Urine Protein Electrophoresis and Immunofixation Electrophoresis Supplement One Another in Characterizing Proteinuria

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Abstract. Urine protein electrophoresis (UPE) is often considered to have limited usefulness in evaluating proteinuria that is not associated with gammopathies. Unusual protein bands that are detected by UPE are commonly characterized by immunofixation electrophoresis (IFE). In this paper, electrophoretic gel patterns are shown to illustrate the greater sensitivity of IFE, compared to UPE. However, UPE remains useful for three applications: (1) UPE provides distinctive patterns that can indicate the source of proteinuria and is useful in assessing renal diseases that are independent of gammopathy; (2) combined use of UPE and IFE can avoid misinterpretations and repeated analyses of urine proteins, and (3) UPE can be used in conjunction with IFE to improve the quantitation of Bence-Jones proteinuria (BJP).

Keywords: Urine proteins, immunofixation, electrophoresis, Bence-Jones protein, renal diseases

Introduction

Urine protein electrophoresis (UPE) is frequently used to screen urine specimens for monoclonal free light chains (Bence-Jones proteins) and is often considered to have limited usefulness in evaluating proteinuria that is not associated with gammopathy. Unusual protein bands that are detected by UPE are commonly characterized by immunofixation electrophoresis (IFE). We and others have pointed out that UPE is less sensitive than IFE for distinguishing small Bence-Jones proteins (BJP) [1,2]. Although IFE is more time-consuming and costly than UPE, IFE has been recommended for screening all urine specimens for which the identification of BJP is important. Nonetheless, UPE remains useful in three applications: First, UPE provides distinctive patterns that can indicate the source of proteinuria [3,5], and is useful in assessing renal diseases that are independent of gammopathy. Second, combined use of UPE and IFE can avoid misinterpretations and repeated analyses of urine proteins. To identify BJP, it is recommended that urine specimens be concentrated 80x - 200x prior to analysis by both UPE and IFE [1,2,6-8]. This degree of concentration can cause problems in interpretation, since the gel patterns may appear overloaded with UPE and BJP bands may show prozoning with IFE. Third, UPE can be used in conjunction with IFE to improve the quantitation of BJP.

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Materials and Methods

Patients, urine collection, storage, and preparation. Urine samples were selected, over a period of several years, from patients at the Veterans' Administration Medical Center or the University of Louisville Hospital who were undergoing urinalysis for suspected plasma cell dyscrasia or to assess the nature of renal proteinuria. All patients with BJP were diagnosed as having multiple myeloma on the basis of serum and urine electrophoresis, bone marrow examination, and radiological and clinical criteria. In some cases, identification of BJP in urine was the first indication of the diagnosis.

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Urine specimens were fresh 24-hr collections that were obtained without the use of preservatives. The urine specimens were refrigerated at 4°C for less than 48 hr, or they were frozen and then thawed prior to analysis. Urine total protein was measured using pyrogallol red-molybdate with a kit (Biotrol Urine Proteins, Biotrol USA, Exton, PA 19341) that was modified in our laboratory to optimize linearity [9]. Prior to electrophoresis, urine samples were mechanically concentrated (Minicon B15, Amicon, Danvers, MA 01923) about 100x, unless otherwise indicated. UPE and IFE were performed by kit techniques (PEP SPE-8-Template procedure and Titan Gel Immunofix procedure, Helena Laboratories, Beaumont, TX 77704). Antisera for free and bound light chains and heavy chains were supplied with the kits; antisera for free light chains were purchased separately (Helena Laboratories).

Results and Discussion

Urine profiles seen with disease. Pathological UPE profiles, first described by Laurell [3] and reviewed elsewhere [5], depend largely on the following physiological processes. Normally, proteins with relatively low molecular weights pass through the glomerulus readily, while those with higher molecular weights do not. About 2-3 g of protein pass through the glomerulus per 24 hr, and all but ~ 0.1-0.2 g are reabsorbed by the renal tubules. Although a molecular weight of approximately 60 kDa is considered to be the threshold below which proteins readily cross the glomerulus, the glomerular pore sizes vary widely; large amounts of very small proteins pass readily, but small amounts of larger proteins also pass. Glomerular tissue is negatively charged so that very negative proteins pass poorly, even those with low molecular weight. Thus, small amounts of albumin normally pass although its molecular weight is about 60 kDa. Polyclonal light chains are synthesized in excess of heavy chains, and, due to low molecular weights (12-24 kDa, depending on whether they are monomeric or dimeric), they readily pass through the glomerulus [10].

