Interference with creatinine assay by IgM-λ monoclonal protein in lithium heparin blood collection tube from a malignant lymphoma patient

Nobuyasu Yukimasa[1*], Wataru Oboshi[1], Keisuke Hayashi[1], Hiroko Kuribayashi[2], Ryuichi Uzawa[3], Kunihiko Fukuchi[3], Takehiro Nakamura[1*]

[1]Department of Medical Technology, Kagawa Prefectural University of Health Sciences, Takamatsu, Kagawa, Japan
[2]Clinical laboratory, Showa University Northern Yokohama Hospital, Yokohama, Kanagawa, Japan
[3]Department of Pathology, Showa University Hospital, Tokyo, Japan

ABSTRACT

Aims: Analytical interference in the clinical laboratory is a well-known phenomenon. When analyzing samples containing monoclonal immunoglobulins (M proteins), various problems could be encountered in the clinical laboratory data.
Presentation of Case: The authors report a patient with false elevated plasma creatinine concentration due to interference of monoclonal IgM in a routine test for creatinine determination. In the present case the interference might be caused by M protein precipitation, due to an increased turbidity and an apparent increase of absorbance in the clinical chemistry creatinine assay. Sia water test can provide a first clue to M protein aggregation; confirmation can be obtained by observing the time/absorbance curves of the analysis. When a Sia water test was performed with changes in the salt concentration, protein aggregation occurred at a higher salt concentration for the lithium (Li)-heparin plasma sample than the serum.
Discussion and Conclusion: These results suggested that the M protein of this patient, when interacted with Li-heparin, is more readily insolubilized. Subsequently, a reaction reproduction experiment was performed by adding the M protein component roughly purified to Li-heparin blood collection tubes. These results might support the aggregation of the M protein of this patient in the reaction solution due to its interaction with Li-heparin.

Keywords: creatinine measurement, lithium (Li)-heparin plasma, monoclonal immunoglobulin (M protein), Sia water test

1. INTRODUCTION

Analytical interference in the clinical laboratory is a well-known phenomenon and has many different causes. In daily laboratory tests, interactions between co-existing substances in samples and the components of reaction reagents sometimes affect measurement values, resulting in values deviating from the actual values. Such interference can be caused by abnormal reactions due to monoclonal immunoglobulins (M proteins) [1]. Concerning test items frequently affected by such reactions, a previous review showed false-positive results on the measurement of inorganic phosphate [2]. Abnormal reactions due to M proteins are often caused by their aggregation and turbidity in reaction solutions, affecting various laboratory test items in addition to inorganic phosphate [3-7]. Interferences caused by immunoglobulins are more difficult to test, however they should be important.

The aim of the study is to encountering a patient with B-cell malignant lymphoma in whom an interaction between IgM-λ type M protein and lithium (Li)-heparin affected the measurement value of creatinine.

2. PRESENTATION OF CASE

A 60-year-old Japanese male visited the Department of Hematology of Showa University Hospital in 1993 for treatment after the excision of tongue cancer, and was admitted and diagnosed with malignant B-cell lymphoma by histopathological examination and cytology. In 1998, the initial treatment was completed (after 5 years he was discharged). In 2006, he was admitted again due to suspected pulmonary infiltration of lymphoma. Table 1 demonstrates the findings of emergency biochemical tests and the measurement of immunoglobulins at the time of re-admission in May 2006. There was dissociation between the urea nitrogen (15.1 mg/dL) and creatinine (3.6 mg/dL) values. Since the previous creatinine value before admission was normal, there was also dissociation between the previous and emergency test values. Therefore, the cause of the high creatinine value shown by the emergency test was investigated.

Table 1. Summary of clinical laboratory data.

