Hemoglobin Interference with Urinary Bence Jones Protein Analysis on Electrophoresis*

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ABSTRACT

Two cases are described which show that on urine protein electrophoresis a paraprotein owing to hemoglobin cannot be distinguished from a Bence Jones protein. A simple method is described for confirming the presence of hemoglobin: a densitometric scan of the electrophoretogram at 415 nm, in which hemoglobin absorbs in a band coincident with the restriction. Furthermore, it is shown that other serum proteins, elevated levels of bilirubin, and Bence Jones protein do not interfere with this detection system.

Introduction

In patients with hemoglobinuria, hemoglobin can be mistaken for a paraprotein in urinary protein electrophoresis. Since hemoglobin normally migrates in the α₂ to β-region, it may be thought to be a Bence Jones protein. In such cases, negative reactions on analysis with antisera against κ and λ light chains by immuno fixation electrophoresis (IFE) usually rules out the paraprotein as a Bence Jones protein.¹ However, it is desirable to identify the exact nature of a paraprotein because antisera against monoclonal immunoglobulins, and especially free light chains, may react peculiarly with abnormal immunoglobulins.¹,²,³,⁴,⁵ In the unusual case, insufficient reaction may cause a monoclonal immunoglobulin to escape detection.⁵ To avoid this type of error, many laboratories maintain supplies of antisera from more than one commercial source for testing suspect sera.⁵,⁶

In urine, low concentrations of hemoglobin may not be obvious on visual inspection. Since most laboratories do not keep antisera against hemoglobin, IFE cannot be used to rule out this type of interference. Furthermore, the migration distance may vary depending on type of hemoglobin and the ionic strength and pH of the urine. Here, it is shown that on electrophoresis of urine a hemoglobin band can be confirmed simply by densitometric scanning of the gel at 415 nm.
Case Reports

Two cases are reported in which hemoglobin was initially thought to be a Bence Jones protein.

Case 1

A 62-year-old, black male with multiple medical problems, including chronic renal failure, insulin dependent diabetes, chronic pancreatitis, congestive heart failure, and chronic obstructive pulmonary disease, was admitted to the hospital because of shortness of breath and chest pain. Myocardial infarction was ruled out by serial electrocardiography, creatine kinase, and lactate dehydrogenase determinations. Clinical and radiological evaluation indicated pneumonia, and the patient improved after treatment with antibiotics.

Abnormal values, with reference ranges in parentheses, were as follows: hemoglobin = 73 g/L (120–160), albumin = 28 g/L (35–48), calcium = 0.069 g/L (0.084–0.117), phosphorus = 0.084 g/L (0.024–0.044), glucose 1.86 g/L (0.7–1.1), chloride of 113 mmol/L (101–111), and carbon dioxide 11 mmol/L (24–31). Urea nitrogen and creatinine were 0.58 g/L (0.05–25), and 0.056 g/L (0.005–0.014), respectively, which were the patient’s usual base line values. Urinalysis revealed elevated protein, trace blood with 2 to 4 red blood cells per high power field, and 1 to 3 white blood cells.

Urine protein electrophoresis resulted in a pattern indicating glomerular failure, with a paraprotein migrating in the β-region (figure 1). Immunofixation with antibody for IgG, IgA, λ, and κ chains showed only polyclonal patterns in the γ-region. On retrospective examination, the urine was red. The paraprotein in the β-region was confirmed as hemoglobin by densitometric scanning at 415 nm, in which a peak migrated coincidentally with the paraprotein (figure 1).

Case 2

A 61-year-old, white male with a history of alcoholism entered the hospital because of hema­temesis. The patient presented with ascites and edema of the extremities.

Abnormal laboratory results were as follows: Heme positive stool, hemoglobin of 73 g/L (120–160), a typical cirrhotic testing profile, with albumin = 20 g/L (35–48), total bilirubin = 0.029 g/L (0.003–0.016) 55% of which was conjugated, alanine transaminase/aspartate transaminase = 0.45, and prothrombin time = 14.5 seconds (11–13 seconds). The patient, also, appeared to be in renal failure with a urea nitrogen of 0.98 g/L (0.05–25), a creatinine of 0.054 g/L (0.005–0.014), uric acid of 0.14 g/L (0.03–0.07) and a phosphorus of 0.077 g/L (0.024–0.044). Urinalysis showed hematuria, 0–5 white blood cells, and trace protein.

Serum protein electrophorese revealed a cirrhotic type profile with a β-γ-bridge, and a superimposed low concentration (<10 g/L) M-component. The UPE showed a pattern that indicated a glomerular lesion, but with a paraprotein migrating in the β-region (Figure 2). Immunofixation with antibody for IgG, IgA, λ and κ chains showed only polyclonal
Specimens were either stored refrigerated and assayed within one week, or they were stored frozen at $-70\,^\circ\mathrm{C}$ and thawed just before being assayed later. Prior to protein electrophoresis and IFE, urine samples were concentrated approximately 80 to 100 fold using gravity-flow concentrators. Protein electrophoresis† for serum and urine was performed according to the manufacturer’s instructions. Proteins were detected by staining with amido black† or by densitometry‡ performed at 415 nm on unstained gels, as previously described.§ IFE (8,9) on serum was performed according to the manufacturer’s instructions.¶ Antiseras supplied with the kit were specific for IgG, IgA, free and bound kappa and lambda chains.

