

CASE REPORT

Application of DTT to Eliminate Interference of Rheumatoid Factor on Serum hCG Detection

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SUMMARY

Background: The accuracy of serum human chorionic gonadotropin (hCG) detection is crucial for the diagnosis of gestational and trophoblastic tumors. Rheumatoid factor (RF), as a common autoantibody in patients with rheumatoid arthritis, often leads to interference in immune detection.

Methods: We report a case of using dithiothreitol (DTT) to successfully eliminate the interference of RF to hCG detection.

Results: After DTT pretreatment, serum RF level decreased from 293.5 IU/mL to 11.4 IU/mL, while hCG level decreased from 39.36 mIU/mL to 4.12 mIU/mL. The validation results of the Roche electrochemiluminescence platform (2.3 mIU/mL) confirmed that the increase of hCG was a false positive.

Conclusions: When the serum hCG of patients with rheumatoid arthritis is abnormally high and there is no clinical manifestation of pregnancy or trophoblastic tumor, we should be alert to the possibility of RF interference. It is recommended to use DTT to pretreat samples. This can easily and efficiently eliminate interference, ensure the accuracy of detection, and avoid misdiagnosis.

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KEYWORDS

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INTRODUCTION

HCG detection is widely used in pregnancy diagnosis and trophoblastic tumor monitoring, but its immune detection is easily interfered by heterophilic antibody and other factors. Rheumatoid factor (RF) is an autoantibody targeting the FC segment of IgG, and the positive rate in patients with rheumatoid arthritis is as high as 70 - 90% [1]. When the concentration of rheumatoid factor is high, it can bridge capture antibody and labeled antibody, leading to interference in immune detection [2]. This paper reports a case of using DTT to eliminate hCG false positive caused by RF.

Table 1. Laboratory related test results.

Test items	Results	Reference value
ALT	12.5	7 - 40.00 U/L
AST	22.5	13 - 35 U/mL
Erythrocyte sedimentation rate	59	< 38.4 mm/hour
CRP	16.59	0 - 6.00 mg/mL
hCG (Chemiluminescence technology)	39.36	0 - 5.00 mIU/mL
hCG (Immune colloidal gold technique)	negative	negative
RF	293.5	0 - 14 IU/mL

Table 2. The results of HCG and RF before and after DTT pretreatment.

	No pretreatment	Physiological Saline pretreatment	DTT pretreatment	Reference ranges
hCG	39.36	43.06	4.12	0 - 5 mIU/mL
RF	293.5	282.6	11.4	0 - 14 IU/mL

Table 3. The results of HCG in different detection platforms.

	Abbott No pretreatment	Abbott DTT pretreatment	Roche No pretreatment
hCG	39.36	4.12	2.3
Reference ranges	0 - 5.00 mIU/mL		0 - 10.00 mIU/mL

CASE PRESENTATION

The patient, female, 54 years old, has been diagnosed with rheumatoid arthritis and Sjogren's syndrome for 11 years. On June 8th, 2025, due to rheumatoid arthritis re-examination and hCG increase, relevant blood tests were carried out. We found that the patient's serum hCG level had increased (39.36 mIU/mL, Table 1). According to the medical history, the hCG level also increased (39.68 mIU/mL) during the hospitalization on November 16, 2024. Since no signs of pregnancy or tumor were found in pelvic CT and abdominal ultrasound, the accuracy of the test results was questionable.

The laboratory staff first checked the internal quality control of the day and showed that the hCG quality control results were under control, thus eliminating the detection errors caused by instrument and reagent factors. However, the possibility of sample specific interference (such as heterophilic antibody) was still not ruled out and needed further verification. Subsequently, we used the colloidal gold strip to conduct hCG qualitative review on the patient samples, and the results were negative. Considering that the patient suffered from rheumatoid arthritis for a long time, and RF was confirmed to interfere with hCG detection, the RF level of the patient sample was additionally detected. The initial test

results showed that the RF concentration exceeded the upper limit of the linear range. After dilution, the RF concentration was as high as 293.5 IU/mL, far exceeding the normal reference value. Based on the above results, it is highly suggested that RF may lead to the immune interference of this hCG detection.

