Case Report

The hook effect in prolactin immunoassays

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ABSTRACT

We describe a young female patient with giant invasive sellar and suprasellar tumor and modest elevation of prolactin to 165 ng/ml (normal range 3-29). A diagnosis was made of non functional pituitary adenoma with stalk effect, causing moderate prolactin elevation. A surgery for the removal of the tumor was advised but the patient declined. Treatment with a dopamine agonist was not offered. The patient presented 2 years later with deterioration of her vision and serum prolactin of >16000 ng/ml. Debulking transsphenoidal surgery was performed. The staining of tissue confirmed prolactinoma. Medical treatment with bromocriptine was initiated. We believe that the discrepancy between the 2 values of serum prolactin, is most probably caused by a hook effect in the initial prolactin assay. The mechanism of the hook effect and its occurrence with prolactin immunoassays and methods to eliminate this effect is discussed. Hook effect needs to be suspected in every patient with a giant pituitary or parasellar mass and serum prolactin <200 ng/ml. Assaying a diluted serum will usually unmask this phenomenon and allow accurate diagnosis and management.

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Giant prolactinoma is defined as >4 cm in diameter or >4 cm of suprasellar extension. It occurs rarely and is usually accompanied by high serum prolactin concentrations up to 50,000-80,000 ng/ml.[1,2] These tumors are not amenable to complete resection and the surgical morbidity and mortality rates may be exceedingly high.[3,4]

In contrast, dopamine agonists, such as bromocriptine, pergolide and cabergoline are extremely effective in lowering serum prolactin levels and inducing considerable tumor shrinkage and therefore, are the treatment of choice for patients with giant prolactinomas.[5] Conversely, a surgical procedure may be indicated as first line therapy for other pituitary and parasellar tumors. Thus, preoperative demonstration of a high prolactin level is of crucial diagnostic and therapeutic importance in distinguishing prolactinomas from other pituitary and parasellar tumors.[6-9]

The magnitude of prolactin secretion in prolactin secreting pituitary adenomas is usually proportionate to the size of the adenoma. Thus, very large prolactinomas are expected to secrete very high levels of prolactin (hundreds to thousands). This will usually aid in distinguishing prolactinomas from other large pituitary and parasellar tumors, in which prolactin is only mildly elevated (usually <100 ng/ml), due to a stalk effect and the loss of the inhibitory effect of dopamine.[6,10-12]

The current immunoassay techniques for the measurement of serum prolactin have been shown to be sensitive, precise and rapid. However, they are limited by the occasional occurrence of the hook effect in which the measured prolactin can be inaccurately low.[13] We present an illustrative case report and a review of this phenomenon.

Case Report. A 25-year-old single saudi female presented with a 2 years history of headaches, left eye ptosis and 8 years of amenorrhea. Menarche was at the age of 15 years. The patient also indicated that she easily gets tired and had become intolerant to cold temperature. Physical examination

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revealed blood pressure of 130/70 mmHg, pulse rate 65/m, left eye III and IV cranial nerve palsy and decreased visual acuity 20/200 in the left eye. The patient had no cushingoid or acromegalic features. Cranial magnetic resonance imaging (MRI), revealed large solid sellar and suprasellar mass with cystic component. The tumor encased both internal carotid arteries, displaced optic chiasm and extended to the third ventricle and sphenoid sinus. (**Figure 1**).

Initial laboratory tests (**Table 1**) revealed an elevated prolactin level of 165 ng/ml. The MRI findings and the modestly elevated prolactin level were interpreted as large non functional sellar/suprasellar pituitary tumor with a stalk effect. The neurosurgeon therefore, offered the patient surgery but the patient declined. Two years later the patient visited the Emergency Room, with a history of grand mal seizures in the previous year, blindness in the left eye and blurred vision in the right eye. Her visual acuity was 20/25 in the right eye and she was blind in left eye with complete left III and IV cranial nerve palsy. She was taking phenytoin 100 mg tid. Repeated cranial MRI showed further increase in the tumor size (**Figure 2**). Her laboratory tests revealed a very high prolactin level >16000 ng/ml, the rest of the lab results are summarized in (**Table 2**). The neurosurgeon, advised and performed debulking procedure through a transsphenoidal approach. The postoperative course was uneventful, with no change in neurological status. The pathological examination of the surgical specimen was a characteristic of a pituitary adenoma, with strong and diffuse immunochemical staining for prolactin. Prolactin level remained very high. A diagnosis of giant prolactinoma was made. Computed tomographic images obtained during the first postoperative week demonstrated minimal decrease in the sellar mass and no change in the suprasellar component.