Free hemoglobin was derived from a whole blood specimen as follows: erythrocytes were pelleted by centrifugation (1000 $\times$ g, 10 minutes) and the plasma poured off. The red cell pellet was washed twice with normal saline, then resuspended in double-distilled water equal to the original volume of plasma. This solution was stored overnight at 4°C, then centrifuged to remove cellular debris. The concentration of hemoglobin was measured by transmittance at 525 nm after conversion to a cyanide containing pigment.¶ This solution was diluted 100 fold with double distilled water. Additional dilutions were made with urine to give hemoglobin concentrations of approximately 1.6, 0.8, 0.4, 0.2, 0.1 g/L. This material was stored for one week at 4°C while experiments were being performed.

Materials and Methods

Urine specimens were collected over 24 hours without the use of preservatives.

* Minicon-B15, Amicon, Danvers, MA 01923.
† Universal II Agarose Film/12, Ciba Corning Diagnostics Corp. Palo Alto, CA 94306.
‡ edc densitometer (Helena, Laboratories, Beaumont, TX 77704)
§ Glycated hemoglobin by Rapid Electrophoresis (REP), also Helena.
¶ Titan Gel Immunofix, also Helena.
* Coulter Counter STKR, Coulter Corporation, Healeah, FL 33010-2458.
formed. Urine samples were evaluated by electrophoresis both before and after concentrating them 80–100 fold.

Controls consisted of two sera, randomly selected, and a urine containing a prominent Bence Jones protein. One of these sera contained a protein concentration of 76 g/L, with an electrophoretic β-γ bridge consistent with cirrhosis of the liver, an elevated immunoglobulin concentration of 30 g/L (7–16), and an elevated bilirubin concentration of 0.027 g/L (0.003–0.016). The other serum sample contained 74 g/L protein and appeared normal.

Results

In figure 1 are shown the protein electrophoresis and IFE patterns, along with the absorbance profile at 415 nm for the urine from Patient 1. A pattern from a patient with a Bence Jones protein in the urine is shown for comparison purposes. Hemoglobin appeared similar to a Bence Jones protein in stained gels.

In figure 2 is shown the stained electrophoretic pattern along with companion absorbance profiles for the urine from Patient 2. Also shown are electrophoresis and absorbance profiles of urine specimens containing indicated hemoglobin dilutions prior to concentration. A paraprotein at 0.2 g/L hemoglobin was found to be detectible after staining. Below 0.2 g/L a paraprotein was not detectible. Similarly, the absorbance profile at 415 nm demonstrated a co-migrating peak at 0.2 g/L but no peak at lower concentrations. When the unconcentrated specimens were assayed at 415 nm a peak could be observed at 0.8 g/L hemoglobin, but not at lower concentrations.

In figure 3 are shown the stained electrophoretic lanes along with absorbance profiles at 415 nm from two sera and a urine containing a prominent Bence Jones protein. It is clear that none of these samples contained an absorbance peak. It is concluded that in urine protein electrophoresis hemoglobin can be identified by scanning the gel at 415 nm, while immunoglobulins and other serum proteins are not detected.

Discussion

A property of hemoglobin is a very intense absorbance in the range of 400 to 425 nm (Soret band) (10). This has been widely used for measuring glycated hemoglobin by electrophoresis.\textsuperscript{11,12} It has been demonstrated that this approach
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can be used for identifying an electrophoretic paraprotein restriction in a patient's urine (figure 1 and figure 2) in which hemoglobin might be mistakenly suspected to be a Bence Jones protein.

Although in most cases hemoglobinuria will be expected because the urine will have a red tint, this is not always the case. Hemoglobin concentrations of 0.1 to 0.3 g/L are difficult to see in urine, especially if the urine is dense. Hemoglobin in unconcentrated specimens could not be detected below 0.8 g/L, which is clearly visible to the eye. However, urine samples are routinely concentrated 100 to 600 fold prior to electrophoresis; in these samples (figure 2), a stained band was detectible at much less than 0.8 g/L hemoglobin. Most clinical laboratories do not keep antisera against hemoglobin for use in confirming a suspect band by immunoelectrophoresis; besides, the present approach is a simpler and cheaper alternative to immunoelectrophoresis.

It was also demonstrated that no absorbance peak was detected when two sera and a urine containing a prominent Bence Jones protein were scanned at 415 nm (figure 3). Thus, non-hemoglobin serum proteins did not interfere with the method. Bilirubin absorbs maximally at 454 nm, and absorbs light into the Soret band. Nevertheless, no interference was observed in two sera which contained elevated levels of bilirubin (figure 1 and figure 2), nor in the urine from one of these patients (figure 1). This would be expected because conjugated bilirubin does not migrate with the serum proteins on electrophoresis. Although not observed in this study, it is possible that very high unconjugated bilirubin concentrations could interfere by absorbing at 415 nm. However, this compound should co-migrate electrophoretically with albumin, and thus not be easily confused with a Bence Jones protein.

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References