In pure tubular proteinuria, the glomerulus behaves normally. Thus, larger proteins cannot penetrate the intact glomerulus, but smaller proteins can. As a result of kidney tubular failure, these smaller proteins are not reabsorbed and appear in the urine. These proteins are largely polyclonal free light chains and α2-microglobulins. This gives rise to the tubular pattern shown in Fig. 1, where dense staining can be seen in the gamma region and double bands in the α2-globulin region; albumin stains lightly, because little albumin passes the intact glomerulus [3].

In pure glomerular disease, larger proteins pass more readily, so that more than 2-3 g/24 hr crosses the leaky glomerulus. Because of the relatively great concentration of serum albumin, glomerular disease produces abundant albuminuria, although transferrin and α1-globulins also pass more readily. The smaller globulins are reabsorbed by the kidney tubules, which behave normally. As a result, the gamma globulin region stains lightly, while the larger proteins give rise to the glomerular pattern that is shown in Fig. 1, with albumin dominating.

Although nephrotic syndrome is typically characterized by urine protein excretion > 3.5 g/24 hr, with low to moderate amounts of protein, it is difficult to decide which type of proteinuria is primarily responsible without electrophoretic analysis. When large amounts of protein are present in urine (ie, >5 g/24 hr), it is reasonable to suspect glomerular origin.

When mixed tubular and glomerular proteinuria occurs, the two patterns are superimposed, as shown in Fig. 1. The presence of double bands in the α2-globulin region, denoting a tubular component, depends on the relative contributions of glomerular and tubular components, as well as the degree of mechanical concentration of the urine specimen [9].

Monoclonal BJP behave similar to polyclonal free light chains, since they pass the glomerulus readily and are reabsorbed if tubular function is normal. The overflow pattern occurs in some cases of BJP, when protein production exceeds the tubular capacity for reabsorption. Significant renal damage is not necessarily present. In other cases, the BJP pattern is superimposed on the pattern associated with renal damage. Two cases of Bence-Jones proteinuria are shown in Fig. 2. In one case, BJP is largely of the overflow type with little evidence of kidney damage. In the other case, the background pattern is largely of the tubular proteinuria type.

Very small BJP. Very small BJP that are seen with IFE may not be apparent with UPE. This problem is shown in Fig, 3, where little BJP is seen by UPE in a specimen

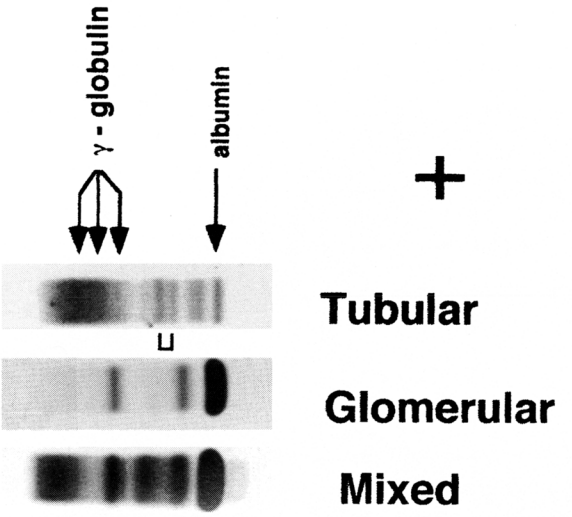

Fig. 1. Pathological urine patterns indicating the source of proteinuria. The patterns were from patients passing: 3 g protein/24 hr with a volume of 2.9 L, tubular; 1.6 g/24 hr, volume 0.7 L, glomerular; and 9 g/24 hr, volume 2.2 L, mixed. The bracket depicts dual bands defining β2 - microglobulins. The polarity of the electrodes is indicated.