Biochemistry	Patient values
total-protein	8.2 g/dl
albumin	3.8 g/dl
total-bilirubin	0.4 mg/dl
direct-bilirubin	< 0.1 mg/dl
BUN	15.1 mg/dl
creatinine	3.6 mg/dl
AST	31IU/l
ALT	15 IU/l
LDH	222 IU/l
CK	25 IU/l
amylase	292 IU/l
Na^+	134.3 mEq/l
K^+	5.0 mEq/l
Cl^-	98.0 mEq/l
CRP	< 0.2 mg/dl
IgG	1129 mg/dl
IgA	320 mg/dl
IgM	2138 mg/dl

BUN: blood urea nitrogen, AST: aspartate transaminase, ALT: alanine transaminase, LDH: lactate dehydrogenase, CK: creatine kinase, CRP: C-reactive protein, Ig: Immunoglobulin

Serum protein fractionation confirmed an M band in the γ fraction (Figure 1-a). Immunoelectrophoresis performed later revealed a reaction due to the anti-IgM and anti-L chain λ antibodies as a retention precipitation line toward the origin (Figure 1-b), and the sample was treated with 2-mercaptoethanol (2-ME). A clear M-bow was observed, and the IgM-λ type M protein was identified (Figure 1-c). Bone marrow puncture showed slight abnormalities such as the accumulation of small lymphocyte-like cells with a narrow cytoplasm and twisted nucleus (Figure 1-d).

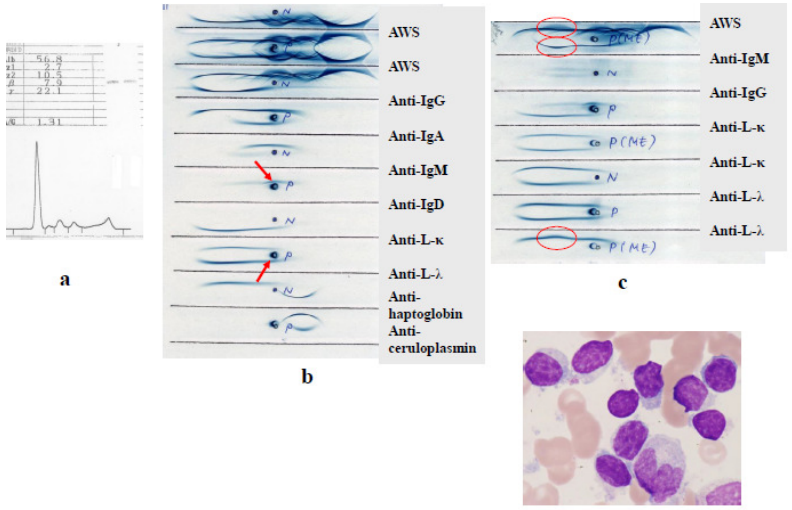

Fig. 1. (a): Serum protein fractionation. (b): Immunoelectrophoretic pattern. (c): Immunoelectrophoretic pattern after 2-mercaptoethanol (2-ME) treatment. (d): Myelogram.

P; patient serum. N; normal serum. AWS; anti whole serum. The arrow indicates an abnormal precipitin. The circle indicates M-bow with monoclonal immunoglobulin. Atypical lymphoid cells can be seen by Wright-Giemsa staining (x 1,000) in (d). These results were detected after the start of therapy; the peak of M protein was attenuated.

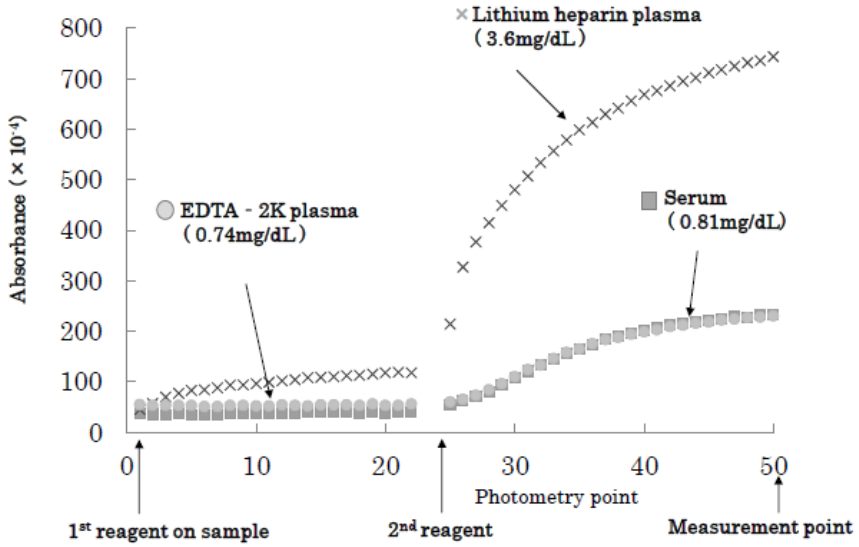

Fig. 2. Reaction monitor plots for creatinine on Hitachi 7350 automated chemistry analyzer.

