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Case Report

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Jerome H. Check
Linda Ubelacker
Carolyn C. Lauer

The University of Medicine and Dentistry
of New Jersey, Robert Wood Johnson
Medical School at Camden, Cooper
Hospital/University Medical Center,
Department of Obstetrics and Gynecology,
Division of Reproductive Endocrinology
and Infertility, Camden, N.J., USA

Falsely Elevated Steroidal Assay Levels Related to Heterophile Antibodies against Various Animal Species

Key Words

Steroidal assays
Heterophile antibodies

Abstract

Two cases of falsely elevated serum estradiol and 1 case of spuriously elevated serum progesterone performed on an automated immunoassay instrument are described. Further testing of same specimens indicated the presence of heterophilic antibodies against rabbit IgG and sheep IgG, respectively.

Introduction

A report is presented of 2 patients exhibiting falsely elevated levels of serum estradiol (E_2) as a result of heterophilic antibodies to rabbit IgG, and 1 patient exhibiting falsely elevated levels of serum progesterone (P) as a result of heterophilic antibodies to sheep IgG. The type of assay used was an enzyme-linked immunosorbent assay (ELISA) based upon competition using streptavidin technology on the ES300 automated immunoassay system (Boehringer Mannheim Corp., Indianapolis, Ind., USA).

Case Report

Patient 1

During the course of method comparison studies for purposes of introducing the ES300 instrument into the laboratory, a 38-year-old female seeking treatment for infertility through assisted reproductive techniques was identified as having markedly disparate levels of E_2 when assayed on the ES300 versus a traditional radioimmunoassay (RIA). The patient's E_2 levels exceeded 1,290 pg/ml on the ES300, requiring dilution. The value on the diluted serum (700 pg/ml) did not correlate, so the specimen was re-tested using RIA (Diagnostic Products, Los Angeles, Calif., USA) and measured 170 pg/ml. There was also some disparity, though not as marked, in P, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) results.

Table 1. Patient 2 E_2 levels by day and method

Day of cycle	E_2 -ES300	E_2 -ES300 (1:4 dil)	E_2 -ES300 (+ R-IgG)	E_2 -RIA
2	258	-	-	-
9	516	-	-	-
12	917	-	-	-
14	>1,290	544	469	430

Though the ultrasound results did not correlate with the very high sera E_2 levels obtained by the ES300, a decision was made to freeze all embryos rather than transfer fresh: she did conceive on her first frozen embryo transfer.

The patient's blood was analyzed for possible contamination by anti-rabbit antibodies, using different test methodologies, i.e., dilution studies, addition of rabbit IgG. All tests indicated the presence of an anti-rabbit antibody in the woman's sera. The patient was contacted and admitted that she had a pet rabbit.

Patient 2

A 29-year-old female had serum drawn for E_2 , P4, LH, and FSH on days 2, 9, 12, and 14 of her cycle (table 1). There was no cause for the laboratory to be suspicious until day 14 when her E_2 level was >1,290 pg/dl and required dilution. When the serum was diluted 1:4 and re-assayed, the result was 544 pg/ml. Because a similar disparity of diluted E_2 results had been noted in the previous patient, this

patient's serum was assayed by the RIA method and found to be 340 pg/ml.

It appeared possible that this patient also had a heterophile antibody, although it seemed unusual for 2 such patients to present in such a short time. The patient was contacted and found to have a pet rabbit. The presence of an anti-rabbit antibody was confirmed by the addition of anti-rabbit IgG to the assay procedure.

Patient 3

A 40-year-old infertility patient undergoing in vitro fertilization (IVF) was unable to suppress the serum P level to <1.5 ng/ml, despite the use of the gonadotropin-releasing hormone agonist (GnRHa), leuprolide acetate (LA). Her P level, as measured on the ES300, was 3.3 ng/ml, after 10 days of LA. When her serum was assayed using the RIA method, the P level was found to be appropriately suppressed (1.1 ng/ml).

The patient's serum P was re-assayed on the ES300 adding anti-sheep IgG to the incubation buffer and confirmed the presence of anti-sheep IgG antibodies. Careful questioning uncovered no previous direct contact with sheep. It is often difficult to establish the origin of heterophile antibodies due to their lack of specificity.

Conclusion

The test kits for the E₂ and P assays on the ES300 have a high specificity, demonstrating negligible cross-reactivity with other hormones. They do, however, utilize biotinylated polyclonal rabbit anti-E₂ and sheep anti-P antibodies, respectively, and are therefore subject to interference from heterophilic antibodies in patient serum. We have previously reported falsely elevated β -human chorionic gonadotropin (hCG) levels also related to a heterophile antibody [1].

Reports differ widely on the incidence of heterophile antibodies and can depend on the use of different test antibodies, different test procedures, detection and decision limits and true differences in the test populations. Although heterophilic antibodies with anti-rabbit specificity have been observed in as many as 25% of laboratory personnel who are in routine contact with laboratory animals, the prevalence in the general population is felt to be relatively low, with reports ranging from 0.01 to 5%. Interference from anti-sheep antibodies has been reported as 0.06 or 7%, dependent upon the analyte being measured [2].

Assay interference by heterophile antibodies can often be corrected by addition of homologous nonspecific immunoglobulin, or nonimmune serum from the animal species that was used to raise the antibody. In cases of suspected heterophile interference in their E₂ assay, Boehringer Mannheim recommends adding 0.1 mg rabbit IgG/ml of incubation buffer. When this step was taken with

patient 2, the E₂ results were consistent with the patient's clinical picture.

Another accepted method of correcting for heterophilic interference is to repeat the assay with a different method which utilizes antibodies from a different animal species. When patient 3's serum was re-assayed by an RIA methodology which utilized antibodies from rabbits rather than sheep, the P results correlated with clinical expectation.

Heterophilic antibody response may be directed against the region of the binding site specific to the species strain. Therefore, it is sometimes possible to abolish the interference by utilizing a different methodology, even though the second methodology's antibody was raised in the same species. Examples of this would be patients 1 and 2, whose sera antibodies interfered with the ES300 assay but not the RIA method. Even though the antibodies utilized in both assays were obtained from rabbits, they were obviously from different strains.

It is normal laboratory procedure to re-assay a specimen using the same methodology if a result seems 'too high' or 'too low'. In cases of heterophilic interference, the lab is simply confirming a still erroneous result. The undetected interference of heterophile antibodies is not trivial and has led to unnecessary testing, chemotherapy and even surgery. The treating physician should always consider the possibility of heterophile antibodies as a cause of spuriously elevated or decreased levels of E₂ and P if the reported values do not fit the clinical picture.

References

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