Medical treatment with bromocriptine was initiated in escalating doses. Thyroid hormone replacement therapy with levothyroxine was started. Serum prolactin level dropped to 4950 ng/ml after one month. Magnetic resonance imaging after 3 weeks postoperatively showed significant reduction of the mass (**Figure 3**). Due to the discrepancy between the initial serum prolactin level on the patient's first presentation (165 ng/ml) and the subsequently high serum prolactin levels, we believe a high dose hook effect is responsible for the falsely low level of prolactin in the first sample.

Table 1 - Initial laboratory results.

Test	Result	Normal Range
Prolactin (ug/ml)	165	3-29
TSH (mu/ml)	3.1	0.35-5.5
FT4 (pmol/L)	12	12-22
FSH (IU/L)	<1	3.4-12.5
LH (IU/L)	<1	2.4-12.6
Estradiol (pmol/L)	<100	90.1-716
ACTH (ug/L)	12	0-46
Cortisol pm (nmol/L)	320	

TSH - Thyroid stimulating hormone, FT4 - Free thyroxine, LH - Leutinizing hormone, FSH - Follicular stimulating hormone, ACTH - Adreno corticotropin hormone

Table 2 - Preoperative laboratory results.

Test	Result	Normal Range
Prolactin (ug/ml)	>16000	3-29
TSH (mu/ml)	3.2	0.35-5.5
FT4 (pmol/L)	11	12-22
FSH (IU/L)	<1	3.4-12.5
LH (IU/L)	<1	2.4-12.6
Estradiol (pmol/L)	<100	90.1-716
Pm cortisol (nmol/L)	188	
Somatomedian-C (ug/L)	117	114-492

TSH - Thyroid stimulating hormone, FT4 - Free thyroxine, LH - Leutinizing hormone, FSH - Follicular stimulating hormone ACTH - Adreno corticotropin hormone

Discussion. This case demonstrates the importance of determining the nature of a large sellar tumor, by accurately measuring prolactin level, for proper medical or surgical management. Our patient's giant prolactinoma was most probably misdiagnosed as non functional tumor due to falsely low level of prolactin, secondary to the hook effect in the prolactin assay. Unfortunately, the specimen could not be retrieved for confirmation of the result, or for assaying a diluted sample. Although, the setting of a giant prolactinoma with an apparent prolactin level of less than 200 is a characteristic of hook effect in the prolactin assay, we cannot completely rule out other possibilities of lab error or analysis of a wrong specimen. Awareness of this phenomenon when our patient first presented and an initiation of a dopamine agonist at that point could have (to a certain degree) improved the outcome for this patient.

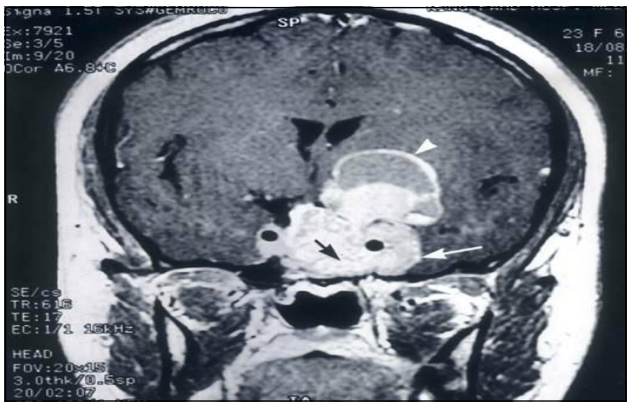

Figure 1 - Magnetic resonance imaging of the sella (coronal view) on patient's firs presentation shows: Large sellar (black arrow) and suprasellar mass with cystic changes (arrow head), encasing carotid vessels (white arrow).

