

Paraproteins: A Potential Source of Fallacy in Bilirubin Assay

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ABSTRACT

Introduction: Plasma cell neoplasms are a heterogeneous group of disorders characterized by the proliferation of clonal plasma cells with or without overproduction of monoclonal proteins. Paraproteins may lead to fallacious results in different biochemical assays.

Objective: The aim of this study was to detect paraprotein-induced fallacy in the direct bilirubin assay in patients with plasma cell neoplasms.

Methodology: This is a year-long observational study conducted in the department of biochemistry at a regional cancer institute in eastern India. Routine biochemistry tests were run on dry chemistry using the Ortho Clinical Diagnostics Vitros 4600® and wet chemistry using the Beckman Coulter DxC 700 AU®.

Results: A total of 82 cases fulfilling the inclusion criteria were included in the study. Out of 82 patients, 79 had normal bilirubin assays, and three cases had fallacious direct bilirubin assay on wet chemistry using the Beckman Coulter DxC 700 AU®. Fallacy was negated when these samples were run on dry chemistry.

Conclusion: Paraproteinemia might interfere with direct bilirubin assay in wet chemistry, leading to spurious results in a few patients. Removal of paraprotein or utilization of dry chemistry might offer a solution to paraproteinemia-induced interference.

Keywords: Bilirubin assay, Dry chemistry, Fallacious results, Paraproteins, Plasma cell neoplasm, Wet chemistry.

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INTRODUCTION

Plasma cell neoplasms are a heterogeneous group of disorders characterized by the proliferation of clonal plasma cells with or without overproduction of monoclonal proteins. Almost all patients with multiple myeloma evolve from an asymptomatic premalignant stage termed monoclonal gammopathy of undetermined significance.¹⁻⁵ When multiple myeloma is suspected clinically, patients should be tested for the presence of M proteins using a combination of tests that should include serum protein electrophoresis (SPEP), serum immunofixation electrophoresis (SIFE), and the serum free light chain (FLC) assay. Besides these, some biochemical, hematological, and radiological investigations are needed for diagnostic, therapeutic, and prognostic monitoring. Plasma cell neoplasm is associated with various interferences in biochemical assays. Paraprotein is associated with interferences in various biochemical assays by virtue of polymerization and precipitation, which may lead to fallacious results.⁶⁻⁸ Very little information is present in the literature. Occasional case reports have been published across the globe. In a literature search, no published data from southeast Asia or the Indian subcontinent were found. Spurious biochemical assays constitute analytical errors, which must be studied to recognize the effects of paraprotein on biochemical assays.

OBJECTIVE

The aim of this study was to detect paraprotein-induced fallacy in the direct bilirubin assay in patients with plasma cell neoplasms.

METHODOLOGY

This is a hospital-based observational study conducted in the department of biochemistry at a regional cancer institute in eastern India from January 2022 to March 2023. All the newly

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diagnosed cases of plasma cell neoplasms, except those who were on treatment, were included in the study using the purposive sampling technique. Relevant clinical history was collected. After that, they underwent routine investigations including complete blood count (CBC), peripheral smear examination, liver function test (LFT), serum urea, serum creatinine, serum calcium, serum lactate dehydrogenase (LDH), and SPEP. Bilirubin assays were run on dry chemistry using the Ortho Clinical Diagnostics Vitros 4600® and wet chemistry using the Beckman Coulter DxC 700 AU®. Electrophoresis and immunotyping were done in a fully automated capillary electrophoresis Capillary 3 Octa Sebia®. Two milliliters of venous blood was collected in red-topped vials and allowed to clot for 30 minutes. Then, it was centrifuged at 4,000 rpm for 10 minutes. Serum was separated, and routine biochemistry parameters were run on the same day. Hemolyzed and grossly lipemic samples were rejected. Descriptive statistical analysis was done using MS Excel 2013.

RESULTS

A total of 82 cases fulfilling the inclusion criteria were included in the study. Details of biochemical assays are described in Table 1. Out of 82 patients, 79 had normal bilirubin assay, and three cases had direct bilirubin more than total bilirubin when analyzed on wet chemistry using the Beckman Coulter DxC 700 AU® (Tables 1 and 2; Figs 1 to 3). Repeat assay was done with dilution 1:3, but it did not improve the results in two patients. The precision of bilirubin assay from these samples was not good. The results of bilirubin assay in these three patients were not clinically correlated. When these samples were run on dry chemistry using the Ortho Clinical Diagnostics Vitros 4600® on the same day, direct bilirubin was within the normal reference range. Details of biochemical assay and electrophoretic pattern of these three patients are given in Table 2.