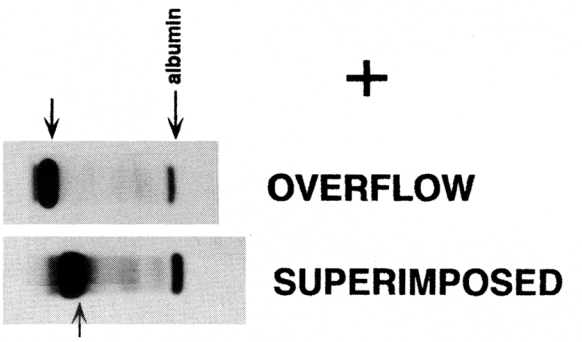

Fig. 2. Bence-Jones proteinuria. The arrows without labels indicate the location of the Bence-Jones proteins. The polarity of the electrodes is indicated.

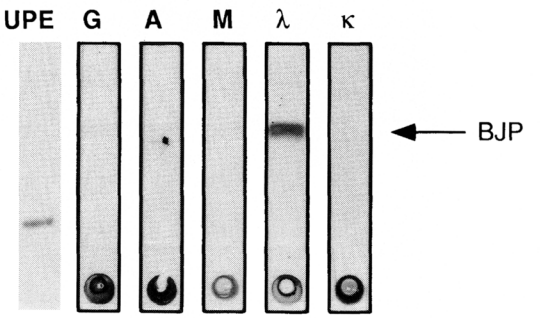

Fig. 3. Insensitivity of UPE for low level Bence-Jones proteins. G, A, M, κ, and λ indicate antisera used to fix appropriate intact immunoglobulins. Total protein in this specimen was 0.15 g/24 hr, essentially a normal level.

that has been concentrated 100-fold. However, a λ-BJP is clearly delineated by IFE. The antibodies that are used to fix the urine proteins cause an increase in total protein mass, compared to unfixed electrophoresis, and thereby enhance the protein staining with IFE.

Some commercial kit manufacturers indicate that urine samples should be concentrated to a defined protein level (eg, 1 g of total protein per dl when BJP is present alone, but to a higher concentration when other proteins are also present). Since the measurement of total urine protein does not predict the amount of Bence-Jones protein relative to other proteins, this seems to be a futile approach. Many studies have concluded that samples should be mechanically concentrated at least 100x regardless of the total protein concentration [1,2,6-9].

Problems of overloading. Because of this requirement for high sensitivity, the patterns may appear overloaded or prozoning may occur with IFE, making interpretation more difficult. A major difficulty with IFE is that it lacks a mechanism whereby dilution and titration produces antibody and antigen equivalency, as compared to the older technique of immuno-electrophoresis which included a diffusion step. More definitive patterns can be obtained by diluting the specimen and repeating the IFE. In practice, this characteristic rarely leads to a mistake in interpretation. In fact, although the older technique of immunoelectrophoresis included a diffusion step, prozoning with need for dilution and repeated assay of urine samples was common [11], while with IFE the simple correspondence between the bands seen on UPE and IFE provides a means by which the correct interpretation can usually be achieved without repeated assay.

Fig. 4 illustrates a case in which routine IFE of a urine specimen, concentrated 100x, demonstrated prozoning in the beta region of the gel, which may be difficult to interpret with free and bound antisera. For demonstration purposes, repeated assay with dilution and fixation with antisera against free κ-light chain clearly indicates that a BJP is present in the beta region. In actual practice, the peculiar pattern on IFE is established as a BJP by a coincidental distinct band on UPE. In this case, the two techniques clearly augment

