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SHORT COMMUNICATION

A Misinterpretable Band on Urine Protein Electrophoresis in Hematuric Patients

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SUMMARY

Background: **Urine protein electrophoresis (PEP) is widely employed to detect proteinuria and monoclonal protein (M protein), which is important for diagnosing and monitoring multiple myeloma. However, the appearance of unusual bands can confound the interpretation.**
Methods: **We described two clinical cases in which a distinct beta-region band in urine PEP associated with hematuria. To investigate this phenomenon, we conducted an experiment by adding both lysed and non-lysed red blood cells (RBCs) to normal urine specimens and then performed urine PEP.**
Results: **In both of our cases, the beta-region band disappeared after hematuria improved. In the experimental setup, the group with RBC lysis at counts above 500/μL produced a marked band in the beta region without gross hematuria, whereas the group with non-lysed RBCs required much higher concentrations to generate faint bands.**
Conclusions: **Our findings demonstrate that hematuria, particularly when red blood cells undergo lysis, can produce a distinct band in the beta region on urine PEP that may be misinterpreted as a monoclonal protein. Careful correlation with urinalysis results is therefore crucial to prevent misdiagnosis, especially in patients with microscopic hematuria.**
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KEYWORDS

urine protein electrophoresis, hematuria, beta-region band, unusual band

INTRODUCTION

Urine protein electrophoresis (PEP) has been widely used to detect proteinuria [1]. It provides information about proteinuria not only in kidney disease but also in monoclonal gammopathy. In detecting monoclonal protein (M protein) via urine PEP, which is important in the diagnosis and treatment of multiple myeloma, the presence of unusual or falsely migrated bands can confuse interpretation [2]. The authors experienced two cases in which a band arising from hematuria could be misinterpreted as a M protein in urine PEP. We investigated the urine PEP patterns of hematuria by adding red blood cells to normal urine. This study was approved by the institutional Review Board of Kyungpook National

University Chilgok Hospital (IRB file No. KNUCH 2024-12-024).

CASE DESCRIPTION

Case 1

A 43-year-old female patient visited a nephrology outpatient clinic with hematuria for two weeks. Her urine stick test showed protein ++, occult blood +++, and the red blood cells were 18,834/μL in the urine sediment test performed with UF-5000 (Sysmex, Kobe, Japan). And one distinct band in beta region, suspected to be M protein, was seen on the urine PEP (Figure 1a). There were no specific findings in the past history, blood test and abdominal CT. Cystogram showed no specific findings except for the left orifice blood jetting. On the follow-up test after treatment, microscopic hematuria was still present, but the band on urine PEP disappeared.

Case 2

A 69-year-old male patient was treated in a urology department for microscopic hematuria lasting for one year, but was recently transferred to the nephrology department due to proteinuria. He had a right nephrectomy 20 years ago and is now being treated in cardiology for heart failure with pulmonary edema. Urinalysis showed proteinuria ++ and hematuria +++, and red blood cell was counted as 14,098/μL in the urine. Serum PEP was unremarkable, while urine PEP showed a large peak in the beta region (Figure 1b). Abdominal-pelvic CT revealed a left renal calyceal stone of approximately 8 mm in size, with no other remarkable findings. The intensity of hematuria decreased after changing his medicine Xarelto (rivaroxaban) to Lixiana (edoxaban), which was used for the treatment of atrial fibrillation.

EXPERIMENTS AND RESULTS

EDTA blood and urine from normal residual specimens in our laboratory were pooled, respectively. The plasma was removed and the remaining RBCs were washed three times with normal saline. Experiments were divided into two groups; 1) “non-lysed group”: the washed red blood cells were transferred with pipette into the urine and RBC counts were adjusted from 64,000 to 256,000/μL, 2) “lysed group”: RBCs were diluted with distilled water (DW) to induce lysis and then spiked into the urine so that the calculated numbers were from 125 to 2,000/μL. A non-spiked urine sample was prepared as a negative control. Urine samples were concentrated and urine PEP was performed on a Helena SPIFE (Beaumont, TX, USA). No clear band was observed in urine with RBC counts below 64,000/μL in the non-lysed group, while a distinct band appeared at calculated RBC counts of 500/μL and above in the lysed group (Figure 2).