DISCUSSION

In the current study, 75% ($n = 59$) of patients diagnosed with plasma cell neoplasms were over 50 years of age. A higher prevalence was noted among male patients, with a male-to-female ratio of

2.15:1. These findings align with global and Asian epidemiological trends.^{9,10} However, contrary data were reported by Hussain et al., who observed a female predominance.¹¹

The major biochemical abnormalities and electrophoretic patterns are presented in Table 1. Clonal plasma cell proliferation leads to excessive production of monoclonal (M) protein in serum and urine, accounting for many of the observed biochemical changes. Approximately 15% of patients had elevated serum creatinine levels. According to the CRAB criteria, renal dysfunction is defined by a creatinine level exceeding 2 mg/dL.^{11,12} The CRAB acronym refers to hypercalcemia, renal dysfunction, anemia, and bone lesions. The findings in this cohort are largely consistent with other studies.^{12,13}

Among the 82 cases, 79 (96.34%) had normal bilirubin assay results, while three (3.66%) demonstrated analytically invalid values where the direct bilirubin exceeded the total bilirubin. As total bilirubin is the sum of direct and indirect fractions, such results are inherently erroneous. These tests were performed on the Beckman Coulter DxC 700 AU®, and repeat assays yielded consistent findings. Notably, these three patients had significantly

Table 1: Summary of patient details and biochemical profile

S. no.	Parameter	Derangement	Patients
1.	Direct bilirubin	Fallacious Normal	3.66% ($n = 03$) 96.34% ($n = 79$)
2.	T protein	>8.5 gm/dL	60%
3.	AG ratio	Altered	64%
4.	Creatinine	>2 mg/dL	15%
5.	M protein	Detectable	100%
6.	IgG	Detectable	80%
7.	IgA	Detectable	10%
8.	IgM	Detectable	10%
9.	Kappa (FLC)	Detectable	85%
10.	Lambda (FLC)	Detectable	15%
11.	Bisalbuminemia	Detectable	2.4%
12.	Mean age of all cases (years)	–	53.1
13.	Mean age of male cases (years)	–	58
14.	Mean age of female cases (years)	–	48.2
15.	Male to female ratio (years)	–	2.15