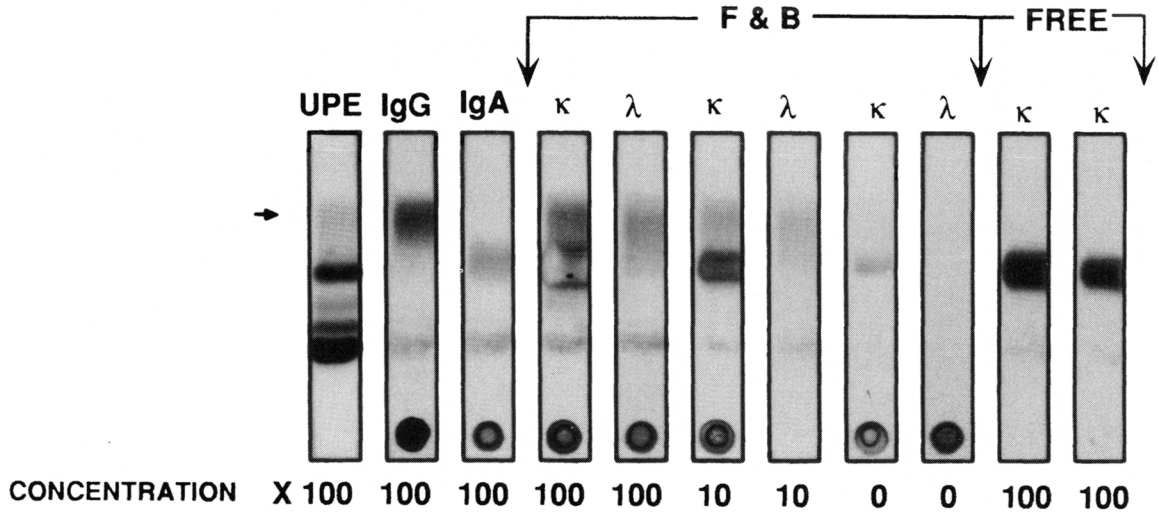

Fig. 4. Augmentation of UPE and IFE for identification of Bence-Jones proteins. F & B signify fixation with antisera against free and bound (intact) light chain, while free indicates antisera against free light chain only. The horizontal arrow indicates the origin.

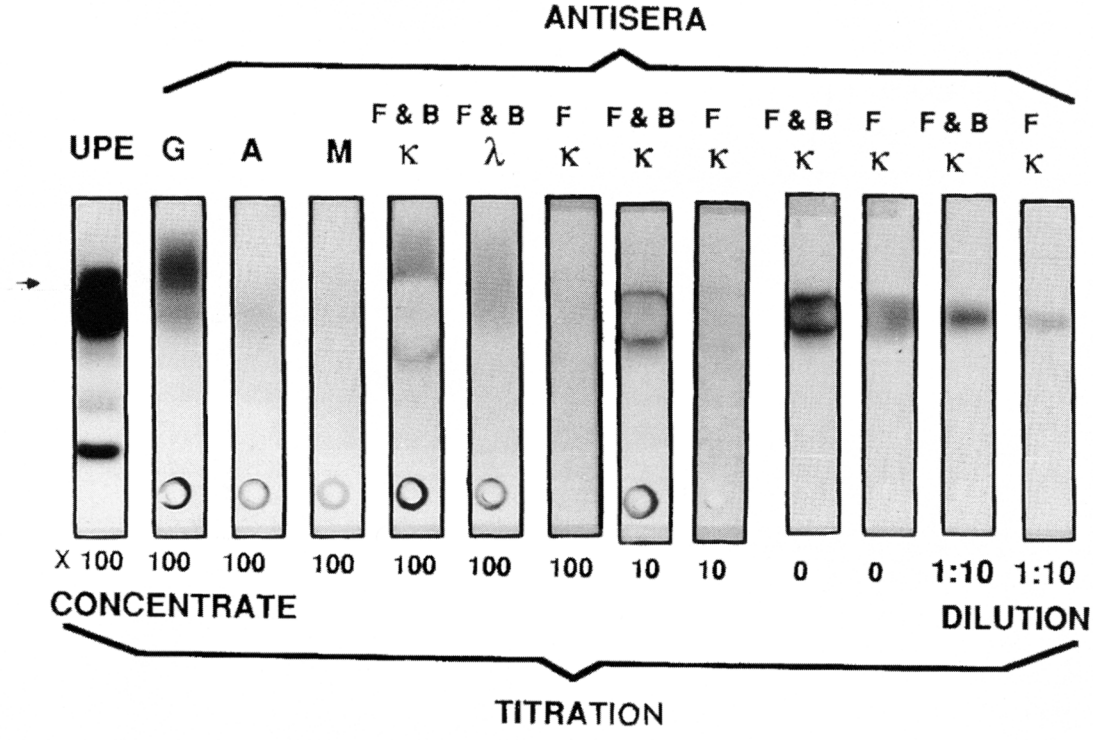

Fig. 5. Verification of BJP by UPE with extreme prozoning on IFE. Antisera used to fix IgG, IgA, IgM, kappa and lambda are indicated. F indicates antisera against free light chain only. Other symbols are defined in the legends to Figs. 3 and 4.

one another. The presence of a monoclonal protein is suspected but unclear with UPE because it is in the beta region where staining for transferrin is expected. A BJP is also uncertain with IFE because of prozoning. But together, the two techniques allow identification. Thus, comparison of the gels seen on UPE and IFE for the 100x concentrate negates the need for further analysis.