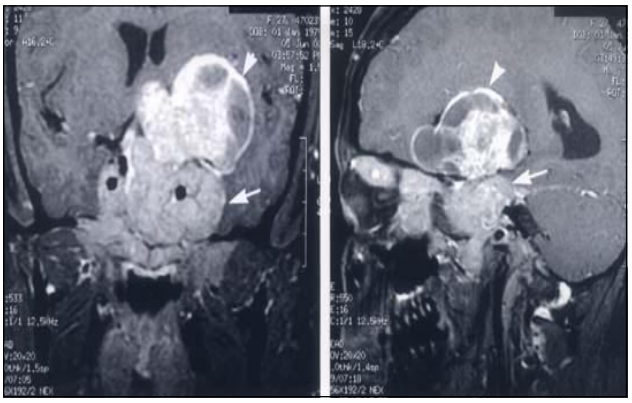

Figure 2 - Magnetic resonance imaging of the sella (coronal and sagittal views) 3 years later shows: Further increase in the size of the mass and it's parasellar (white arrow) and suprasellar (arrow head) components.

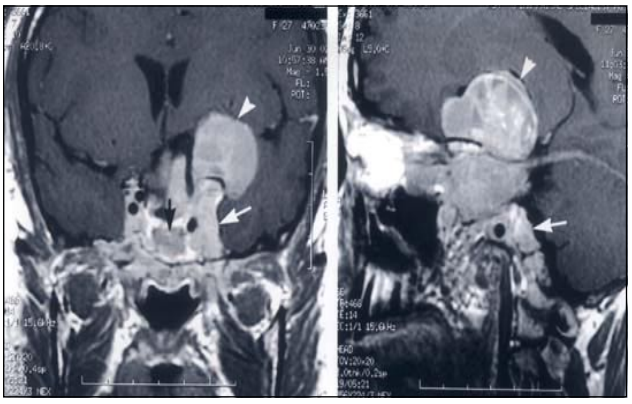

Figure 3 - Magnetic resonance imaging of the sella (coronal and sagittal views) 3 weeks post operatively and after initiation of bromocriptine shows: significant decrease in all of the components of the mass.

Currently, serum prolactin can be measured by 3 immunoassay techniques. Immunoradiometric (IRMA), immunochemiluminometric and enzyme immunoassay (EIA). All of these 3 immunoassay techniques utilize 2 antibodies to measure the serum prolactin level. The first antibody is the capture antibody, which is directed against a certain site of the antigen (namely, prolactin) and is attached to a solid surface. The second (signal) antibody, which is labeled, tagged, or enzyme linked, is directed against another antigen site and is not attached to a solid surface. The 2 antibodies bind to the antigen (prolactin), "sandwiching" the prolactin in between them, and attaching it to the surface. When the liquid phase with any excess signal antibodies is then discarded, the antigen (prolactin) can be measured by the activity of the signal antibodies.[13]

The hook effect can occur when an extremely high antigen concentration would saturate not only the captured antibodies on the surface, but have an excess to bind to the free signal antibodies as well, preventing them from binding to the prolactin attached to the captured antibodies and, therefore, preventing the formation of the "sandwiches." When the liquid phase is later discarded, the antigen that is only bound to the signal antibodies or that is still free will be lost as well. This will result in a falsely low measurement. The hook effect can occur in all the different prolactin immunoassays, but it is commonly reported with IRMA.[13]

This phenomenon is not specific for prolactin or hormones. It has been described in 2 site IRMAs, including thyroid stimulating hormone,[14] beta human chorionic gonadotropin,[15] immunoglobulin E,[16] luteinizing and follicle stimulating hormones[17] and prostate specific antigen.[18] Comtois et al[7] were the first to report the hook effect in prolactin IRMA and subsequently described its occurrence in 5-8% of patients with large pituitary tumors (3.3-6 cm in diameter).[8] The absolute prolactin level, above which a hook effect occurs, depends on the particular assay.[19] St. Jean et al[8] observed the hook effect with serum prolactin concentrations of 10,000-30,000 ng/ml, whereas it was observed with serum prolactin as low as 1320 ng/ml by Mendes et al.[20]

There are 4 methods described in the literature to eliminate the hook effect. The first is to convert the assay into a 2 step technique, but this is time consuming.[17,19] The second method is to add more labeled antibodies, however, this does not always eliminate the hook effect.[21] The third is to dilute the sample, this seems to be the preferred one for its efficacy and simplicity.[17,22-24] The last method is a computerized kinetic rate analysis in order to establish the need for dilution.[22]

Hook effect in prolactin assays is uncommon, but can lead to incorrect diagnosis and management of

sellar tumors, with serious consequences. Some investigators suggest that in any patient with a large pituitary tumor, prolactin should be measured in an undiluted serum sample, as well as, after a 1:100 dilution.[12]

This phenomenon should be suspected when serum prolactin is <200 ng/ml in the presence of giant pituitary or parasellar tumor.

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