DISCUSSION

The detection of M protein in either serum or urine PEP is important for diagnosis and treatment of multiple myeloma [3-5]. M protein is most commonly detected in the gamma region on PEP. However, it can occasionally appear in the beta or beta-to-gamma region, where it can be easily missed. This often necessitates the use of immunofixation electrophoresis (IFE) for accurate identification [6,7]. Other proteins can mimic M protein bands such as haptoglobin-hemoglobin complexes, C3, β-lipoprotein, transferrin, fibrinogen, immune complexes, CRP, and occasionally α2-macroglobulin [8]. Therefore, it is crucial to interpret these bands with caution when analyzing PEP. In the presence of renal dysfunction or lower urinary tract abnormalities, bands unrelated to M proteins may also be detected in urine PEP [9]. Hematuria, for example, has been documented as a factor contributing to the M-protein-like band in urine [10]. The band resulting from hematuria is usually in a beta region, and could be misinterpreted as migrated immunoglobulin. Both cases in this report showed a distinct band in the beta region, which could be mistaken for M protein. However, urinalysis revealed hematuria in both cases, and the bands disappeared as the hematuria improved. According to Tapp et al. [10], lysed RBCs in urine can produce M protein-like bands on urine electrophoresis. Based on this finding, we inferred that hematuria might be the cause of the distinct band in our cases and conducted an investigation to explore this further.

In both groups in the result, the band was predominantly observed in the beta region, consistent with findings from previous studies [10,11]. In the lysed group, a band appeared even at a smaller number of RBCs in urine compared to that in the non-lysed group. Furthermore, the intensity of the bands increased as the RBC count in the sample increased. Lysis of RBCs in urine can be induced by urinary tract infections, pH changes, or chemical factors, among others. Therefore, abnormal findings in urinalysis, such as pH, specific gravity, pyuria, and high nitrite, can provide useful information related to abnormal band in urine PEP in hematuria patients. Both of our cases presented with gross hematuria. Despite the absence of gross hematuria in the non-lysed group, a band was detected on PEP in the urine when RBC counts exceeded 64,000/μL. This finding underscores that even microscopic hematuria can yield M protein-like bands in the beta region of urine electrophoresis, potentially leading to misdiagnosis. Therefore, careful correlation with urinalysis results, including RBC counts, pH, specific gravity, and potential lysis factors, is essential to distinguish hematuria-induced bands from true M proteins.

A limitation of this experiment is the lack of osmolality measurements to compare the degree of lysis. Changes in the osmolality of the medium containing RBCs can cause the cell membrane to shrink or swell, leading to lysis. As a result, hemoglobin from lysed RBCs is re-

