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Original article

DOI: https://doi.org/10.3329/jacedb.v4i2.83569

Journal of Association of Clinical Endocrinologist and Diabetologist of Bangladesh
July 2025, Vol. 4, No. 2, pp. 66-71

ISSN (Online): 2959-8176
ISSN (Print): 2958-0307

Underestimation of prolactin in pituitary macroadenoma: Serial dilution may provide diagnostic insights

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Abstract

Background: Serum prolactin (PRL) level is important for differentiating prolactinomas from non-functioning pituitary adenomas (NFPAs). However, extremely high PRL levels in patients with large pituitary tumors can lead to the high-dose hook effect, resulting in falsely low assay readings. PRL measurement after serial dilution may overcome this problem.

Objective: To evaluate the implications of PRL measurements before and after serial dilution in patients with pituitary macroadenomas.

Methods: This cross-sectional study included 73 patients with pituitary macroadenomas admitted to the Department of Neurosurgery at the National Institute of Neurosciences, Dhaka. Clinical, biochemical, and radiological characteristics were recorded. Serum PRL was measured by chemiluminescent immunoassay after 1:10 and 1:100 dilutions. Agreement between PRL levels pre- and post-dilution was assessed using the kappa statistic.

Results: The mean age of the participants was 42.0±12.3 years; 28.8% were female. Headache and visual disturbances were common symptoms. Tumor size averaged 3.3±1.0 cm (maximal diameter), with 28.8% classified as giant tumors. Dilution assays revealed that three patients (4.1%) initially categorized as having PRL <200 ng/mL exhibited significantly elevated levels (>200 ng/mL) post-dilution. All affected patients had tumors with suprasellar extension. In addition, seven patients who were reported to have PRL levels>200 ng/mL in undiluted serum had markedly high PRL (606-12582 ng/mL) when measured after dilution. Moderate agreement was found between pre- and post-dilution PRL levels (κ=0.557, $p<0.001$).

Conclusion: Serial dilution uncovered clinically meaningful underestimation of serum PRL in a subset of patients, emphasizing the persistent relevance of the hook effect even with modern assays.

[J Assoc Clin Endocrinol Diabetol Bangladesh, July 2025;4(2)*: 66-71]*

Keywords: Prolactin, Pituitary Macroadenoma, Hook Effect, Immunoassay Interference, Prolactinoma, Bangladesh

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Introduction

Prolactin (PRL) is a hormone of the anterior pituitary gland that is secreted from lactotroph cells. The hormone is essential for humans as it is needed for lactation. PRL is a 199-amino-acid polypeptide that circulates in the blood in various sizes. There are monomeric or little PRL, dimeric or big PRL, and polymeric or big, big PRL with the molecular weight of

23 kDa, 48-56 kDa, and >100 kDa, respectively. The most biologically active form of PRL is the monomeric form.[1]

Elevation of PRL level may be physiological, drug-induced, or pathological. Serum PRL concentration may be elevated at the diagnosis of clinically non-functioning pituitary macroadenomas if there is compression of the pituitary stalk, thus interrupting the delivery of dopamine, which exerts a tonic inhibitory effect on PRL release from the anterior pituitary.[2-4] Serum PRL level more than 500 µg/L is pathognomic of pituitary macroprolactinoma, and a level >200 µg/L is likely to be a prolactinoma.[5] It is important to distinguish between a macroadenoma causing disconnection hyperprolactinemia and a macroprolactinoma because non-functioning pituitary adenomas causing compressive symptoms are optimally treated with transsphenoidal surgical debulking, while the majority of prolactinomas respond favorably to dopamine agonist therapy.[6]

