Original Article

Replacing Acid-Heat Precipitation with Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis Improves Detecting Bence-Jones proteinuria

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Abstract

Background: Detecting Bence-Jones protein in urine is essential for determining plasma cell dyscrasia and multiple myeloma. Conventionally, acid-heat precipitation assay is used for detecting Bence-jones protein in most medical laboratories; however, because of the low accuracy of this test, other more sensitive tests like urine electrophoresis are recommended. **Materials and Methods:** In this study, the presence of Bence-jones protein in the urine of patients suspected to monoclonal gammopathies were compared using acid-heat precipitation, capillary immunoelectrophoresis and sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Moreover, the subsets of light chain (κ & λ) in capillary immunoelectrophoresis were determined. **Results:** Our data showed high false negative results (77.7%) using acid-heat precipitation assay in comparison with polyacrylamide gel and capillary immunoelectrophoresis (0%). **Conclusion:** Collectively, in spite of advantages like easy performance and low cost, acid-heat precipitation assay is not reliable for determining Bence-jones proteinuria in medical laboratories due to its low sensitivity. Therefore, it is recommended to be replaced with more sensitive assays like electrophoresis.

Keywords: Bence-Jones protein, Monoclonal gammopathies, Capillary immuno-electrophoresis, Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

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Introduction

Bence-Jones protein (BJP) is the first tumor marker, discovered by Dr. Henry Bence Jones more than 150 years ago. In fact, BJP is an immunoglobulin free-light chain excreted in urine. It has been used as a bio-marker for B-cell dyscrasia like multiple myeloma (MM)(1). This protein is detected in the urine of 75% of MM patients and is one of the major laboratory features of this disease (2, 3). Therefore, assays that detect BJP in the urine play a critical role in the diagnosis and monitoring of MM.

Conventionally, the acid-heat precipitation test is established as the first assay for detection of BJP in

urine and routinely utilized in most medical laboratories due to its low cost and simplicity (4). Urine protein electrophoresis (UPE) is another method for detection of BJP on the basis of charge and molecular size of proteins. Moreover, immuno-electrophoresis (IEF) is applied to urine electrophoresis to specifically identify the light chain and its subsets (5). Apart from IEF, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) has been introduced as another reliable method for qualitative analysis of urinary proteins. SDS-PAGE separates proteins according to their molecular size, and free light chain monomers are visualized as a band of 23000-25000 KDa, easily distinguished from intact immunoglobulins (150000 KDa) (6).

In most of the clinical laboratories, heat-precipitation is the well known method routinely used for BJP detection; however, high discrepancies between urinary BJP results using acid-heat precipitation and IEF and SDS-PAGE have been reported (7, 8). Therefore, in this study we compared the results of acid-heat precipitation assay as a conventional test with capillary IEF and SDS-PAGE in urine samples to compare the sensitivity and specificity of these methods in detection of BJP.

Methods

Patients

The patients studied were those attending the Noor Pathobiology Laboratory (Tehran-Iran) for BJP test. One-hundred twenty three patients suspected by physicians to monoclonal gammopathies were investigated. All measurements were done on random urine samples without any preservatives.

Toluene-Sulfonic Acid (TSA)-precipitation assay

One milliliter of TSA reagent (12 gr of p-toluene Sulfonic acid/100 ml acetic acid (Merck, Germany)) was added to 4 ml of urine sample in a test tube. If turbidity was visualized within 15 minutes in 40-60°C, the test would be positive for BJP. The heat-precipitation test was performed in boiling and cooling back the urine samples in order to confirm the TSA method. The appearance of turbidity was found as positive between 40-60°C.

SDS-PAGE

All the TSA-heat tested urine samples were also analyzed using SDS-PAGE as described previously with slight modification (9, 10). Briefly, after casting 4 layer discontinuous of polyacrylamide gels (5-20%, stacking 4%) with mini protean II cell system (BioRad, USA), the equal volume of urine and sample buffer (40 µl) were loaded into wells and electrophoresis was conducted at 30 mA for 150 minutes. Following electrophoresis, the gels were stained with 2% silver nitrate (Merck, Germany) for 10 minutes. The bands were analyzed using Ultroscan GSX software (Pharmacia, USA).

Urine capillary IEF and free light chain subsets determination

The UPE was performed for samples that were positive for SDS-PAGE and carried out using automated capillary IEF apparatus (Sebia, France) according to manufacturer's instructions. The subsets of free light chain (FLC) were determined using polyclonal κ and λ antisera (Sebia, France).

