CASE REPORT

Monoclonal gammopathy may disturb oestradiol measurement in the treatment and monitoring of in-vitro fertilization

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A 31 year old woman had her treatment for infertility by in-vitro fertilization (IVF) cancelled because a highly elevated serum concentration of oestradiol was detected, contrary to the clinical picture and that observed by vaginal ultrasound. The immunoassay for measuring oestradiol had been affected by circulating heterophilic antibodies in the form of an elevated immunoglobulin (Ig) G-kappa M component. This may often be associated with a haematological malignancy of lymphoid origin, but this patient had a benign monoclonal gammopathy. Monoclonal gammopathy has not been described in IVF patients previously, nor has monoclonal gammopathy been reported as a cause of erroneously elevated oestradiol concentration. This sort of interference in oestradiol analysis is probably very rare, but may lead to unnecessary cancellation of the treatment.

Key words: analytical interference/heterophilic antibodies/immunoassay/monoclonal gammopathy/oestradiol

Introduction

In-vitro fertilization (IVF) treatment includes ovarian stimulation. This consists of gonadotrophin-releasing hormone (GnRH) downregulation with an agonist and gonadotrophin stimulation, where the ovarian effect is monitored by vaginal ultrasound and in many cases by the serum concentration of oestradiol. In general in our clinic, we rely on ultrasound interpretation for monitoring the ovarian response, and only when ovarian hyperstimulation syndrome (OHSS) develops do we use oestradiol measurements and eventually a 'prolonged coasting' (Waldenström et al., 1999), or cancellation of the treatment. The number and size of the follicles detected by ultrasound gives for all practical purposes an impression of the oestradiol concentration. We report a case where oestradiol concentrations were at least five times higher than expected compared with the size of the ovaries, and with the number and size of the follicles. We discuss this very rare situation, which led to further investigation.

Case report

A 31 year old Hispanic woman was accepted for IVF due to tubal infertility. Sterilization had been performed in connection with her third Caesarean section at the age of 15 in Brazil. She had had a normal menstrual pattern since the age of 9 years, and 1 year prior to this IVF treatment, physical and gynaecological examination, including hormonal assessment, were found to be normal.

In the IVF treatment, pituitary suppression was obtained by daily 800 mg nafareline nasal spray (Synarel, Searle, Morpeth, Northumberland, UK) from day 19 in the previous cycle and for 16 days before starting ovarian stimulation with recombinant follicle stimulating hormone (FSH) 150 IU/day (Gonal-F, Ares-Serono, Geneva, Switzerland). She was monitored on day 10, after 9 days of FSH treatment. She appeared healthy, with no symptoms of OHSS. The ovaries measured 52×40 mm and 50×44 mm, with a total of two follicles of 18 mm, four of 15 mm, two of 14 mm and ~10 follicles <12 mm in diameter. The dose of FSH was reduced to 100 IU, and the serum oestradiol measurement was 56.50 nmol/l. From experience, we expected oestradiol concentrations to be <10 nmol/l. The treatment was cancelled, and the patient continued with the GnRH agonist. Daily monitoring for the following week showed the largest size of the ovaries to be 62×54 mm and 64×54 mm, and the oestradiol concentrations were 66.67, 52.05, 43.33, 20.19, 17.07 and 19.40 nmol/l. The patient never had any signs of OHSS, which would have been expected with these high oestradiol concentrations. The disagreement between the clinical and the biochemical observations led us to suspect a laboratory error or an oestradiol-producing tumour (Rey et al., 1996; Aboud, 1997). Therefore, on two separate occasions oestradiol measurements were performed elsewhere using different methodology. The comparison of samples drawn on the same day, showed our laboratory and the reference laboratory values to be 20.19/0.19 nmol/l and 19.40/0.17 nmol/l respectively. The latter values of oestradiol were in concordance with the clinical situation, and this resulted in further examinations. Both oestradiol methods were automatic immunoassays utilizing polyclonal rabbit antibodies: ours was Elecsys Immunoassay (Boehringer Mannheim, Mannheim, Germany) and in the reference laboratory the method was AutoDeFIA (LKB Wallac, Turku, Finland). The Elecsys Immunoassay is an automated immunochemical electrochemi-
Our patient had an anomalously elevated oestradiol result in a routine laboratory examination. We suspected a laboratory error. Clinically, the patient should have had a much lower oestradiol concentration at the time of investigation, and therefore additional serum samples were taken and analysed elsewhere. Fortunately, in the referring hospital the possible cause of interference in hormone analysis was studied. Further examinations revealed a monoclonal gammopathy in a symptomless and healthy patient.

