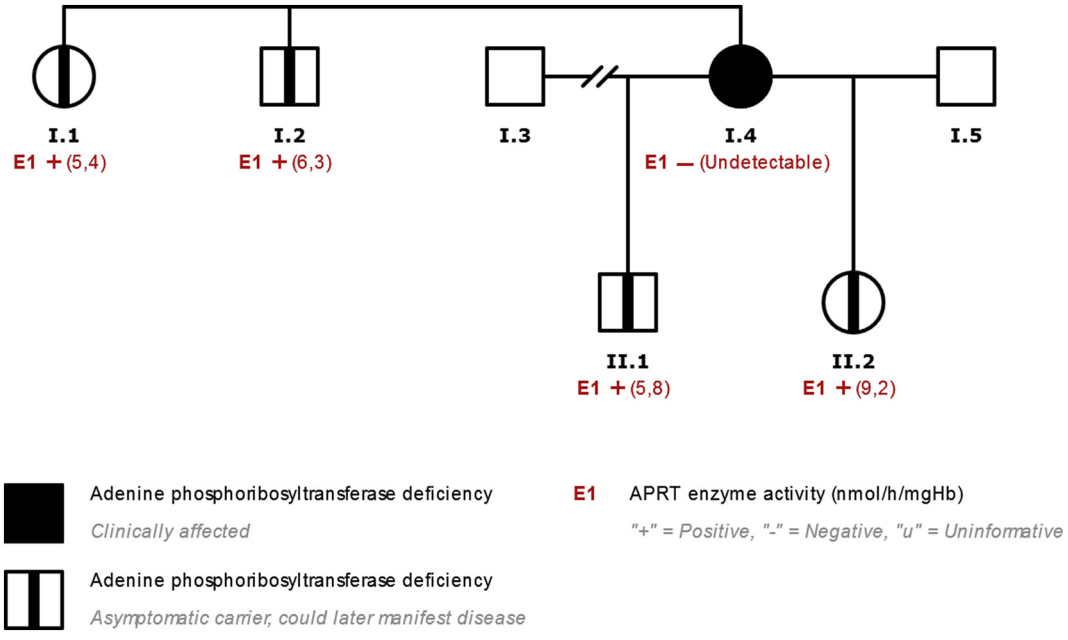

Fig. 2. Familial genetic and metabolic study.
I.4 Patient; I.1 and I.2 Siblings; II.1 and II.2 Children. Circle: Woman; Square: Man. E1: APRT enzyme activity in brackets (normal reference value: 19–38 nmol/h/mg Haemoglobin).

deficiency. The modest proteinuria detected could be explained by the fact that 2,8–DHA molecules have amino groups that may also react with PRM method but show less chromogenicity than albumin or globulins.

Deficiency of APRT is a rare autosomal–recessive disorder that causes a recurrent crystalline nephropathy and urolithiasis. Progression to renal insufficiency is a real risk in homozygotes who are totally deficient in the enzyme [5,6]. 2,8–DHA is the end-product of the adenine oxidation by xanthine oxidase and is insoluble in urine at any pH producing crystalluria. The deficiency of APRT causes the adenine produced during purine metabolism to accumulate and metabolized into 2,8–DHA, rather than return to the adenine monophosphate (AMP) pool. Several studies have highlighted that APRT deficiency can present at any age. In fact, age of diagnosis varies from infancy to more than 70 years due to the lack of symptoms for decades or because patients remain misdiagnosed despite recurrent nephrolithiasis. At the time of diagnosis, decreased renal function had been detected in one third of the patients from several European and Japanese cohorts [7]. Our patient had her first urolithiasis episode in her fifth decade of life. There were no other events in her past medical history that suggested APRT deficiency.

Given that the condition is treatable, early diagnosis is crucial in reducing the morbidity and the progression of the illness. 2,8 – DHA's lack of solubility at pH 7 makes urine alkalization useless and not recommended. However, a low purine diet, has been shown to be beneficial. Allopurinol and Febuxostat, both xanthine oxidase inhibitors, are the treatment of choice. In fact, based on our laboratory findings, our patient was immediately prescribed allopurinol which reduced serum creatinine levels and crystalluria in five months.

The enzyme APRT activity was measured in a red cell haemolysate in our patient, her children and her siblings (normal reference value: 19–38 nmol/h/mg Haemoglobin). Additionally, we performed genetic tests in the APRT gene located on chromosome 16q24 (Fig. 2). Our patient had an homozygotic substitution in exon 2 c.82 G > C that correlated with an undetectable activity of the enzyme (type I deficiency). Her children and her siblings had the same mutation but in heterozygote status, showing approximately 25% of normal APRT activity (type II deficiency) (Fig. 2) [8]. Although reduced, enzyme levels in these patients are adequate for adenine metabolism so they should not suffer from nephrolithiasis.

APRT deficiency has an estimated prevalence between 1/50,000 and 1/100,000 and remains an underdiagnosed condition due to the lack of awareness between clinicians and because of the difficulty of finding and identifying these rare radiolucent 2,8 – DHA crystals, sometimes confused with uric acid crystals [9].

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