CASE REPORT

A Case of Pseudoelevation of Serum CA19-9 Level

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SUMMARY

Background: Carbohydrate antigen 19-9 (CA19-9) is usually synthesized by pancreatic and bile duct cells and is present in small amounts in serum. During the period of tumor disease, its serum level significantly increases, and it is the most widely used serum tumor marker for diagnosis and monitoring therapy of pancreatic cancer.

Methods: We reported a case of abnormal elevation of serum CA19-9. Considering the possibility of detection interference, we used heterophilic antibody blocking analysis, detection by different analysis systems, and polyethylene glycol (PEG) precipitation to evaluate the reliability of abnormally elevated CA19-9 concentration.

Results: Repeated measurements on the Roche Cobas 8000 system of another hospital significantly reduced the CA19-9 concentration, as did PEG precipitation. Therefore, the abnormally elevated level of CA19-9 in this patient is considered to be pseudoelevation caused by interferences.

Conclusions: We suggest considering the presence of detection interference in cases with elevated CA19-9 levels but no related clinical manifestations to prevent false positives. PEG precipitation may be a simple and feasible solution to eliminate interference.

KEYWORDS

carbohydrate antigen 19-9 (CA19-9), polyethylene glycol (PEG), interference

INTRODUCTION

Carbohydrate antigen 19-9 (CA19-9) is a tumor related antigen, which exists in the form of mucoprotein in the serum. The level of CA19-9 in patients with pancreatic cancer, gastric cancer, colorectal cancer and other gastrointestinal malignant diseases can be significantly increased, and the level of CA19-9 in patients with pancreatitis, cholecystitis and other benign diseases can also be slightly increased [1]. We found a case of abnormal elevation of serum CA19-9 due to interference of immune detection without obvious clinical symptoms. The details are shown below.
CASE PRESENTATION

The patient is a 32-year-old female. During physical examination in Shangyu Second People's Hospital in November 2022, it was found that serum CA19-9 was abnormally elevated, reaching 2,014.78 U/mL. On March 17, 2023, she was admitted to our hospital due to elevated serum CA19-9 level. After admission, common tumor markers were rechecked on our hospital's Abbott i2000SR system as shown in Table 1: CA19-9 significantly increased (786.87 U/mL), CA12-5 slightly increased, and other markers were within the normal range. At the same time, routine biochemical tests were conducted as shown in Table 2, and no abnormalities were found. Gastroscopy showed that the patient had chronic antral gastritis, and colonoscopy showed no abnormalities. At the same time, the patient underwent abdominal ultrasound and enhanced CT examination of the upper abdomen, and no abnormalities were found in the pancreas, gallbladder, etc. Other examinations (pelvic CT, thyroid ultrasound, lung CT) showed no significant abnormalities. The patient did not take medication, did not experience any discomfort, and did not have any other chronic diseases.

Due to the inconsistency between the CA19-9 level and the clinical assessment, the clinical doctor questioned the results and contacted our department. For this question, the laboratory staff first retested the sample and the results were consistent with the previous ones. After analysis and discussion, we suspect that there may be detection interference. So, we preprocessed the patient's serum with heterophilic blocking tube (HBT) to study the interference of heterophilic antibodies, and the result was 480.24 U/mL, a decrease of 39%, indicating that HBT eliminated some interference from the sample. At the same time, the sample was sent to another hospital for testing which was using the Roche Cobas 8000 system, and the results are shown in Table 3. We found that the serum CA19-9 level of the Roche Cobas 8000 system significantly decreased to 5.66 U/mL (Table 3), within the normal range. At the same time, the patient's serum sample was pretreated with PEG and tested in our Abbott i2000SR system, with a result of 8.46 U/mL, which is consistent with the result of the Roche Cobas 8000 system. After discussion with clinical doctors, the patient's clinical diagnosis and presentation are inconsistent with the abnormal elevation of CA19-9. Therefore, we ultimately determined that the CA19-9 level should be normal, while the abnormal increase in the result was a pseudoelevation caused by unknown interference.