However, PRL is known to be sensitive to various analytical interferences, including the high-dose hook effect. PRL level is subjected to large amplitudes of variation in concentration. Consequently, extremely high PRL levels can interfere with immunoassay systems, paradoxically resulting in falsely low readings that fall within the normal or mildly elevated range. This is attributable to 'the hook effect', which describes the inhibition of immune complex formation by excess antigen concentrations during two-site immunometric assays. This is an important consideration in patients with large pituitary tumors, when the clinical suspicion of prolactinoma is strong. Appropriate serum dilution in such cases is essential for accurate PRL quantification.[7,8]

The relevance of the hook effect with modern assay reagents remains under investigation. While much of the literature describing this phenomenon consists of case reports and case series, a recent study evaluating various PRL measurement reagents still observed the hook effect. The authors emphasized the importance of including this analytical interference in clinical guidelines to prevent potential misdiagnosis and subsequent inappropriate treatment.[9]

While there is recognized analytical interference in PRL measurements, the frequency and clinical implications of this phenomenon in patients with pituitary macroadenomas in the Bangladeshi population remain underexplored. Hence, this study aimed to evaluate the serum prolactin levels in patients with pituitary macroadenomas awaiting surgical intervention and to determine the impact of serial dilution on the accuracy of prolactin measurement. By comparing prolactin levels before and after sample dilution and examining the clinical and radiological profiles of patients with significantly elevated levels, we sought to assess the frequency and implications of the hook effect in this population.

Methods

Study design and subjects

The present cross-sectional study was carried out from January 2022 to June 2023. The study population comprised patients with pituitary macroadenoma who were consecutively recruited via non-probability sampling upon admission to the Neurosurgery department at the National Institute of Neurosciences (NINS) and Hospital, Dhaka. Exclusion criteria included a history of pituitary tumor apoplexy or adrenal crises.

Study procedure

After obtaining informed written consent, the investigators clinically assessed all participants, and laboratory and imaging data were collected from medical records. Five milliliters of blood were collected from each participant and stored at -20°C until analysis. Serum prolactin was measured in ng/mL by chemiluminescent immunoassay (Architect plus I1000, USA) in the department of Biochemistry, NINS, after 1:10 and 1:100 dilutions. The detection range of PRL in the undiluted serum was 0.6-200 ng/mL.

Statistical analysis

Analyses will be performed with SPSS software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Normally distributed continuous data were summarized as the mean and standard deviation. Categorical data were summarized in frequency and percentages. Agreement between the PRL levels before and after dilution was assessed by the kappa test. A two-sided p-value of less than 0.05 was considered to indicate statistical significance.

Ethical aspects

The Institutional Review Board (IRB) of NINS granted ethical approval for this research. Written informed consent was obtained from all participants after a detailed explanation of the study's purpose, design, and procedures. Participants were informed of their right to refuse or withdraw from the study without consequence. The confidentiality of their data was ensured, and no physical or psychological risks were involved. Standard clinical management was provided to all participants during their hospitalization.

Result

In this study, 73 patients diagnosed with pituitary macroadenoma and waiting for surgery in the department of neurosurgery were included consecutively. The age of the participants was 42.0±12.3 years (mean±SD), and 28.8% were female. Headache and visual disturbance were the most common presenting symptoms. The maximal diameter of the tumor was 3.3±1.0 cm (mean±SD), with 30.8% giant tumors (maximal diameter >4cm). Suprasellar extension was present in 94.5% of participants, and parasellar extension was present in 49.3% (Table-I).

Table-I: Characteristics of the participants (n=73)

Variable		Values
Age (years, mean±SD)		42.0±12.3
Gender	Female	21 (28.8)
	Male	52 (71.2)
Presenting problems	Headache	62 (84.9)
	Visual disturbance	65 (89.0)
	Sexual dysfunction in males (n=52)	16 (30.8)
	Menstrual disturbance (n=21)	19 (90.5)
	Galactorrhea	2 (2.7)
Maximal tumor diameter (Mean±SD)		3.3±1.0
Giant tumor (>4cm)		21 (28.8)
Suprasellar extension		69 (94.5)
Parasellar extension		36 (49.3)