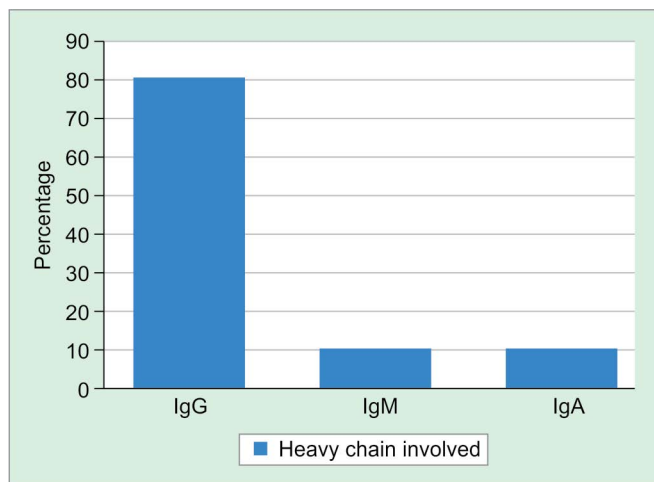


Fig. 1: Types of heavy chains

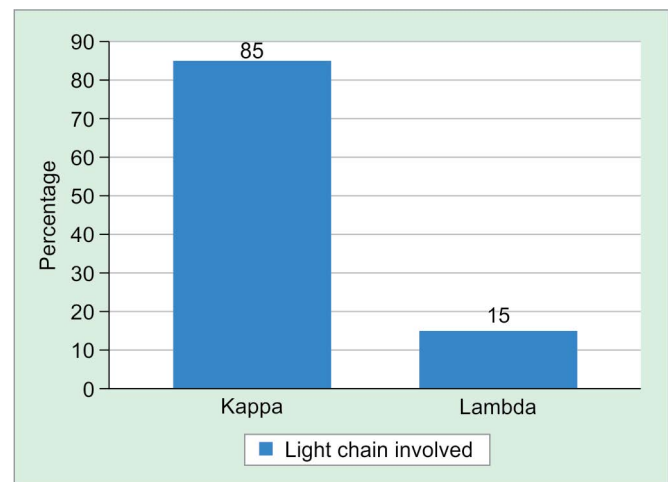
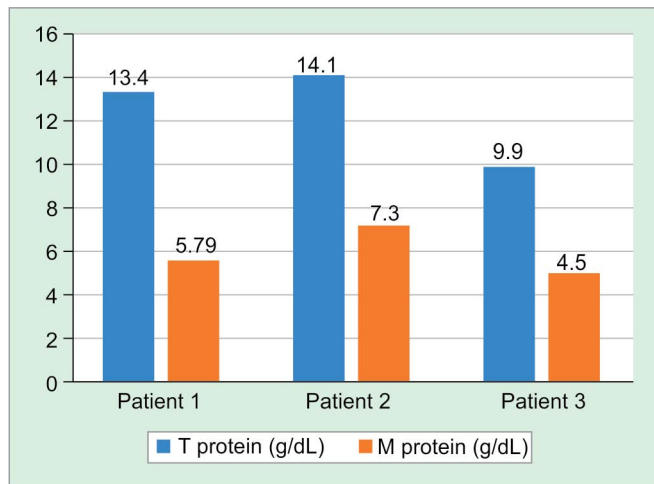
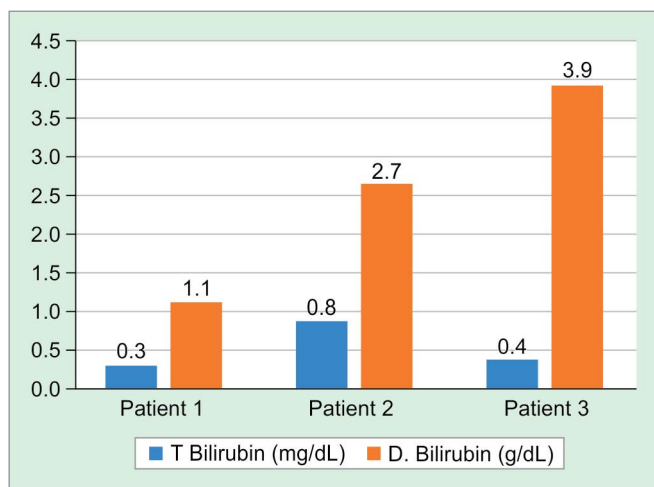


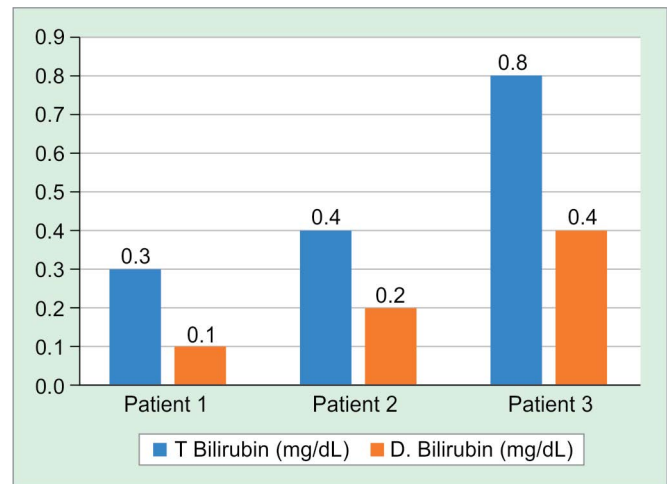
Fig. 2: Types of light chains

Table 2: Detail of patients having fallacious bilirubin assay

Parameter	Patient 1	Patient 2	Patient 3
Age	69	49	62
Gender	Female	Male	Male
Total protein (gm/dL)	13.4	14.1	9.9
Albumin (gm/dL)	2.6	2.2	2.6
Globulin (gm/dL)	11.0	11.7	8.5
AG ratio	0.23	0.18	0.31
M protein (gm/dL)	5.79	7.30	4.50
(Heavy and light chains involved) Immunotyping pattern	IgG lambda	IgG kappa	IgG kappa
Total bilirubin (wet chemistry) (mg/dL)	0.3	0.8	0.4
Conjugated bilirubin (wet chemistry) (mg/dL)	1.1	2.7	3.9
Total bilirubin (wet chemistry with dilution 1:3) (mg/dL)	0.4	0.5	0.5
Conjugated bilirubin (wet chemistry with dilution 1:3) (mg/dL)	0.1	2.1	1.2
Total bilirubin (dry chemistry) (mg/dL)	0.3	0.4	0.8
Conjugated bilirubin (dry chemistry) (mg/dL)	0.1	0.2	0.4