DISCUSSION

CA19-9 immunoassay has good specificity and high sensitivity, but due to the complexity of antigen antibody reaction, immunoassay cannot completely avoid the influence of various interferences [2]. In recent years, it has been reported many times that immunoassays were interfered with, including HCG, CA125, AFP, etc., resulting in inaccurate test results and unnecessary examination and treatment for patients [3]. Because the CA19-9 level of the patient in this case do not match their imaging data and clinical manifestations, it is suspected that this result is falsely elevated. When there is suspicion of interference in the detection, identification and confirmation can be carried out by using heterophilic blocking tube (HBT) analysis, PEG precipitation pretreatment, replacing different detection systems, etc. [4].

We first preprocessed the patient's serum with HBT, but the interference was not completely eliminated. Analyzing the reason, it may be that the interference is not entirely caused by heterophilic antibodies. At the same time, we retested CA19-9 on the Roche testing system in another hospital and found that the result was within the normal range. Chemiluminescence Immunoassay technology is used in both detection systems. Why is only Abbott detection system disturbed? We reviewed the reagent instructions and found that Abbott's detection system uses two-step chemiluminescence microparticle immunoassay and uses specific mouse monoclonal antibodies labeled with acridinium ester. The Roche detection system uses electrochemical luminescence method and uses specific mouse monoclonal antibodies labeled with ruthenium (Ru). In this case, the Roche detection system eliminated interference, possibly due to the use of different labeled antibodies. According to reports, polyethylene glycol (PEG) can precipitate large molecule immunoglobulins through steric hindrance [5]. Previous studies have shown that PEG pretreatment of specimens can eliminate the interference of endogenous antibodies [6]. We pretreated the sample with PEG and then tested CA19-9 on the Abbott testing system. The result of CA19-9 showed a significant decrease, indicating that the interference may be caused by macromolecular immunoglobulins. The detection result is similar to that of the Roche detection system, consistent with the patient's clinical manifestations and imaging data, indicating that PEG effectively eliminates interference from the sample. The polyethylene glycol precipitation method is easy to do, low-cost, and easy to operate. It can be used as a screening method for suspicious immune detection interference, and is worthy of application and promotion in clinical basic laboratory.

In summary, this case emphasizes that when laboratory staff find that the test results do not match clinical manifestations, we should consider the possibility of interference and take corresponding corrective measures to avoid unnecessary examination and treatment of patients.

Acknowledgment:
Not applicable.
Table 1. Test results of common serum tumor markers from the patient.

<table>
<thead>
<tr>
<th>Test items</th>
<th>Results</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate antigen 19-9 (CA19-9)</td>
<td>786.87 U/mL</td>
<td>≤ 37.00 U/mL</td>
</tr>
<tr>
<td>Carbohydrate antigen 125 (CA125)</td>
<td>43.06 U/mL</td>
<td>≤ 35.00 U/mL</td>
</tr>
<tr>
<td>Carbohydrate antigen 15-3 (CA15-3)</td>
<td>9.4 U/mL</td>
<td>≤ 31.30 U/mL</td>
</tr>
<tr>
<td>Carbohydrate antigen 242 (CA242)</td>
<td>2.9 IU/mL</td>
<td>≤ 10 IU/mL</td>
</tr>
<tr>
<td>Carbohydrate antigen 50 (CA50)</td>
<td>4.21 IU/mL</td>
<td>≤ 25 IU/mL</td>
</tr>
<tr>
<td>Alpha fetoprotein (AFP)</td>
<td>1.85 ng/mL</td>
<td>≤ 13.4 ng/mL</td>
</tr>
<tr>
<td>Carcino-embryonic antigen (CEA)</td>
<td>0.86 ng/mL</td>
<td>≤ 5 ng/mL</td>
</tr>
<tr>
<td>Carbohydrate antigen 742 (CA742)</td>
<td>0.20 IU/mL</td>
<td>≤ 6 IU/mL</td>
</tr>
</tbody>
</table>

Table 2. Test results of common biochemical report from the patient.