Within parentheses are percentages over the column total, if not mentioned otherwise

Table-II presents a cross-tabulation of serum prolactin levels before and after sample dilution, categorized into four concentration ranges: <30 ng/mL, 30–100 ng/mL, 101–200 ng/mL, and >200 ng/mL. All seven samples originally measured as >200 ng/mL were confirmed to be in the same range after dilution. However, three additional samples measured >200 ng/ml after dilution, which were <200 ng/dl before dilution. The agreement between prolactin levels before and after dilution, as assessed by Cohen's kappa, was moderate (κ=0.557, p<0.001).

In the seven participants who were reported to have serum PRL levels >200 ng/mL in the undiluted serum, subsequent dilution assays confirmed markedly elevated PRL concentrations, ranging from 606.0 to 12582.5 ng/mL. All of them had tumors with suprasellar and

Table-II: Comparison of PRL levels before and after dilution (n=73)

PRL level (After dilution)	PRL level (Before dilution)				
	<30 ng/mL	**30-100 ng/mL**	**101-200 ng/mL**	**>200 ng/mL**	**Total**
<30 ng/mL	43 (86.0)	6 (46.2)	0	0	49 (67.1)
30-100 ng/mL	6 (12.0)	6 (46.2)	1 (33.3)	0	13 (17.8)
101-200 ng/mL	0	0	1 (33.3)	0	1 (1.4)
>200 ng/mL	1 (2.0)	1 (7.7)	1 (33.3)	7 (100.0)	10 (13.7)
Total	**50**	**13**	**3**	**7**	**73**

Within parentheses are percentages over the column total, if not mentioned otherwise

Table-III : Comparison of PRL levels before and after dilution (n=73)

S/N	Age	Sex	Headache and visual problem	*Sexual dys-function	Galactorrhea	Tumor diameter (cm)	Suprasellar and parasellar extension	PRL level after dilution
1	39	Male	Yes	No	No	5.4	Yes	12582.5
2	36	Male	Yes	No	No	5.0	Yes	4666.1
3	18	Female	Yes	Yes	No	2.5	Yes	3193.7
4	45	Male	Yes	No	No	4.2	Yes	3125.8
5	35	Male	Yes	Yes	No	3.8	Yes	4761.0
6	30	Male	Yes	No	No	4.7	Yes	1687.0
7	25	Male	Yes	No	No	5.5	Yes	606.0

*Erectile dysfunction in male and menstrual irregularity in female
PRL: Prolactin