The index (×) lithium heparin plasma had creatinine measured as 3.60 mg/dL. The control serum (□) and EDTA plasma (○) had creatinine measured as 0.81 mg/dL and 0.74 mg/dL, respectively.

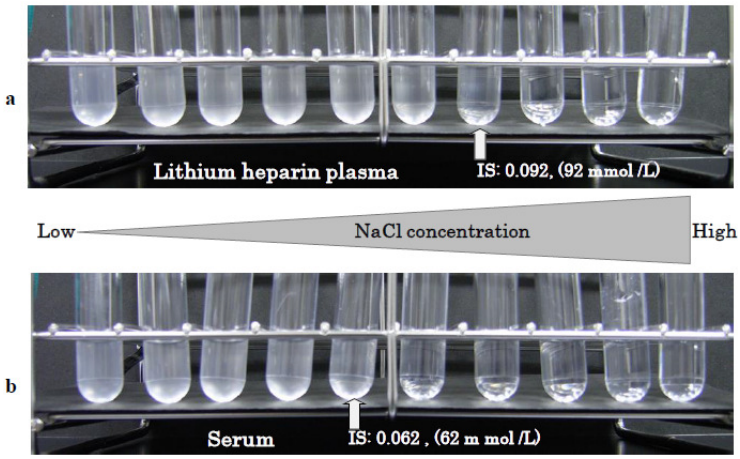

Fig. 3. Sia euglobulin precipitation test: precipitate formation with the gradient of ionic strength (IS) and sodium chloride concentrations.

(a): Lithium heparin plasma. (b): Serum. attenuated.

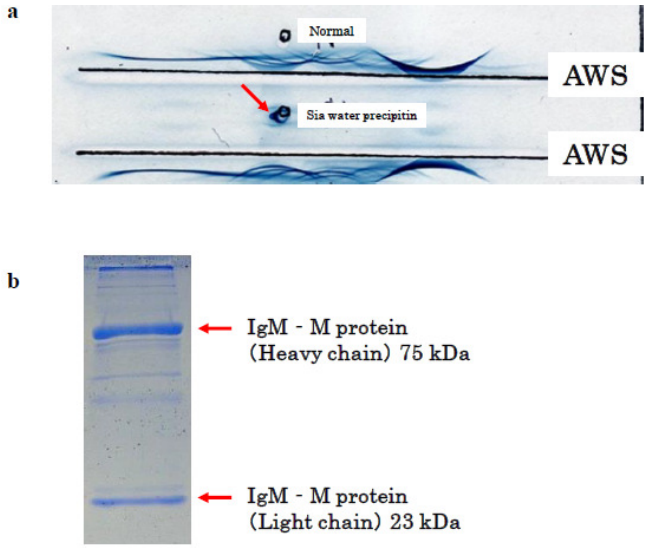

Fig. 4. The main components of the sia water test purification product.

(a): Immunoelectrophoresis (IEP). (b): Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The arrow indicate the M protein components.

2.1 Evaluation of abnormal reactions

2.1.1 Measurement of creatinine using other types of specimen

In Showa University Hospital, blood collection tubes with Li-heparin (Venoject Ⅱ; Terumo Co.) were used for emergency biochemical tests. To determine whether this dissociation in the creatinine value depends on the plasma a component, measurement was performed using an EDTA-2K plasma sample presented for the complete blood counting (CBC) test. The creatinine concentration in this sample was 0.75 mg/mL, approximating to that in the serum sample.

Creatinine was measured using the enzyme method (Aqua-auto Kinos CRE-Ⅱ test kit; Kainos Laboratories, Inc.) on an automatic analyzer (7350 Clinical Analyzer; Hitachi High-Technologies, Ltd.).

2.1.2 Changes in absorbance in the measurement of creatinine

The reaction process was compared between the Li-heparin plasma sample and the other 2 types of sample. The reaction process did not differ between the serum and EDTA-2K plasma samples. However, in the Li-heparin sample, absorbance increased after the addition of the first reagent and after the addition of the second reagent (Figure 2). When the creatinine measurement reaction was reproduced in test tubes, turbidity occurred in the Li-heparin plasma sample after the addition of the second reagent, confirming that the cause of the increase in absorbance was protein aggregation. The ionic strength of the second reagent is 0.070 in this kit.

