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Case Report

Fibrinogen interference mimicking monoclonal band in serum protein electrophoresis of hemodialysis patient

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Abstract

Serum protein electrophoresis (SPE) is an important laboratory technique in the diagnosis of multiple myeloma. Analytical interference affects SPE as well as many other laboratory methods. Here, we have presented the fibrinogen interference that mimics the monoclonal band in the SPE in the sample sent in the gel biochemistry tube. A female patient presented to the emergency department with abdominal pain, nausea, and decreased urine output. In patients with high serum creatinine and blood urea nitrogen levels, a complete blood count has been found to be compatible with the presence of pancytopenia. Although an abnormal band similar to the M-spike protein was observed in the β/γ-region on the SPE evaluation, no monoclonal immunoglobulin was found in immunofixation electrophoresis. It was assured that the sample sent for SPE was collected in the correct tube (gel biochemistry tube). It was noted that the patient was receiving hemodialysis treatment while the sample was sent for SPE. It was considered that the specimen of the patient was contaminated with heparin. To test this theory, we added 1 ml of heparin to 5 ml of blood taken from a healthy subject in a gel biochemistry tube. Similarly, we observed that a band formed between the beta-gamma region in the SPE. Interferences are one of the most important causes of laboratory errors. Because they can have clinically important consequences such as misdiagnosis and treatment, laboratory specialists need to recognize these interferences and inform clinicians about them.

Keywords: Hemodialysis, laboratory interferences, m-spike protein, serum protein electrophoresis

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Multiple myeloma (MM) is a plasma cell neoplasm characterized by hypercalcemia, renal failure, anemia, and bone lesions, resulting from the proliferation of malignant cells in the bone marrow [1]. Serum protein electrophoresis (SPE) is an important laboratory technique for the diagnosis of MM. In SPE, separated according to their charge and molecular weight in the agarose carrier environment. After the separation of the proteins, the presence of the M-spike protein is examined. Subsequently, monoclonal immunoglobulins can be typed by immunofixation electrophoresis (IFE) [2].

Interferences are an important cause of laboratory errors. Because they can have clinically important consequences such as misdiagnosis and treatment, laboratory experts recognize these interferences and inform clinicians. Analytical interference affects many laboratory methods, and SPE and IFE are no exceptions [3].

Here, we sought to identify the abnormal band in the beta-gamma region in SPE performed to exclude the diagnosis of MM in a patient hospitalized with pancytopenia and acute renal failure. Trap interference mimicking the monoclonal band due to the presence of fibrinogen was presented to the laboratory in the sample, accepted with a serum separator tube (SST) containing no anticoagulant EDTA, citrate, etc.

Case Report

A 31-year-old female patient presented to the hospital with abdominal pain, nausea, and decreased urine output. A complete blood count with high serum creatinine and blood urea

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nitrogen levels revealed that pancytopenia was present. The hospitalization of the patient for evaluation and treatment was considered necessary. Additional tests performed during the patient's follow-up are presented in Table 1.

In addition to the tests in Table 1, SPE was requested. On the evaluation of the SPE, an abnormal band resembling M-spike protein was observed in the β/γ-zone. No monoclonal immunoglobulin was found in the IFE performed to identify this band in the SPE (Fig. 1a). It was assured that the sample sent for SPE was placed in the correct tube (SST).

When the laboratory team spoke with the patient's physician and examined the clinical course, it was determined that the patient was receiving hemodialysis treatment while the sample was sent for SPE. It was noted that the sample was taken directly from the hemodialysis catheter. During hemodialysis, anticoagulants such as heparin may be used to prevent clotting in dialysis sets and membranes. In addition, the length of the patient's Apt t-test suggested that the patient sample was contaminated with heparin. To test this theory, we added 1-mL heparin to 5-mL blood obtained from a healthy subject. Similarly, we observed that a band was formed between the beta-gamma region in the SPE (Fig. 1b). It has been understood that this band is fibrinogen with heparin effects.