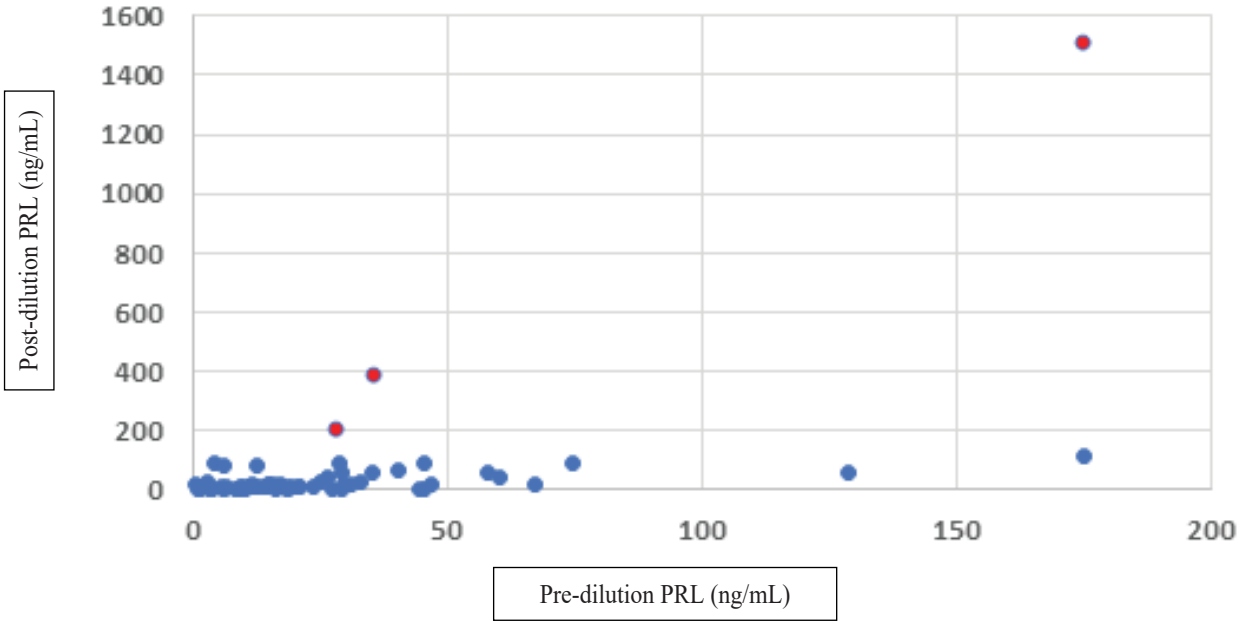

Figure-1: Correlation between pre and post-dilution PRL levels in patients with pituitary macroadenoma having pre-dilution PRL <200 ng/mL (n=66)

PRL: Prolactin

Table-IV : Clinical and radiological characteristics of patients with higher PRL after dilution of sample

S/N	Age	Sex	Headache and visual problem	*Sexual dys-function	Galactor rhea	Tumor diameter (cm)	Suprasellar extension	Parasellar extension	PRL level	PRL level after dilution
1	60	Male	Yes	No	No	2.5	Yes	No	27.9	212.3
2	30	Female	Yes	Yes	No	4.8	Yes	Yes	35.5	392.5
3	45	Female	Yes	Yes	No	2.2	Yes	No	174.5	1517.6

parasellar extension. Maximal tumor diameters ranged from 2.5 to 5.5 cm. Clinical presentations included headache and visual disturbances in all cases, while two patients reported sexual dysfunction (erectile dysfunction in males, menstrual irregularity in females), and none had galactorrhea (Table-III).

The relationship between basal and post-dilution PRL levels in patients with pituitary macroadenoma having pre-dilution PRL <200 ng/mL is shown in Figure-1. While most patients had basal PRL levels below 200 ng/mL and correspondingly low post-dilution values, 3 cases (highlighted in red) exhibited basal PRL levels above 200 ng/mL. Table-IV presents clinical, biochemical, and radiological data of these three patients. The cohort included two females and one male, aged between 30 and 60 years. All reported headaches and visual disturbances. Menstrual disturbance was present in both female patients, while galactorrhea was absent in all cases. MRI findings revealed varying tumor diameters (2.20–4.76 cm), with suprasellar extension observed in all three and parasellar extension noted in one. Following serial dilution, significantly elevated PRL levels were detected in all cases, ranging from 212.31 to 1517.57 ng/mL.

Discussion

This study evaluated the utility and clinical implications of measuring serum PRL estimation after serial dilution among patients with pituitary macroadenomas admitted to a referral neuroscience hospital in Bangladesh. It was observed that undiluted serum PRL levels may underestimate true hormone concentrations in a subset of patients, highlighting the diagnostic value of serial dilution assays in such cases.