2.1.3 Sia water test (Sia euglobulin precipitation test)

The Sia water test [8] is a classical method for the identification of IgM type M protein in macroglobulinemia. Its usefulness is recognized even at present [9]. The Sia water test using samples from this patient was positive for all of the Li-heparin plasma, EDTA plasma, and serum samples. However, a serum sample presented around the same time from another patient (multiple myeloma) with the IgM-λ type M protein for the control was negative, showing no influences on the creatinine measurement.

In this patient, since both the plasma and serum samples were positive for the Sia water test, evaluation was performed to determine whether or not the salt concentration causing turbidity differs between the Li-heparin plasma and serum samples. In Figure 3, the left end indicates a salt concentration of 0 mmol/L, and the right end indicates the maximum salt concentration (139 mmol/L). The upper photograph shows the addition of a Li-heparin plasma sample, and turbidity was observed at a salt concentration of 92 mmol/L (ionic strength: 0.092). The lower photograph shows the addition of a serum sample, and turbidity was observed at a salt concentration of 62 mmol/L (ionic strength: 0.062). Thus, the salt concentration causing turbidity differed between the Li-heparin plasma and serum samples.

2.1.4 Reproduction of the interaction between purified M protein and Li-heparin

The protein component was washed and the roughly purified M protein component of the patient was re-dissolved in physiological saline. This solution (1 mL) was added to a Li-heparin blood collection tube, and creatinine was measured. As a result, no reaction was observed in the measurement of creatinine using a control sample without Li-heparin (measurement value: 0.03 mg/dL), but a measurement value was obtained using a sample with Li-heparin (0.64 mg/dL). These results suggest that the interaction between the M protein component of this patient and Li-heparin affected the measurement of creatinine. The main component of the purification product was confirmed to be IgM type M protein by immunoelectrophoresis (IEP) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Figure 4).

3. DISCUSSION

In daily laboratory tests, abnormal values that do not reflect the pathological condition are often encountered. As a cause of such abnormal values, interference by M proteins has frequently been reported. In particular, the aggregation reaction of M proteins in the reaction solution is known to be a problem [1-7].

The accurate mechanism of the protein aggregation reaction remains unclear. However, a previous study suggested that differences in the isoelectric point among proteins are associated with solubility [10]. The acidity of the reaction reagent affects the protein conformation, resulting in an aggregation reaction [11]. We considered the possible influences of the ionic strength, and performed a Sia water test. Both the serum and plasma samples were positive. Our results suggested that the M protein of this patient, when interacted with Li-heparin, is more readily insolubilized. Subsequently, a reaction reproduction experiment was performed by adding the M protein component roughly purified using the Sia water test to Li-heparin blood collection tubes. After the addition of the roughly purified M protein to Li-heparin, a creatinine value was obtained. However, no reaction was observed using only the roughly purified M protein component as a control. These results support the aggregation of the M protein of this patient in the reaction solution due to its interaction with Li-heparin.

Concerning the ability of ions to promote the aggregation of high molecular weight colloids, Hofmeister series are known, and among cations, Li has the strongest ability [12]. Therefore, there is a possibility that the aggregation ability of the patient's M protein was increased by Li ions, resulting in abnormal reactions. The influences of M protein aggregation on measurement values are often associated with the IgM type M protein. Regarding its mechanism, the charge state rather than molecular hydrophobicity is involved. Inhibition of aggregation was reported to be possible using charged molecules such as EDTA [13]. The serum of the myeloma patient with the IgM-λ type M protein as a control was negative for the Sia water test. However, when M proteins which are produced and increased as monoclonal clones due to cell changes into tumors, are charged molecules with a marked tendency to become insoluble, their possible influences on the measurement systems of laboratory tests are considered.

Li-heparin blood collection tubes are used for emergency biochemical tests because of advantages such as reductions in fibrin deposition and the time from sample presentation until tests. Using Li-heparin tubes, the measurement values of potassium and lactate dehydrogenase (LD) were reported to increase [14]. This was a rare case of abnormal measurement reactions due to the combination of the M protein and Li-heparin. The overlooking of examination data due to abnormal reactions sometimes leads to serious medical malpractice [15]. When Li-heparin blood collection tubes are used, the possibility of such abnormal reactions should be recognized.