Discussion

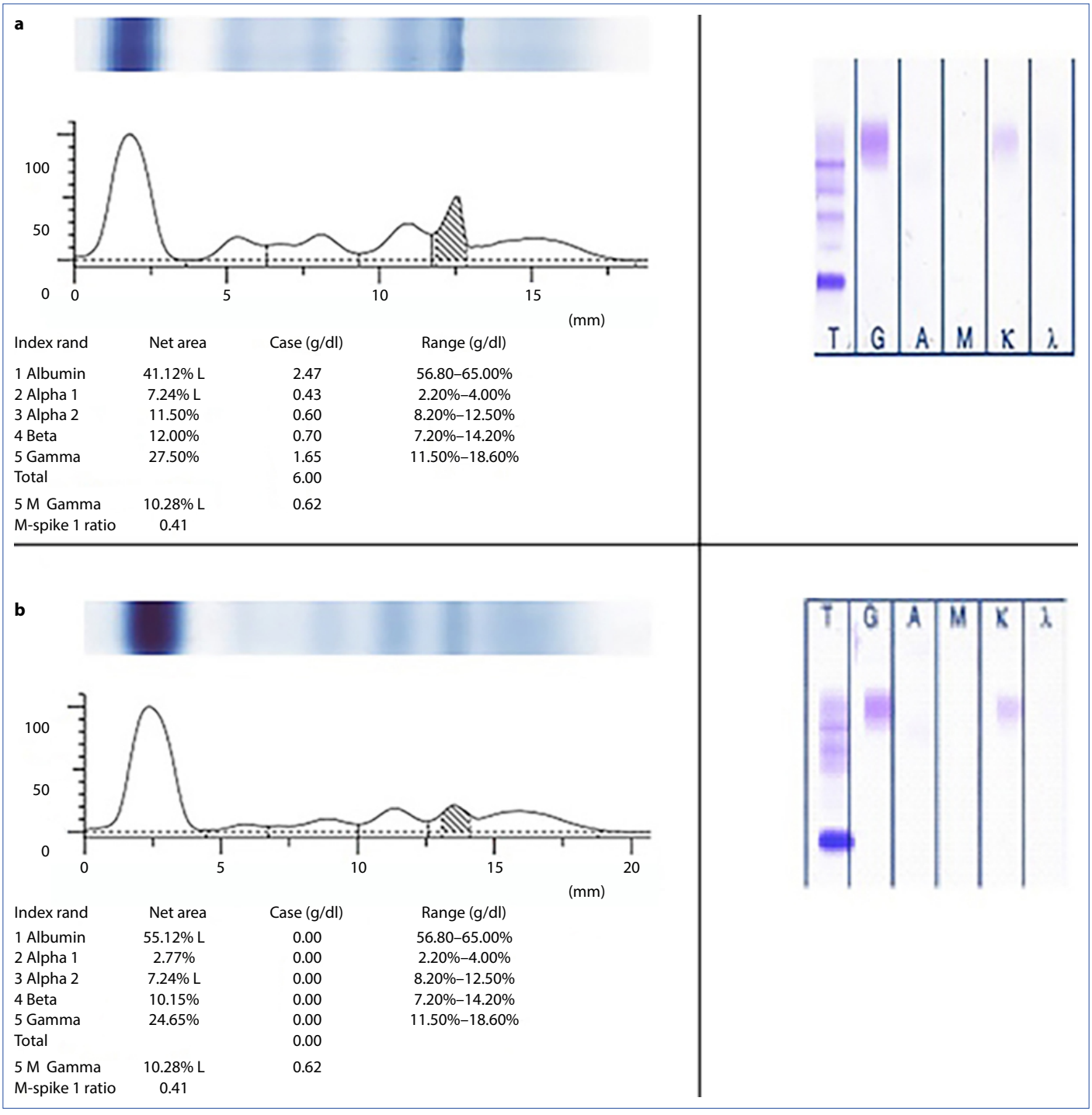
Pre-analytical interferences are an important cause of laboratory errors. Laboratory experts must recognize these interferences because they may have clinically important consequences, such as misdiagnosis and treatment. In this study, a band resembling the M-spike protein was observed in the β/γ-region of a female patient who presented to our hospital with complaints of abdominal pain, nausea, and decreased urine output. No monoclonal immunoglobulin was found in the IFE. Based on our subsequent investigations, we found that this band in the sample was formed by fibrinogen.