Artfactually increased heterophilic antibodies in the patient’s serum with specificity for immunoglobulin class G-kappa were found. These heterophilic antibodies formed complexes with the antisera to human oestradiol that are used in the enzyme-linked immunosorbent assay (ELISA) system, in particular blocking the binding of the second antibody. When it was measured immunometrically with sera of rabbit origin, the concentrations measured were significantly decreased. Protein electrophoresis was done to characterize the possible amount and immunoglobulin subclass of circulating protein. Then an M-component was found and it was further characterized by immunofixation.

In large multicentre evaluation of two new enzyme-linked immunosorbent assays for FSH and LH, no interference by heterophilic antibodies was found (Thijssen et al., 1991). These methods are similar to our oestradiol analysis. These methods are known to be reproducible (coefficient of variation <5%), highly specific, and sensitive enough to measure the hormones directly in almost all patients’ samples. However, many endogenous antibodies exhibit a potential for interference with immunoassays (Kohse and Wissner, 1990). Interference by heterophilic antibodies can be abolished by the addition of non-immune serum. Investigations with our samples revealed an M-component cross-reacting with some polyclonal rabbit antibodies. This is possible, because the methods used for oestradiol determination used different polyclonal anti-rabbit antibodies. The possibility of anti-streptavidin antibodies was excluded using a binding assay. In clinical chemical analysis, autoantibodies, antibodies to foreign antigens, antibodies administered for therapeutic purposes, and monoclonal gammapathies are possible sources of interference. Differences between various immunometric assays and radioimmunoassays and their vulnerability to interference from heterophilic antibodies have been reported (Seth et al., 1989). It is known from the laboratory experience of various manufacturers, although not reported, that heterophilic antibodies seldom disturb oestradiol analyses. No specific analyses of oestradiol assays have been reported, but we believe that this sort of disturbance must be very rare (perhaps <1/10 000 analyses) (Thijssen et al., 1991; Seth et al., 1999). No specific data have been reported regarding monoclonal gammopathy as a source of laboratory errors. We know theoretically that it is possible, extremely rare, and that the prevalence is much lower than that of heterophilic antibodies.

There are various options available to avoid interference by heterophilic antibodies; alternatively, the sample can be studied elsewhere. The immunoassay itself has to be validated, as we have reported, and which led us to conclude that our analysis could not give the right result because of the clonality of the M-component. The sample can be preincubated with serum from other species, but this did not give an adequate result in
our case. A separation procedure other than the double-antibody method, such as ether or polyethylene glycol extraction, may give better results (Dericks-Tan et al., 1984). However, all these techniques are very time consuming for automated routine laboratory analyses and require manual laboratory methods. Additionally, the respective hormone can always be measured in urine.

This case demonstrates how important it is to identify the nature of heterophilic antibodies, to delineate the processes that produce them, to examine the mechanisms by which these antibodies cause interference, and to explore how this information can be used to reduce immunoassay interference. This procedure may reveal another disease without any clinical significance so far, as it did in our case. Interference of this type has not been previously reported and its incidence is low, but also poorly characterized, because routine hormone laboratories do not often perform electrophoresis analyses. In fact, virtually nothing has been reported about monoclonal gammopathy as a source of laboratory errors. In our case the treatment of the patient was unnecessarily cancelled but on the other hand the procedure could have revealed a haematological cancer.

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References


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