Factitious Proteinuria: Diagnosis and Protein Identification by Use of Isoelectric Focusing

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Factitious proteinuria is an unusual finding. We present a case in which clinical suspicion was aroused by the disparity between the clinical history and findings and the 24-h excretion of protein in urine. Electrophoresis of the patient's serum and urine confirmed the presence of an unusual protein. By isoelectric focusing we identified it as egg-white, a finding confirmed by immunofixation with antiserum to egg-albumen. In the past, confirmation of the identity of such a protein has required specific antisera for immunofixation or immunodiffusion. Such antisera may not always be available. However, isoelectric focusing gives sufficient resolution for positive identification of exogenous proteins, even in the presence of true proteinuria.

Additional Keyphrases: egg-albumen · forensic medicine

There are few reported cases of the addition of protein to urine specimens in an attempt to deceive. Mitas (1) made a presumptive diagnosis of factitious proteinuria based on clinical and biochemical findings, but identification of the exogenous protein was not attempted. Adner et al. (2) confirmed the presence of egg-white in a patient's urine by immunodiffusion. Irrefutable identification may be of value when the patient is confronted and in medico-legal situations, but it may be hindered by lack of suitable specific antiserum. We believed that the high resolution afforded by isoelectric focusing could be of value in this situation. We therefore describe the first case in which the exogenous protein has been so identified.

Case Report

A female patient was referred for investigation of proteinuria by her general practitioner. The patient's daily medication at the time of presentation consisted of warfarin, ranitidine 300 mg, furosemide 80 mg, apironolactone 100 mg, and up to eight "Distalgesic" tablets (each containing 32.5 mg of dextro-propoxyphene and 325 mg of acetaminophen). On examination the only abnormalities were from the scars of previous surgery and other injuries sustained beforehand. Urine testing with Ames "Labstix" (Miles Laboratories, Slough, U.K.) showed protein +++ (i.e., about 3 g/L) but no blood. Some initial relevant data were: hemoglobin 124 g/L, erythrocyte sedimentation rate 25 mm/h, plasma creatinine 0.112 mmol/L, urea 11.6 mmol/L, potassium 4.3 mmol/L, albumin 44 g/L, globuline 31 g/L. Subsequent investigations demonstrated on occasion a very intense proteinuria. Also there were large fluctuations in the 24-h urine volume and creatinine excretion (Figure 1). Subsequently, it was noted that the discrepancies between the measured creatinine clearances and those calculated from the patient's age, sex, weight, and plasma creatinine by use of a nomogram (3) were greater than expected. Plasma urea and creatinine concentrations were above normal and ranged from 10.1 to 13.4 mmol/L and 0.103 to 0.145 mmol/L, respectively. The greater increase in the plasma urea relative to the creatinine concentration suggested a degree of saline depletion (4). Despite the severe proteinuria, the albumin concentration in serum ranged between 41 and 49 g/L; the serum cholesterol concentration was 7.0 mmol/L. These results were suggestive of manipulation of the urine specimens by the addition of protein, which we then attempted to identify.

Materials and Methods

Electrophoresis: Urine proteins and a number of sources of possible exogenous protein were resolved on cellulose diacetate ("Electrafor"; Shandon Southern Products Ltd., Astmoor, Cheshire, U.K.), by applying 200 V for 30 min in barbitral buffer (50 mmol/L, pH 8.6), followed by fixing and finally staining with nigrosine.

Isoelectric focusing: For protein separation we used an agarose medium containing 0.33 g of "Agarose IEF" (Pharmacia AB, Uppeala, Sweden), 2.1 mL of "Pharmalyte" pH range 3–10 (Pharmacia), and 4 g of sorbitol (BDH Ltd., Poole, Dorset, U.K.) in 30 mL of a 100 mL/L aqueous solution of glycerol. This suffices for a 22 cm × 11 cm gel. The anode and cathode strips (Pharmacia) respectively contained 60 mmol of sulfuric acid and 1.0 mol of sodium hydroxide per liter. Applied samples were electrophoresed for a total of 1500 V·h. The proteins were made visible by fixing (with sulfosalicylic acid, 10 g/L, and trichloracetic acid, 100 g/L, in distilled water), followed by staining with Coomassie Blue (5 g/L in methanol/acetic acid/water, 35/10/
55 by vol). The gel was destained in the stain solvent.

**Immunofixation electrophoresis:** Cellulose acetate electrophoresis was done as described above. The separated serum proteins were reacted with antisera to either egg-albumen (Janssen Life Sciences Products, Wantage, Oxon, U.K.), human albumin (Atlantic Antibodies, Winnerah, Berkshire, U.K.), or human immunoglobulin moieties (Dako Ltd., High Wycombe, Bucks, U.K.). Insoluble antibody-antigen complexes so produced were stained with nigrosine (0.2 g/L in 50 mL/L aqueous solution of glacial acetic acid).

**Results**

Electrophoresis of the patient's urine consistently produced a pattern in which the albumin zone migrated less anodally than normal and had an appearance usually associated in serum with bisalbuminemia. The normal β1 zone appeared shifted towards the cathode, and a further zone was present far beyond the cathodal end of the usual gamma region. Overall, there was little resemblance to the pattern produced with urine of nephrotic patients. The patient's serum gave a normal electrophoresis pattern, containing none of the features described above (Figure 2). Immunofixation of the patient's urine with antisera to human albumin and human immunoglobulin chains showed no reaction. The electrophoretic pattern obtained from the patient's urine was compared with the patterns obtained on electrophoresis of some protein-containing preparations that would be easily available to a person who did not have access to a chemistry laboratory (Figure 3). The pattern for the patient's urine was found to resemble solutions of egg-white and Maxipro protein supplement (Scientific Hospital Supplies Ltd., Liverpool, U.K.). These sources of protein and the patient's urine were then subjected to isoelectric focusing. The patient's urine gave a pattern identical with egg-white (Figure 4) but dissimilar from Maxipro (Figure 5). Final confirmation that egg-albumen was present in the patient's urine was obtained by immunofixation with anti-egg-albumen antiserum.

**Discussion**

Cellulose acetate electrophoresis is adequate for routine screening of urines for abnormal proteins. However, it lacks the discriminatory power necessary for specific, abnormal proteins to be identified with confidence. The advantage of
isoelectric focusing is the greater resolving power, which produces many more bands and, therefore, more points for comparison between a solution containing unknown proteins and reference proteins. This technique enables the source of abnormal proteins to be identified with some certainty, although the proteins themselves may be unknown. If a specific antiserum is available, the application of cellulose acetate immunofixation, or isoelectric focusing followed by immunoblotting, enables positive identification of the unknown protein. By a combination of these techniques we made a diagnosis of factitious proteinuria and identified the exogenous protein source as egg-white.

Observations that might lead one to suspect factitious proteinuria include inordinately large day-to-day variations in urine protein concentration; the maintenance of serum albumin concentration within the normal range despite the presence of excessive, apparent proteinuria; or unusual patterns produced when urine proteins are separated by electrophoresis.

Failure to recognize proteinuria as factitious may lead to further laboratory investigations that will be wasteful of both time and money. The patient may also attempt to deceive more than one physician. A warning of this tendency may prevent unnecessary clinical investigations, e.g., renal biopsy with its attendant risk. Also, electrophoresis is often used to analyze urine for the presence of a paraprotein, which could easily be masked by exogenous protein and thereby delay diagnosis of an underlying disease. Finally, evidence of factitious proteinuria will be useful in medico-legal situations in which iatrogenic renal disease is postulated.

References