Interferences can originate both from naturally occurring endogenous substances and exogenous compounds due to medical treatments. Endogenous interferences that may

Table 1. Laboratory tests of the patient

Test name	Value	Reference value	Unit
WBC	2.2	4–10.5	10^3 mm^3
RBC	3.8	4.2–5.4	10^6 mm^3
HGB	10.2	12.5–16	g/dl
PLT	62.2	150–450	10^3 mm^3
NEU	0.22	2–7.15	10^3 mm^3
Creatinine	6.6	0.66–1.09	mg/dl
BUN	43	8–20	mg/dl
LDH	1564	<248	U/L
CRP	105.2	0–5	mg/dl
Kappa/Lambda (free)	0.72		
Kappa light chains (free)	134.0	6.7–22.4	mg/L
Lambda light chain (free)	187.0	8.3–27.0	mg/L
Immunoglobulin IgA	2.67	0.7–4	g/L
Immunoglobulin IgG	11.30	7–16.	g/L
Immunoglobulin IgM	0.91	0.04–2.30	g/L
Beta-2 microglobulin	51.30	1.09–2.53	mg/L
C4 complement	0.33	0.1–0.4	g/L
C3 complement	1.34	0.9–1.8	g/L
aPTT	72.2	25–37.5	s
Prothrombin time	11.1	9.5–13.2	s
INR	0.97	0.8–1.2	
ANCA	Negative		
U1-snRNP/sm/dsDNA/riposomal p-proteins	Negative		
Nucleosom/histon/centromer protein B	Negative		
SS-A/SS-B/Scl-70 Jo-1/mi-2/Ku/	Negative		

WBC: White blood cell; RBC: Red blood cells; HGB: Hemoglobin; PLT: Platelets; NEU: Neutrophils; BUN: Blood urea nitrogen; LDH: Lactate dehydrogenase; CRP: C-reactive protein; aPTT: Active partial thromboplastin time; INR: International normalized ratio; ANCA: Antineutrophil cytoplasmic antibodies.



Index rand	Net area	Case (g/dl)	Range (g/dl)
1 Albumin	41.12% L	2.47	56.80–65.00%
2 Alpha 1	7.24% L	0.43	2.20%–4.00%
3 Alpha 2	11.50%	0.60	8.20%–12.50%
4 Beta	12.00%	0.70	7.20%–14.20%
5 Gamma	27.50%	1.65	11.50%–18.60%
Total		6.00	
5 M Gamma	10.28% L	0.62	
M-spike 1 ratio	0.41		

Index rand	Net area	Case (g/dl)	Range (g/dl)
1 Albumin	55.12% L	0.00	56.80–65.00%
2 Alpha 1	2.77%	0.00	2.20%–4.00%
3 Alpha 2	7.24% L	0.00	8.20%–12.50%
4 Beta	10.15%	0.00	7.20%–14.20%
5 Gamma	24.65%	0.00	11.50%–18.60%
Total		0.00	
5 M Gamma	10.28% L	0.62	
M-spike 1 ratio	0.41		

Figure 1. SPEs and IFEs. (a) SPE and IFE of the patients, (b) SPE and IFE of the expriment sample.
SPEs: Serum protein electrophoresis; IFEs: Immunofixation electrophoresis.

