1 INTRODUCTION

In clinical chemistry, pre- and post-analytical factors are the main and the most important sources of errors compared with analytical aspects. Pre-analytical errors are even more common than the post-analytical ones; thus, prompt detection and effective correction of pre-analytical interferences are necessary to achieve reliable results. In laboratory tests, interference is the effect of substances other than the proposed analyte, which reacts with the analytical method’s reagents or detection system. The interference by hemolysis, icterus, paraproteinemia, and lipemia is of great concern in laboratory tests. Laboratory results that are unacceptable can indicate the presence of an interfering factor.1–3

2 CASE PRESENTATION

Before obtaining routine biochemistry results from a DIRUI 1200 autoanalyzer, we observed an unacceptable bilirubin result; surprisingly, the conjugated/direct bilirubin was significantly greater than the total bilirubin. This problem was limited only to 1 patient. Since conjugated bilirubin is just a component of total bilirubin, the result was unacceptable. The test was repeated, and further investigation was conducted. Considering satisfactory internal quality control findings for both conjugated and total bilirubin at 2 levels (normal and high), we focused on the same sample. The same results were obtained after repeating the test. Visually, the serum sample was icteric without definite evidence of lipemia or hemolysis. After taking the patient’s history, we learned that the specimen belonged to a 41-year-old woman, a new case of hilar cholangiocarcinoma (according to liver trucut biopsy), referred for preoperational hematologic and biochemistry evaluation. Table 1 shows the initial laboratory results of the patient. We suspected excess paraproteins in the serum because of the high serum total protein and high erythrocyte sedimentation rate (ESR), which could interfere with clinical assessments. To eliminate the effect of
possible interference, we repeated the total and conjugated bilirubin tests on a diluted patient sample (1/2, 1/4, 1/8, 1/10) with distilled water. As shown in Table 2, from 1/2 to 1/8 dilution, there was a gradual decline in conjugated bilirubin without any significant change in the total bilirubin value. Finally, there was no significant change between 1/8 and 1/10 dilution; therefore, those results were considered as a reliable value. Further analysis of the specimen by serum protein electrophoresis revealed IgG polyclonal gammopathy, which could be explained by chronic inflammation due to liver malignancy. On follow-up laboratory testing (6 months following surgical treatment and chemotherapy), the serum total protein and ESR were in normal ranges, and there was no evidence of a problem in bilirubin measurement. The institutional review was not required for this study because the study was performed using deidentified discarded blood samples obtained for patient care and not for research purposes.

### Table 1 Initial laboratory results of the patient

| Test               | Reference range (unit) |
|--------------------|------------------------|
| Total bilirubin    | 11.29 0.2–1 (mg/dl)    |
| Direct bilirubin   | 15.35 0.1–0.3 (mg/dl)  |
| Total protein      | 10.1 6.5–8.5 (mg/dl)   |
| Albumin            | 4.1 3.5–5.5 (mg/dl)    |
| BUN                | 6 6–20 (mg/dl)         |
| Cr                 | 0.8 0.5–1.3 (mg/dl)    |
| AST                | 72 3–40 (IU/L)         |
| ALT                | 45 3–40 (IU/L)         |
| ALP                | 1313 80–306 (IU/L)     |
| LDH                | 536 200–400 (IU/L)     |
| ESR                | 111 0–30 (mm/hr)       |
| TSH                | 3.96 0.3–5 (mIU/L)     |
| WBC                | 15.6 4.5–11 (x10^3/μl) |
| RBC                | 2.71 4.5–5.5 (x10^3/μl) |
| HB                 | 10.0 12–16 (g/dL)      |
| HCT                | 30.4 36–48 (%)         |
| MCV                | 112.2 80–96 (f)        |
| MCH                | 36.9 27–33 (pgr)       |
| MCHC               | 32.9 32–36 (g/dL)      |
| PLT                | 534 150–450 (x10^3/μl) |

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; ESR, erythrocyte sedimentation rate; HB, hemoglobin; HCT, hematocrit; LDH, lactate dehydrogenase; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PLT, platelet; RBC, red blood cell; TSH, thyroid-stimulating hormone; WBC, white blood cell.

### DISCUSSION

Total and conjugated bilirubin concentrations are routinely measured by automated chemistry analyzers based on the endpoint chromogenic Jendrassik–Grof method. In this method, diazotized sulfanilic acid reaction with 2 pyrrole rings of bilirubin produces phenyl azo at low pH, with the difference that in the total bilirubin assay, a protein-stabilizing detergent is used to stabilize unconjugated bilirubin and accelerate the reaction. The concentration of bilirubin is directly proportional to the formation and absorption of phenyl azo at 540/600 nm. According to the manufacturers’ instructions, hemolysis and lipemia, in high concentrations, can interfere with the bilirubin test, but there are no documented data about excess protein. Proteins can cause several effects in the clinical biochemistry analysis. These analytical interferences may result from sample turbidity, chemical or immunological reaction with assay constituent, hook effect, and hyperviscosity. Some chemical analytes, including conjugated bilirubin, are sensitive to proteins’ interference. While there are many reports on monoclonal paraproteins’ interference in biochemistry autoanalysers’ results, the interference by polyclonal immunoglobulin is not yet well known. Individual proteins have unique amino acid sequences and different set points for conformational changes in response to changes in pH and ionic strength. So, each specific protein may precipitate under a particular and unpredictable assay condition, whether basic or acidic, or even neutral pH. Thus, the consequent false results appear to be instrument-, method-, kit-, and protein concentration-dependent, and maneuvers for elimination (such as serial dilution, precipitation, and ultrafiltration) may not always be practical.

Conjugated bilirubin is a part of the total bilirubin and should never exceed the total bilirubin concentration. In our case, unacceptable elevated conjugated bilirubin (higher than the total bilirubin) might be caused by a high level of protein. It seems that with the use of the Biorex kit on a DIRUI 1200 analyzer, high protein concentrations could interfere with the conjugated bilirubin assay, even in the absence of a monoclonal protein and surfactant, which is used for total bilirubin measurement, and could prevent protein precipitation in the total bilirubin assay. In this case, there was no evidence of hemolysis and lipemia, and the high total protein, ESR, and serum protein electrophoresis pattern were in favor of excess proteins. In addition, icterus has no effect on bilirubin measurement. So, the problem could be attributed to excess protein. We could eliminate the interference by sample dilution and achieve reproducible results for both total and conjugated bilirubin concentrations. In addition, after treatment of the underlying disease, the ESR and total protein returned to normal, and no similar problem was observed in the bilirubin measurement. To
our knowledge, this is the first report of polyclonal immunoglobulin interference with a conjugated bilirubin assay, which was successfully removed by dilution.

4 | CONCLUSION

In the clinical laboratory, irrational results can signal the presence of analytical interferences. In these situations, monitoring reaction curves and measuring free hemoglobin, bilirubin, triglyceride, and total protein levels could aid in identifying the culprit. Although specimen dilution or the use of various measurement methods may not totally resolve the interference, they can assist in diminishing it and obtaining a reproducible result.

AUTHOR CONTRIBUTION

Sahand Mohammadzadeh and Neda Soleimani contributed to the writing and revision of article. Dr. Saeideh Khaleghpanah contributed to data collection.

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None.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL

The research has been carried out by the World Medical Association Declaration of Helsinki. The study was approved by the Ethics Committee of Shiraz University of Medical Science.

CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal’s patient consent policy.