Our findings reinforce the relevance of the high-dose hook effect in clinical practice. Among the 73 participants, three patients (4.1%) who initially had PRL levels <200 ng/mL showed markedly elevated levels (>200 ng/mL) after serial dilution, consistent with the

hook effect. This underlines the risk of misclassifying prolactinomas as non-functioning pituitary adenomas (NFPAs) if dilution protocols are not systematically employed. Additionally, PRL levels could be specified to be very high after dilution in the seven patients who had initial PRL above the upper limit of detection by the assay (>200 ng/mL). This is useful because the magnitude of PRL elevation is important in determining the etiology of hyperprolactinemia. All these patients had tumors with suprasellar extension, and most had parasellar involvement, further emphasizing the relationship between tumor size and PRL level. These results align with previous literature that has described the hook effect in large pituitary tumors.[10,11] The serum prolactin level to tumor size ratio is observed to be able to differentiate prolactinomas from the stalk effect of NFPA, calculation of which needs true quantification of the PRL level.[12] The moderate agreement between PRL levels before and after dilution further emphasizes the clinical significance of performing serial dilutions, as a substantial proportion of patients could have their PRL classification altered, potentially impacting treatment decisions. Moreover, differentiating patients exhibiting the hook effect based on clinical characteristics alone remains challenging.[13]

Although modern immunoassay systems have improved sensitivity and specificity, our findings suggest that the hook effect persists despite advances in assay technology. Notably, the analyzer and reagent used in our study were susceptible to this phenomenon, affirming earlier suggestions that no immunoassay is completely immune to such interferences. Compared to studies from Western settings, this research provides new insight into the South Asian context, which is relatively underrepresented in endocrine literature. The frequency of the hook effect among susceptible patients with pituitary adenoma, in whom prolactin levels may pose a diagnostic challenge, was observed to be around 4%. This finding offers a new perspective on a rarely recognized problem, although confirmation through larger multicenter studies is warranted.

A key strength of this study lies in its real-world clinical context and systematic evaluation of PRL levels before and after dilution in all patients, regardless of baseline values. Furthermore, correlating these findings with clinical and radiological data provides a more comprehensive understanding of the potential clinical implications of this analytical interference. However, some limitations should be acknowledged. First, the cross-sectional design precludes assessment of longitudinal outcomes, such as response to dopamine agonists or surgical interventions, which would have helped establish the clinical impact of correcting PRL levels through dilution. Second, while providing initial insights, the sample size could be expanded in future studies to delineate further the prevalence and associated factors of the hook effect in this population. Additionally, we used a single chemiluminescent immunoassay; evaluating the hook effect across different assay platforms could provide further valuable information.

Conclusions

In conclusion, this study observed that serial dilution may add additional information when assessing serum PRL in patients with pituitary macroadenomas. The high-dose hook effect, though infrequent, has significant clinical implications, potentially leading to misdiagnosis and inappropriate management. We advocate routine dilution protocols in all patients with radiologically large pituitary tumors and discordantly low PRL levels. Clinical guidelines should incorporate awareness of assay-related interferences, particularly in resource-constrained settings where diagnostic errors may have greater consequences. Future research involving larger, multi-center cohorts and assay comparisons will help refine diagnostic thresholds and improve clinical decision-making in patients with suspected prolactinomas.

Conflict of interest

The authors have no conflicts of interest to disclose.

Acknowledgements

Department of Biochemistry, NINS (For biochemical measurements).

Financial Disclosure

The study was funded by a grant from Bangladesh Medical Research Council.

Data Availability

Any queries regarding this study should be directed to the corresponding author, and supporting data are available from the corresponding author upon reasonable request.

Ethical Approval and Consent to Participate

This study was approved by the Institutional Review Board (IRB) of NINS. All procedures performed in this study involving human participants were following the ethical standards of the IRB and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed written consent was obtained from each of the participants included in the study.

How to cite this article: Atiqur-Rahman M, Hasan M, Islam KMA, Ahmed MF, Ratul RH, Chowdhury S, Islam MJ. Underestimation of prolactin in pituitary macroadenoma: Serial dilution may provide diagnostic insights. J Assoc Clin Endocrinol Diabetol Bangladesh 2025; 4(2): 66-71

Publication History

Received on: 12 Jun 2025

Accepted on: 28 Jun 2025

Published on: 01 Jul 2025

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