affect the SPE/IFE test are primarily hemolysis and fibrinogen, whereas exogenous compounds include radiocontrast dyes, antibiotics, and recently used monoclonal antibodies [4]. Other interferences may also occur in the evaluation of SPE. For example, hemolysis causes hemoglobin and hemoglobin-haptoglobin complexes to appear as separate bands in the a 2 and β regions [4]. IgG4-related disease can also be seen similar to monoclonal bands between SPE and the β and γ-fractions [5]. Most monoclonal therapeutics may also appear as monoclonal IgG kappa by IFE at pharmacological concentrations. These drugs may cause M proteins to appear in the system because of their use, thus leading to a misinterpretation of the disease's response [4]. Similar to our case, Hryszko et al. [6] demonstrated fibrinogen interference in two hemodialysis patients. Rade et al., in their study to avoid this hemodialysis-induced interference,

found that the interference in the sample was minimized by transferring the blood collected in the standard SST to the rapid separator tube. However, although the method found by Rade et al. [7] is less expensive than IFE, it cannot be said to be more practical. Since it is not known how much heparin the samples are exposed to, standardization of the method seems difficult.

Fibrinogen interference is well-known in theory but often overlooked in routine clinical practice. Considering and measuring fibrinogen interference with laboratory techniques are extremely important in preventing misdiagnosis and delayed or under diagnosis of monoclonal gammopathies. In this publication, a serum sample of a hemodialyzed patient that contains residual heparin we have been reported as a cause of fibrinogen interference (incomplete clotting and consequently fibrinogen interference in SPE). Fibrinogen interference is seen as M paraprotein in the beta-gamma region in the SPE test. At this point, the serum immunofixation test, accepted as a reflex test for distinguishing monoclonal proteins from other proteins such as fibrinogen, has a critical role as we presented in this case.

It is important to evaluate SPE in the first step in the diagnosis of MM. If M-spike is detected, M-spike protein is typed using IFE [3]. Interference in SPE causes confusion and loss of time and money in the evaluation of the results in the diagnosis of MM. This is because the more sensitive IFE test performed to rule out possible confusion is a more costly test than both time-consuming and SPE. Therefore, we wanted to emphasize the importance of following the correct rules for blood collection to avoid interferences that falsely impact the diagnostic process and delay the treatment.

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References

1. Bladé J, Bruno B, Mohty M. Multiple Myeloma. In: Carreras E, Dufour C, Mohty M, Kröger N, editors. The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies. 7th ed. Cham (CH): Springer; 2019. Chapter 80. [CrossRef]
2. Morrison T, Booth RA, Hauff K, Berardi P, Visram A. Laboratory assessment of multiple myeloma. Adv Clin Chem 2019;89:1–58. [CrossRef]
3. Iceede F, Mbah HA, Dakata A, Gwarzo DH, Abdulrahman SA, Kuliya-Gwarzo A. Evaluating laboratory request forms submitted to hematology and blood transfusion departments at a hospital in Northwest Nigeria. Afr J Lab Med 2016;5(1):381.
4. McCudden CR, Jacobs JFM, Keren D, Caillon H, Dejoie T, Andersen K. Recognition and management of common, rare, and novel serum protein electrophoresis and immunofixation interferences. Ann Clin Biochem 2018;51:72–9. [CrossRef]
5. Jacobs JF, van der Molen RG, Keren DF. Relatively restricted migration of polyclonal IgG4 may mimic a monoclonal gammopathy in IgG4-related disease. Am J Surg Pathol 2014;142(1):76–81. [CrossRef]
6. Hryszko T, Brzosko S, Rydzewska- Rosolowska A, Mysliwiec M. Abnormal serum protein electrophoresis in hemodialysis patients. Clin Kidney J 2010;3:201. [CrossRef]
7. Rade A, Uras A, Kocijan I, Banković Radovanović P, Turčić A. Simple thrombin-based method for eliminating fibrinogen interference in serum protein electrophoresis of hemodialysed patients. Biochem Med (Zagreb) 2020;30(2):020705. [CrossRef]