Fig. 5 shows a more extreme case of prozoning. Here, the pattern developed by reaction with antisera against free light chain shows no band when the 100x concentrate is analyzed. The prozoning pattern seen with fixation of the 100x concentrate using free and bound antisera is coincident with a distinct band on the UPE pattern. The two profiles demonstrate a BJP, and additional analysis is not needed.

Quantitation of the amount of Bence-Jones protein. It may be useful to quantitate the amount of BJP, because the amount of monoclonal light chain may correlate with the tumor burden [8]. Previous work indicated that BJP cannot be accurately measured using routine immunochemical techniques, since the reaction of BJP with the assay antibodies differs from that of the polyclonal calibrators, which contain intact light chains [12].

Theoretically, the amount of BJP can be estimated from a densitometer tracing as a percentage of the total protein. There are two problems associated with quantitating BJP by densitometry: First, most laboratories use precipitation or dye binding methods to quantitate total protein. Although these methods measure intact globulins and albumin to a similar extent, it is unclear to what extent they measure BJP. Few laboratories use the biuret method, which reacts with the peptide bonds in proteins and measures all proteins equally. Except for the biuret techniques, many methods for total protein estimation are unreliable for quantitation of free light chains. Second, even when the biuret method is used, BJP may be superimposed on a pathological protein band, causing inaccuracy in quantitation. For example, in Fig. 2, the BJP shown in the overflow pattern could be quantitated fairly accurately, while the superimposed BJP could be estimated less accurately, since some of the staining is from underlying proteins. This problem is more evident in Fig. 4, where it is unclear whether or not the BJP band in the beta region of the UPE is superimposed on other protein bands.

In the author's laboratory, semiquantitative results are provided to clinicians based on the profiles obtained from both IFE and UPE. For example, the bands representing BJP in Figs. 2 and 5 would be described as a "large amount of BJP," while that in Fig. 4 would be described as "moderate amount," and those in Fig. 3 as a "small amount." If a "large" or "moderate" amount of BJP becomes a "small" amount with treatment and clinical improvement, it may indicate that the tumor burden has declined. Also, based on clinical evidence and other testing, "large" or "moderately large" BJP bands may indicate a worse prognosis than "small" ones.

Conclusions

The most important questions asked when assessing proteinuria by electrophoresis are: "What is the nature of the kidney defect?" and "Is a BJP present?" UPE is often considered merely a screening test for BJP, and IFE is considered to be more reliable. The author's experience indicates that UPE complements IFE, and that the two techniques together can usually answer the two important questions without repeat testing.

Since BJP at low concentrations, or migrating coincidentally with other proteins, may escape detection by UPE, IFE should be performed routinely on all specimens for which Bence-Jones proteinuria is suspected. To obtain sufficient sensitivity for detecting low-level BJP, mechanical concentration of the samples to between 100x - 200x is recommended [1,2,6-8]. Furthermore, the urine specimens should preferably be 24 hr collections [6,7]. First morning collections may also be adequate, but random collections are unsuitable [6]. The amount of total protein in the specimen should not dictate the amount of mechanical concentration, since the proportion of BJP to other proteins is unknown. In spite of low sensitivity, UPE has an important role in insuring that misinterpretation does not occur on overloaded gels due to prozoning of bands on IFE. In this regard, UPE is also helpful for reducing the need for dilution and repeat analysis. In the author's experience, it is unusual to misinterpret the result when the two procedures are used in conjunction.

Finally, although it may be useful to quantitate the amount of BJP in order to assess tumor burden

and judge prognosis, quantitation is difficult because of the poor assays for total protein that are performed in most laboratories and because of coincidental electrophoretic migration of urine proteins. Semi-quantitation on the basis of the UPE and IFE profiles is a reasonable substitute.

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