4. CONCLUSIONS

The present report could be important for an abnormal phenomenon in daily laboratory tests. We are going to apply this case as a document for group working discussion in the lectures of clinical immunology curriculum. The present case might be beneficial for especially clinicians working in Hematology, Oncology, Emergency, and Biochemistry Departments.

ACKNOWLEDGEMENTS

The authors thank Prof. Richard F. Keep (University of Michigan) for the useful discussion.

COMPETING INTERESTS

The authors declare no conflict of interests associated with this manuscript.

AUTHORS' CONTRIBUTIONS

Nobuyasu Yukimasa designed the study, wrote the protocol, and wrote the first draft of the manuscript. Wataru Oboshi, Keisuke Hayashi, Hiroko Kuribayashi, Ryuichi Uzawa, Kunihiko Fukuchi and Takehiro Nakamura managed the analyses of the study. All authors read and approved the final manuscript.

ETHICAL APPROVAL

The present study was performed in compliance with the guiding principles of "Opinions of the Japanese Society of Laboratory Medicine about Utilization of Specimens after Laboratory Examinations for Laboratory Work, Education, and Clinical Studies (2006)", and written consent was obtained from all participating subjects in accordance with the Declaration of Helsinki.

REFERENCES

1. Berth M, Delanghe J. Protein precipitation as a possible important pitfall in the clinical chemistry analysis of blood samples containing monoclonal immunoglobulins: 2case reports and a review of the literature. Acta Clinica Belgica 59: 263-273,2004

2. Larner AJ. Pseudohyperphosphatemia. Clin Biochem 28: 391-393, 1995

3. Hullin DA. An IgM paraprotein causing a falsely low result in an enzymatic assay for acetaminophen. Clin Chem 45: 155-156, 1999

4. Hummel KM, von Ahsen N, Kühn RB, Kaboth U, Grunewald RW, Oellerich M. Pseudohypercreatininemia due to positive interference in enzymatic creatinine measurements caused by monoclonal IgM in patients with Waldenström's macroglobulinemia. Nephron 86: 188-189, 2000

5. Tokmakjian S, Moses G, Haines M. Excessive sample blankings in two analyzers generate reports of apparent hypoglycemia and hypophosphatemia in patients with macroglobulinemia. Clin Chem 36: 1261-1262, 1990

6. Langman LJ, Allen LC, Romaschin AD. Interference of IgM paraprotein in the Olympus AU800 uric acid assay. Clin Biochem 31: 517-521, 1998

7. Smogorzewska A, Flood JG, Long WH, Dighe AS. Paraprotein interference in automated chemistry analyzers. Clin Chem 50: 1691-1693, 2004

8. Franglen G. A modification of the water-dilution test for screening macroglobulinaemic sera, Clin Chim Acta, 14: 559-561, 1966

9. Mockli GC. The sia euglobulin precipitation test revisited. Clin Chem 44: 1190, 1998

10. Chi EY, Krishnan S, Randolph TW, Carpenter JF. Physical stability of proteins in aqueous solution: mechanism and driving forces in nonnative protein aggregation. Pharm Res 20: 1325-1336, 2003

11. Jiskoot W, Bloemendal M, van Haeringen B, van Grondelle R, Beuvery EC, Herron JN, Crommelin DJA. Non-random conformation of a mouse IgG_{2a} monoclonal antibody at low pH. Eur J Biochem 201: 223-232, 1991

12. Curtis RA, Ulrich J, Montaser A, Prausnitz JM, Blanch HW. Protein-protein interactions in concentrated electrolyte solutions. Biotechnol Bioeng 79: 367-380, 2002

13. Sharma L, Baker J, Brooks AM, Sharma A. Study of IgM aggregation in serum of patients with macroglobulinemia. Clin Chem Lab Med 38: 759-764, 2000

14. Dimeski G, Solano C, Petroff MK, Hynd M. Centrifugation protocol: tests to determine optimal lithium heparin and citrate plasma sample quality. Ann Clin Biochem 48: 218-222, 2011

15. Rotmensch S, Cole LA. False diagnosis and needless therapy of presumed malignant disease in women false-positive human chorionic gonadotropin concentrations. Lancet 355: 712-715, 2000