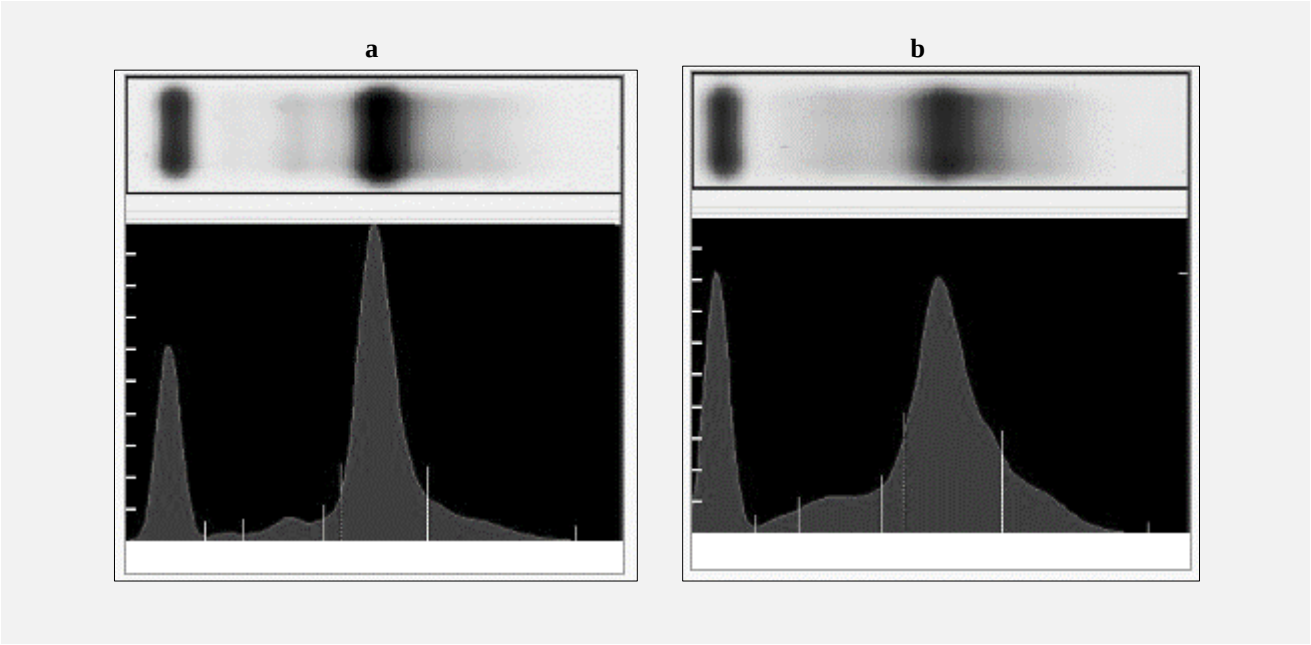

Figure 1. Urine electrophoresis in hematuria cases.

a - case 1 and b - case 2.

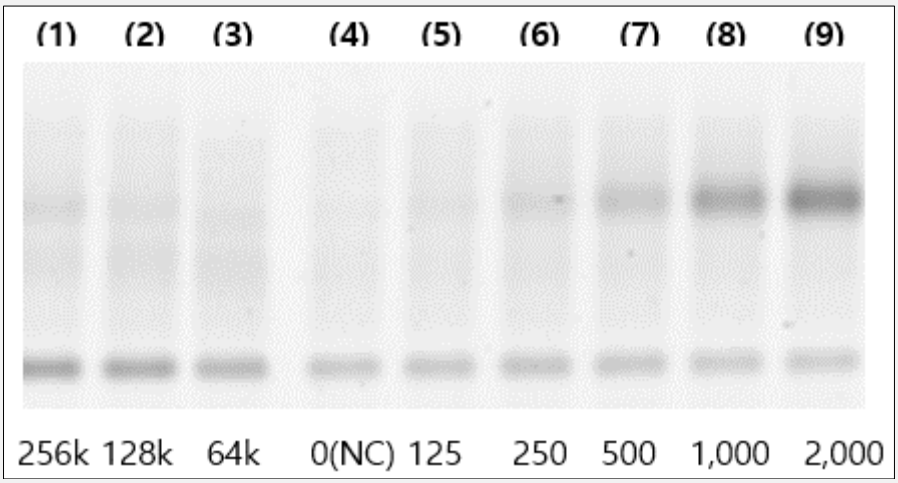

Figure 2. Patterns of urine PEP in RBC-spiked samples.

1) - 3) Non-lysed group, 4) Negative control, 5) - 9) Lysed group.

leased and becomes detectable on electrophoresis. Further investigation into this relationship could provide deeper insights into how the degree of lysis correlates with the appearance of a band on urine PEP.

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