Fig. 3: Total protein and M protein among patients with fallacious bilirubin assay

Fig. 4: Fallacious bilirubin assay performed in wet chemistry

elevated total protein and M protein levels (Figs 3 to 5). Paraprotein-related interference in bilirubin assays is uncommon, as demonstrated by its occurrence in only 3 of 82 cases. The


Fig. 5: Normal bilirubin assay performed in dry chemistry

literature acknowledges that monoclonal gammopathies may rarely produce unreliable biochemical results.¹⁴

Interference in bilirubin assays performed *via* wet chemistry can be attributed to matrix effects—alterations in analyte detection due to the presence of interfering substances.^{7,8} These may include blood cells, high sample viscosity, or plasma components such as heterophilic antibodies,¹⁵ human antianimal antibodies (HAAA)¹⁶ or proteins including albumin, lysozyme, fibrinogen, and paraproteins.¹⁷ Paraproteins tend to polymerize and precipitate.^{1–5,16} Such effects may compromise the assay's precision by altering the analyte's detection profile. In the present study, paraproteins and increased viscosity were likely responsible for the lack of precision in bilirubin measurements.

Despite attempts at dilution (1:3 to 1:4 ratios), valid results could not be obtained. When bilirubin assays were conducted using dry chemistry on the Ortho Clinical Diagnostics Vitros 4600®, the results were consistent with the clinical findings and other laboratory values.

Bilirubin, a byproduct of hemoglobin degradation, is conjugated in the liver and eliminated *via* bile. Both conjugated (direct) and unconjugated (indirect) bilirubin circulate bound to albumin. Total bilirubin is measured *via* a modified diazo reaction using reagents that include caffeine (2.1 mmol/L), a surfactant, and 3,5-dichlorophenyldiazonium tetrafluoroborate

(0.31 mmol/L). These react with bilirubin to form azobilirubin, measured at 570/660 nm.^{18,19} Direct bilirubin is assessed using a variation of the Van den Bergh and Mueller method, also with DPD, but under highly acidic conditions without caffeine or surfactants.¹⁹ Under such acidic environments, particularly in the presence of elevated proteins, protein precipitation can occur. If the protein-stabilizing capacity of the reagent is overwhelmed, turbidity results, impairing assay precision.²⁰ Manufacturer documentation notes that rare cases of monoclonal gammopathy—particularly those involving immunoglobulin M (IgM) (e.g., Waldenström's macroglobulinemia)—may yield unreliable bilirubin measurements.^{19,21–23}

In contrast, the Vitros 4600® uses multilayered slides for bilirubin assays. A drop of the patient's sample is evenly distributed through a spreading layer to the underlying analytical layers, where bilirubin reacts with diazonium salt [4-(*N*-carboxymethylsulfonyl) benzenediazonium hexafluorophosphate] to form azobilirubin chromophores, detected at ~520 nm.^{24–26} The method includes diaphylline to dissociate bilirubin from albumin. Interferences are minimized as the spreading layer filters out molecules larger than 15000 Da,^{16,24–26} preventing paraprotein-induced turbidity and enhancing accuracy.

Documented mechanisms of paraprotein interference in wet chemistry include precipitation, turbidity, prozone and hook effects, and hyperviscosity.^{27–31} In this study, precipitation and turbidity appeared to be the main causes of assay interference.

Our institution's access to both wet and dry chemistry platforms enabled accurate bilirubin quantification. However, many laboratories lack dual-platform capacity. In such cases, strategies for mitigating paraprotein interference include ultrafiltration, deproteinization, polyethylene glycol (PEG) treatment, or sample dilution.^{27,32–38}

Limitations of the Study

We need further studies to know what factors led to interference in a few patients and not in all cases. This study did not find the interfering effect of paraproteins on other biochemical parameters.

CONCLUSION

Paraproteinemia might interfere with direct bilirubin assay in wet chemistry, leading to spurious results in a few patients. Removal of paraprotein or utilization of dry chemistry might offer a solution to paraproteinemia-induced interference.

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