Dithiothreitol (DTT) is a disulfide reducing agent, which can reduce the disulfide bond of IgM RF, destroy its pentamer structure, and eliminate its non-specific binding ability. To verify the interference, we mixed the patient's serum sample with 20 mmol/L DTT solution at a volume ratio of 1:1, left it at room temperature for 30 minutes, then centrifuged and took the supernatant for detection. At the same time, the same amount of normal saline was mixed with the sample as the control (Table 2). In addition, the samples were sent to another detection platform (Roche Electrochemiluminescence Method) in parallel for detection. The results showed that after DTT pretreatment, the serum hCG level of the patient decreased significantly from 39.36 mIU/mL to 4.12 mIU/mL. However, the detection results of samples without DTT pretreatment on Roche electrochemiluminescence platform were 2.3 mIU/mL (Table 3), which were similar to the results after DTT pretreatment, and both were significantly lower than the initial detection value. This shows that DTT processing can ef-

fectively eliminate the interference of RF on hCG detection. At the same time, after DTT pretreatment, the serum RF level of the patient also decreased from 293.5 IU/mL to 11.4 IU/mL (Table 2), which further confirmed that DTT could significantly destroy RF activity. In conclusion, the false elevation of serum hCG in this patient is caused by RF interference, and DTT pretreatment can effectively eliminate the interference of RF on hCG detection.

DISCUSSION

Serum hCG detection is a key indicator for pregnancy diagnosis and trophoblastic disease monitoring, but its immunological methods are easily affected by heterophilic antibody, rheumatoid factor (RF) and other interfering substances, which may lead to false positive results, leading to misdiagnosis and unnecessary clinical intervention [3-5]. In this case, the patient's serum hCG level was significantly increased, but no signs of pregnancy or tumor were found in imaging examination, and the hCG colloidal gold method test result was negative, which highly suggested the presence of detection interference.

In depth analysis found that the patient was a long-term rheumatoid arthritis patient, and the serum RF concentration was significantly increased. As an IgM type autoantibody, RF can nonspecifically bind to the FC segment of the antibody in the detection reagent, bridging the captured antibody with the labeled antibody, resulting in detection interference. Commercial reagent research has confirmed that RF positive serum is more likely to react strongly with mouse derived monoclonal IgG antibody and interfere with immunoassay [6]. The detection platform used in our laboratory is based on mouse derived monoclonal antibody, which is more vulnerable to RF interference. It is worth noting that the sample was retested by Roche electrochemiluminescence platform (using different antibody design and anti-interference technology), and the hCG result was only 2.3 mIU/mL, which further confirmed the false positive of the original test result. This also shows that different detection platforms have different sensitivity to RF interference. The Roche platform has more anti-interference ability for this sample. In order to solve the RF interference, DTT pretreatment was used in this study. The mechanism is that DTT, as a powerful disulfide reductant, can reduce the disulfide bond in RF, destroy the pentamer structure of IgM RF, and lose its ability to nonspecifically bind to the FC segment of IgG [7,8]. In this case, after DTT treatment, the serum RF level dropped sharply from 293.5 IU/mL to 11.4 IU/mL, while the HCG value decreased from 39.36 mIU/mL to 4.12 mIU/mL, which was highly consistent with the validation result of the Roche platform. This result not only confirmed that RF interference was the direct cause of hCG false positive, but also confirmed the efficiency and reliability of DTT pretreatment. Compared with the

cumbersome operation of the detection platform replacement or the expensive xenophilic antibody blocker, DTT pretreatment has the advantages of simple operation and low cost, especially suitable for use in primary hospitals.

In conclusion, this case emphasizes that DTT can effectively destroy the immune activity of RF and eliminate its interference on immune detection. The laboratory should strengthen the recognition awareness of detection interference. It should incorporate DTT pretreatment into the routine verification and correction process of high-risk samples (such as RA patients' serum), so as to avoid misdiagnosis and subsequent unnecessary diagnosis and treatment operations.

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Declaration of Interest:

All authors declare that they have no competing interests.

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