False proteinuria in patients with alkaptonuria

Sirs,

Alkaptonuria (AKU) is a rare autosomal recessive disorder characterised by the inability to metabolise homgentisic acid (HGA). In the absence of HGA oxidase, HGA is rapidly oxidised to benzoquinone acetic acid, which polymerises to a melanin-like pigment and deposits in connective tissue. Musculoskeletal involvement (ochronosis) is the most serious complication of this condition (1-3). Affected individuals excrete HGA in the urine, causing a characteristic dark colour when the urine is alkalinised or oxidised. This metabolic impairment has not been associated with kidney diseases, except for an increased occurrence of stone formation (4). However, as kidneys play a critical role in eliminating HGA, preserving renal function is crucial in alkaptonuria patients. Conversely, altered renal function might lead to a faster progression of ochronosis (5).

Herein we describe the case of a 45-year-old woman affected by alkaptonuria/ochronosis, with asymptomatic, unexpected "*proteinuria*".

The patient was referred to our Department for a routine check-up. Her past history was unremarkable except for her metabolic disorder, diagnosed when she was 35 years old. Over the previous 4 years she had complained of a non-specific back pain and lumbar stiffness in the flexion-extension movements, due to ochronotic involvement of the spine. No peripheral joint involvement was observable. Brownish spots of the sclerae were also evident. She had been taking ascorbic acid 1gr daily and analgesics occasionally.

Biohumoral analyses including renal functional parameters and the urine dipstick test were within normal limits. However, 3 g/lt of protein were detected in the 24-hour urine sample using the benzethonium chloride method. On the basis of these conflicting results, a urine electrophoresis assay was performed and revealed that, in line with the dipstick test, proteins were absent. Renal ultrasonography did not show either parenchymal abnormalities or kidney stones. Similar results were recorded in another two ochronotic patients, who had 2.8 and 3.5g/lt of protein respectively in their 24-hour urine as measured by the benzethonium chloride method, but no protein according to the dipstick test or electrophoresis assay. In order to check the possibility of falsely elevated total urinary protein, a urine sample from a healthy volunteer was analysed by the dipstick and benzethonium chloride methods. In basal conditions both tests resulted negative for proteinuria. However, the addition of HGA (5mg/ml) to the same sample confirmed the absence of protein using the dipstick test, while the benzethonium chloride method found 1 g/lt of protein.

Taken together, these observations suggested that HGA might interfere with the benzethonium chloride assay. This widespread laboratory method relies on an increase in absorbance when urinary protein is precipitated by benzethonium chloride in an alkaline medium. To date, only one other report in literature describes the oxidation of HGA by the alkaline conditions in the benzethonium chloride method as causing a positive interference (6).

Our observation prompted us to draw attention to the fact that HGA might interfere with urinary protein assays, yielding false positive results. This occurrence should be kept in mind to avoid subjecting AKU patients to unnecessary invasive diagnostic procedures.

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References

1. ZANNONI VG, LOMTEVAS N, GOLDFINGER S: Oxidation of homogentisic acid to ochronotic pigment in connective tissue. *Biochem Biophys Acta* 1969; 177: 94-105.
2. SELVI E, MANGANELLI S, MANNONI A, BENUCCI M, MINACCI C, MARCOLONGO R: Chronic ochronotic arthritis: clinical, arthroscopic and pathologic findings. *J Rheumatol* 2000; 27: 2272-4.
3. IANNUCCELLI C, COARI G, MASTANTUONO M, OSIMANI M, VALESINI G, DI FRANCO M: Joint and tendineous involvement in ochronosis: clinical history and imaging of a case. *Clin Exp Rheumatol* 2009; 27: 696-7.
4. PHORNPHUTKUL C, INTRONE WJ, PERRY MB *et al*.: Natural history of alkaptonuria. *N Engl J Med* 2002; 347: 2111-21.
5. INTRONE WJ, PHORNPHUTKUL C, BERNARDINI I, MCLAUGHLIN K, FITZPATRICK D, GAHL WA: Exacerbation of the ochronosis of alkaptonuria due to renal insufficiency and improvement after renal transplantation. *Mol Genet Metab* 2002; 77: 136-42.
6. SYKES E, GIBSON M, DMUCHOWSKI C: Homogentisic acid interference in the measurement of urinary protein using benzethonium chloride. *Ann Clin Biochem*1996; 33: 86-8.