<table>
<thead>
<tr>
<th>Test items</th>
<th>Results</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylase (AMY)</td>
<td>58.8 U/L</td>
<td>40.0 - 132.0 U/L</td>
</tr>
<tr>
<td>Total bilirubin (TBil)</td>
<td>19.2 µmol/L</td>
<td>5.0 - 21.0 µmol/L</td>
</tr>
<tr>
<td>Direct bilirubin (DBil)</td>
<td>2.6 µmol/L</td>
<td>0.0 - 3.4 µmol/L</td>
</tr>
<tr>
<td>Total protein (TP)</td>
<td>67.8 G/L</td>
<td>65.0 - 85.0 G/L</td>
</tr>
<tr>
<td>Albumin</td>
<td>40.2 G/L</td>
<td>40.0 - 55.0 G/L</td>
</tr>
<tr>
<td>Alanine aminotransferase (ALT)</td>
<td>15 U/L</td>
<td>0.0 - 40.0 U/L</td>
</tr>
<tr>
<td>Aspartate transaminase (AST)</td>
<td>16.3 U/L</td>
<td>13.0 - 35.0 U/L</td>
</tr>
<tr>
<td>Alkaline phosphatase (ALP)</td>
<td>77.9 U/L</td>
<td>35.0 - 100.0 U/L</td>
</tr>
<tr>
<td>Glutamine transpeptidase</td>
<td>13.3 U/L</td>
<td>7.0 - 45.0 U/L</td>
</tr>
<tr>
<td>Adenosine deaminase (ADA)</td>
<td>5.6 U/L</td>
<td>0.0 - 24.0 U/L</td>
</tr>
<tr>
<td>Lactic dehydrogenase (LDH)</td>
<td>148.0 U/L</td>
<td>120.0 - 250.0 U/L</td>
</tr>
<tr>
<td>Hydroxybutyrate dehydrogenase (HBD)</td>
<td>87.4 U/L</td>
<td>72.0 - 182.0 U/L</td>
</tr>
<tr>
<td>Blood urea nitrogen (BUN)</td>
<td>4.72 mmol/L</td>
<td>2.86 - 8.20 mmol/L</td>
</tr>
<tr>
<td>Creatinine (CRE)</td>
<td>53.1 µmol/L</td>
<td>45.0 - 84.0 µmol/L</td>
</tr>
<tr>
<td>Blood glucose (BLU)</td>
<td>4.47 mmol/L</td>
<td>3.90 - 6.10 mmol/L</td>
</tr>
<tr>
<td>Rheumatoid Factor (RF)</td>
<td>6.8 IU/mL</td>
<td>≤ 14 IU/mL</td>
</tr>
<tr>
<td>Immunoglobulin G (IgG)</td>
<td>13.63 G/L</td>
<td>7.00 - 16.00 G/L</td>
</tr>
<tr>
<td>Immunoglobulin M (IgM)</td>
<td>2.31 G/L</td>
<td>0.40 - 2.80 G/L</td>
</tr>
<tr>
<td>Immunoglobulin A (IgA)</td>
<td>1.59 G/L</td>
<td>0.70 - 5.00 G/L</td>
</tr>
</tbody>
</table>

Table 3. The results of CA19-9 in different detection system and the results of CA19-9 with and without PEG pretreatment using Abbott i2000SR system.

<table>
<thead>
<tr>
<th></th>
<th>Abbott i2000SR No pretreatment</th>
<th>Abbott i2000SR PEG pretreatment</th>
<th>Roche Cobas 8000</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA19-9 (U/mL)</td>
<td>786.87</td>
<td>8.46</td>
<td>5.66</td>
</tr>
<tr>
<td>Reference ranges</td>
<td>≤ 37.00 U/mL</td>
<td></td>
<td>≤ 27.00 U/mL</td>
</tr>
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</table>
Sources of Support:
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Declaration of Interest:
All authors declare that they have no competing interests.

References: