CASE REPORT

The 'Hook Effect' on Serum Prolactin Estimation in a Patient with Macroprolactinoma

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Summary

Large quantities of antigen in an immunoassay system impair antigen-antibody binding, resulting in low antigen determination. This is called the 'high dose hook effect'. We report this phenomenon in a patient with a large macroprolactinoma. In this patient, the correct estimate of serum prolactin (PRL) was obtained only after appropriate dilution of serum. We suggest that in order to avoid the high dose hook effect, the serum PRL be estimated in appropriate dilution in all patients with large pituitary tumours. This is particularly important when the clinical suspicion of high PRL is strong, as in women with amenorrhoea-galactorrhoea and men with long standing hypogonadism.

Key words : Prolactin, Hook effect, Immunoassay.

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Introduction

Very high prolactin (PRL) concentrations can interfere with immunoassay systems resulting in falsely low PRL determination.[1] This is attributable to 'the hook effect', which describes the inhibition of immune complex formation by excess antigen concentrations.[2,3] This is an important consideration in patients with large pituitary tumours,[4,5] when the clinical suspicion of prolactinoma is strong, as in patients with amenorrhoea-galactorrhoea or longstanding hypogonadism. Appropriate dilution of the serum in such cases helps in accurate estimation of serum PRL concentration.[4]

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We report the occurrence of the high dose hook effect in a patient with an underlying macroprolactinoma. In this case, the high level of serum PRL was not identified when the assay was intially performed on the undiluted serum sample.

Case Report

A 27 year old male presented with diminished vision in both eyes of two months duration. He developed visual loss suddenly after a day-long intense headache. The visual loss had been progressive since then. He also experienced impairment in memory and erectile dysfunction. Physical examination revealed a pulse rate of 89/minute and blood pressure of 120/80 mm Hg. Pectoral and facial hair were scanty, however, external genitalia were normal and there was no gynaecomastia. Central nervous system evaluation

Fig. 1 : MRI of the brain (post gadolinium) showing an enhancing sellar mass with suprasellar extension.

revealed impairment of remote memory. He had normal visual acuity in the right eye however acuity in the left eye was reduced to finger counting at a distance of seven feet. There was left sided ptosis, a left relative afferent pupillary defect and bilateral primary optic atrophy. Visual fields by confrontation showed a left temporal field defect. Rest of the systemic examination was normal.

Investigations revealed that haemogram, electrolytes, creatinine and blood sugar were normal. Visual field testing using the Humphrey's field analyzer showed a right superior temporal quadrantanopia and a left temporal hemianopia. Magnetic resonance imaging (MRI) (Fig. 1) showed a sellar mass of about 5.5 x 4 x 3.5 cm in size with extension into the cavernous sinus, sphenoid sinus and the basal ganglia, thalamus and both temporal lobes. His hormonal profile was as follows: serum total thyroxine - 8.3 μg%, free thyroxine - 0.93 ng%, TSH - 0.87 μIU/ml, cortisol - 15.3 μg/dl (8 am) (normal: 9-25 μg/dl) and 5.89 μg/dl (4 pm) (normal: 2-9 μg/dl), luteinising hormone - 3.55 mIU/ml (normal: 0.5 - 10 mIU/ml), follicle stimulating hormone - 4.85 mIU/ml (normal: 1.7 - 11 mIU/ml), testosterone - 5.5 ng/ml (normal: 3-9 ng/ml).

The serum prolactin (PRL) was moderately elevated at 211 ng/ml (normal: 5-20 ng/ml) with chemiluminescence technology (Chiron Diagnostic Corp., USA). This was considered to be consistent with pituitary stalk compression by a nonfunctional tumour (Pituitary stalk compression would deprive the inhibitory hypothalamic influence on PRL secretion, mediated by dopamine, leading to modest hyperprolactinemia). However, in view of the clinical suspicion of a macroprolactinoma, the PRL assay was repeated in 1 in 200 dilution of the serum, which was reported as 12, 266 ng/ml.

In view of the ongoing visual loss, the patient underwent transphenoidal decompression of the tumour. Biopsy confirmed the diagnosis of pituitary adenoma. This patient subsequently underwent external radiation. He was also started on Tab. Bromocriptine 2.5 mg thrice daily. At follow up after 6 months, his serum prolactin levels had come to 87 ng/ml and he had noticed considerable visual improvement in both eyes, which was confirmed on visual field assessment.

Discussion

The intensity of an antigen-antibody interaction depends primarily on the relative proportion of the antigen and the antibody. A relative excess of either will impair adequate immune complex formation.[2] This is called the 'high dose hook effect' or the 'prozone phenomenon'. This has been classically described in serological tests for diagnosis of brucellosis.[6] All immunoassays are based on antigen-antibody interactions. The high dose hook effect often interferes with the assay result. In addition to hormonal assays, the high dose hook effect has also been demonstrated in immune-based techiques used in the measurements of CA 125, IgE and prostrate specific antigen.[3,4]

At our laboratory, PRL is assayed by the double antibody sandwich technique using direct chemiluminiscent particles that facilitate separation in a magenetic field. The signal antibody that binds to PRL has acridine esters that produce luminiscence. These antibodies together help quantitate PRL depending on the relative light units (RLUs) detected by the system.

Extremely high levels of PRL can interfere with the assay and produce low readings. This high dose hook effect may occur because there is not enough anitbody to bind to both ends of all antigenic (prolactin) peptides. Most of the PRL is now complexed to a single antibody. Only the few remaining PRl peptides are 'sandwiched' and therefore detectable.[4] This results in a falsely low PRL value. Hence as the antigen concentrations increase, there is proportional increase in assay titres upto a certain level. Antigen

concentrations above this threshold level would 'hook' down the assay values resulting in very low measurements.[5] In addition, high antigen titres can directly dissolve the antigen-antibody complex.[7]

To overcome the hook effect, the serum sample is diluted and PRL assayed.[4] In our patient, a 1:50 dilution did not change the PRL estimate significantly. Considering that this dilution may be inadequate, higher dilutions of 1:100 and 1:200 were tried to get accurate values. Other suggested remedies for the hook effect include the use of excess antibody, a cumbersome two step procedure and the use of a computer to predict the head to dilute serum samples.[4]

Though repeatedly demonstrated in other immunoassays, the high dose hook effect has only occasionally been observed in chemiluminescence assay systems for PRL estimation.[5] The characteristics of the 'high dose hook effect' in patients with prolactinomas have been reported.[4] St-Jean et al[4] studied 69 patients with pituitary macroadenomas in order to characterise patients with the high dose effect. Patients demonstrating the high dose hook effect are more likely to be males who have significantly large tumours as compared to other with macroprolactinomas. Four patients with the hook effect accounted for 5.8% of all macradenomas. Three of the four patients underwent surgery on a mistaken impression of a nonfunctioning adenoma without improvement and the high dose hook effect was demonstrated only post operatively. All subjects on bromocriptine responded well with respect to visual fields and tumour shrinkage. The excellent prospect of medical cure makes it mandatory to get accurate estimate of PRL prior to surgery. Surgery in a macroprolactinoma is not always necessary and may be even risky as these tumours are sometimes very vascular.[5]

We suggest that in order to accurately estimate PRL in patients with large pituitary tumours, PRL should be assayed in 1:100, 1:200 or even higher dilutions of serum in order to get an accurate estimate of serum PRL.

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