Results

One hundred and twenty three consecutive suspected patients (female; n=44, male; n=79) were

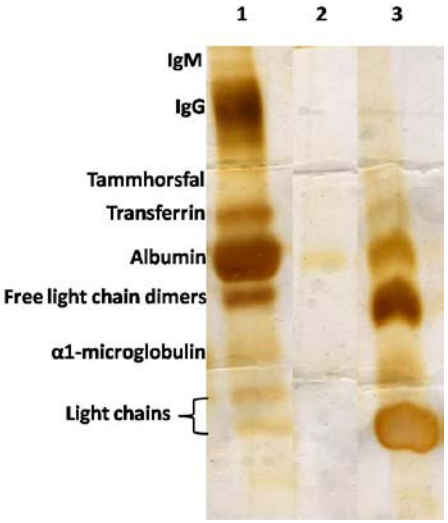

Fig. 1. SDS-PAGE analysis of unconcentrated urine in 4 layer resolving gel (5-20%, stacking 4%) that stained with 2% silver nitrate stain. Lane 1, Pooled urine molecular weight marker; Lane 2, Unconcentrated normal control urine; Lane 3, Unconcentrated urine suspected to multiple myeloma (MM).

Table 1: Distribution of comparison of TSA and SDS-PAGE test results in 123 suspected patients to monoclonal gammopathy. TSA, *p*-tolouene sulphonic acid assay. SDS-PAGE, sodium dodecyl sulphate poly-acrylamide gel electrophoresis.

Total patient:123		SDS-PAGE	
		positive	negative
TSA	positive	8	0
	negative	28	87
	Total	36	87

enrolled in this study (November 2014-15). The SDS-PAGE of a urine sample suspected to MM and normal control in comparison with pooled urine molecular weight marker was shown in figure 1. As depicted in table 1, using TSA assay, 8 of 123 individuals were positive for BJP, while 36 of 123 individuals were detected positive using SDS-PAGE. On the other hand, all the SDS-PAGE positive samples were also positive for FLC using capillary IEF (100%). Moreover, of 36 samples, κ light chain was detected in the urine of 22 individuals, while λ light chain was shown in the rest of them (n=14). In this regard, it was determined that the TSA assay had a high number of false negative results (77.7%) and low sensitivity (22.3%), while the SDS-PAGE results showed no false negative in comparison with capillary IEF (table 2).

Discussion

The results of this study determined the low accuracy of TSA-heat precipitation assay in comparison with SDS-PAGE and capillary electrophoresis. According to false negative and sensitivity results, the TSA assay also showed lower sensitivity than SDS-PAGE and confirmed that precipitation assay could not be a powerful test for detection of BJP in urine. Historically, the TSA assay is still being the main test in most medical laboratories for detection of BJP in urine samples of patients suspected to B cell dyscrasia like MM; however, although this test is easy in performance and cost effective, since not all positive samples could be detected upon heating, the results are not reliable. Therefore, more sensitive methods seems to be essential for detection of BJP.

Table 2: Diagnostic accuracy of TSA and SDS-PAGE in comparison with capillary IEF.

Parameter	TSA	SDS-PAGE
False negative (%)	77.7	0
False positive (%)	0	0
Sensitivity (%)	22.3	100
Specificity (%)	100	100

UPE method is one of the sensitive tests based on both the charge and molecular size of proteins. This assay could also determine the proteins specifically using antisera in capillary IEF (11). Moreover, because of its potential, rapid and high efficiency separation, capillary electrophoresis is used as a reference method in determining the patients suspected to MM. SDS-PAGE is also the other sensitive method for the study of the excreted proteins in urine (12, 13). Previous reports have shown that the 4 layer discontinuous SDS-PAGE is a powerful tool to separate the proteins of unconcentrated urine samples with high resolution and also distinguishes proteinuria of glomerular, tubular, or mixed origin (10, 14). Therefore, this is a powerful method for assessing kidney function, which is an important prognostic factor in MM (15, 16).

Although, various kinds of electrophoresis, unlike TSA-heat precipitation test, are technically demanding and time consuming, the sensitivity as well as specificity of these tests are very high that they are recommended as powerful approaches for replacing traditional method. The results obtained using the two techniques were similar and both of methods would be suitable. Although, the advantages of capillary electrophoresis include rapid analysis as well as light chain subtypes identification, SDS-PAGE is reliable to perform without professional instrumental automation, which is cost effective in most of the medical laboratories.

Collectively, our data suggest that utilizing the acid-heat precipitation as traditional approach does not optimally detect BJP in urine and it would rather to be replaced with SDS-PAGE for identification of patients suspected to monoclonal gammopathies.

Conflicts of Interest

The authors declare that there are no conflicts of interest

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