

A STUDY TO ESTABLISH AND VALIDATE DILUTION PROTOCOL FOR HIGH SERUM PROLACTIN LEVELS: A PILOT STUDY

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Abstract

Background: Hyperprolactinemia is a prevalent endocrine disorder affecting the hypothalamic-pituitary axis. Immunological assays can yield false-low results in hyperprolactinemia due to the high dose hook effect. To counteract this, dilutions are often employed, but potential errors introduced by this process necessitate a thorough validation of the dilution protocol to ensure result accuracy. This study aimed to compare serum prolactin levels in patients with suspected hyperprolactinemia with and without dilution. **Materials and Methods:** In this validation study, we analysed serum prolactin levels directly and after 1:10, 1:100, and 1:1000 serial dilutions in 30 patient samples with suspected hyperprolactinemia. A two-proportion Z test was conducted to compare the variation in prolactin levels between different dilution methods and the direct method. **Results and Conclusions:** Our study revealed that a 1:10 dilution did not significantly alter the prolactin estimate. Higher dilutions of 1:100 and 1:1000 were explored, but no significant difference was observed between the two. Notably, 100% of results were obtained with a 1:100 dilution. In conclusion, sample dilution proves to be a straightforward method for detecting falsely low concentrations, with the 1:100 dilution being particularly effective in mitigating the high dose hook effect. While modern assays have enhanced reliability, physicians should remain vigilant about the high-dose hook effect and consider appropriate sample dilution techniques when necessary.

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Introduction

Hyperprolactinemia, characterized by elevated prolactin levels exceeding 30 ng/mL, represents a disorder within the hypothalamic-pituitary axis¹, predominantly affecting women. The prevalence of hyperprolactinemia varies from 0.4% in an unselected adult population to as high as 9-17% in women with reproductive diseases. This condition is treatable, and accurate prolactin values are crucial for clinicians to monitor and titrate treatment effectively. Notably, values surpassing the assay measurement range (AMR) are reported as greater than the upper limit of AMR or falsely low values due to the hook effect.

The high-dose hook effect is a well-documented occurrence in immunological and serological assays, leading to false-negative reports. This phenomenon arises from a limited quantity of antibodies in the coated wells compared to very high concentrations of the antigen in the sample, resulting in decreased antigen-antibody complex formation and false low analyte values. The hook effect, often overlooked, can lead to erroneous results with potential serious medical implications. Alternatively, analysing a pooled sample that has been drawn over a short period of time, is found to be useful in detecting falsely low concentrations which could arise due to high dose hook effect.²

In recent times, there has been a surge in requests for prolactin estimation, with an additional directive to perform the assay at a 1:1000 dilution. While published data for 1:10 and 1:100 prolactin dilutions are available, the 1:1000 dilution remains undocumented and unvalidated. Consequently, it is imperative for laboratories to establish and validate dilution protocols to ensure the reliability of prolactin values.

Mandatory validation of dilution protocols for prolactin is essential for providing reliable, accurate, and absolute values, facilitating effective treatment, follow-up, and monitoring of patients with hyperprolactinemia. Moreover, such validation instills confidence in physicians regarding the analytical performance of the laboratory. This study was undertaken to estimate serum prolactin levels with and without dilution in patients with suspected hyperprolactinemia, aiming to establish and validate the dilution protocol for prolactin assays.

Materials And Methods

This validation study was conducted in the department of Biochemistry, St. John's Medical College and Hospital, following the approval of the Institutional Ethics Review Board. Thirty patients suspected of hyperprolactinemia were enrolled for the analysis of serum prolactin levels using serial dilutions of 1:10, 1:100, and 1:1000.

Inclusion criteria encompassed adult patients, irrespective of gender, within the age range of 18 to 80 years, exhibiting signs of suspected hyperprolactinemia. Exclusion criteria were defined to exclude pregnant and lactating women, patients currently on antipsychotics and antidepressants, as well as samples that were haemolysed, lipemic, icteric, or of insufficient quantity.

Blood samples were collected from each participant under aseptic precautions, and following centrifugation, serum samples were divided into two aliquots. One aliquot was processed without any dilution, while the other underwent serial dilution to achieve 1:10, 1:100, and 1:1000 portions. The estimation of serum prolactin levels was performed using the Siemens ADVIA Centaur® XP chemiluminescent immunoassay. The assay's assay measurement range (AMR) for prolactin was 0.37 to 200 ng/mL, with intra-assay and inter-assay coefficients of variation consistently below 10%.

Storage of the serum samples was carried out at -20°C until the time of analysis, ensuring the preservation of sample integrity.

The comprehensive methodological approach employed in this study aimed to assess the impact of serial dilutions on serum prolactin levels in patients with suspected hyperprolactinemia, providing valuable insights into the accuracy and reliability of the dilution protocol.

Statistical Analysis

Descriptive statistical analysis was used to describe the mean and standard deviation for the study population.

To assess the variation in prolactin levels between different dilution methods and the direct method, a Two Proportion Z test was conducted.

This statistical test enabled the comparison of proportions, providing insights into the significance of the observed differences in prolactin results.

Results

In this validation study, 30 patient samples with suspected hyperprolactinemia were analysed for serum prolactin levels and in serial dilutions 1:10, 1:100 & 1:1000.

Table 1 and 2 depicts the age and gender wise distribution among the population studied.

Table 1: Age distribution among subjects

Age (Yrs)	No	%
18-25	7	23.33
26-35	5	16.66
36-45	13	43.33
46-60	5	16.66
Total	30	100
Mean + SD: 36.26 ± 11.08		

Table 2: Gender distribution among subjects

Gender	Number	%
Male	17	56.66
Female	13	43.33

Data were analysed to establish the dilution protocol and validate the test results.

Figure 1, 2, 3 and 4 shows the comparison of prolactin levels in different dilutions (direct, 1:10, 1:100 & 1:1000)

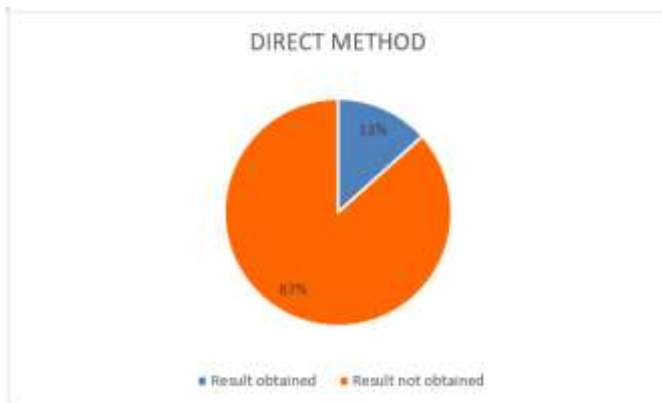


Figure 1

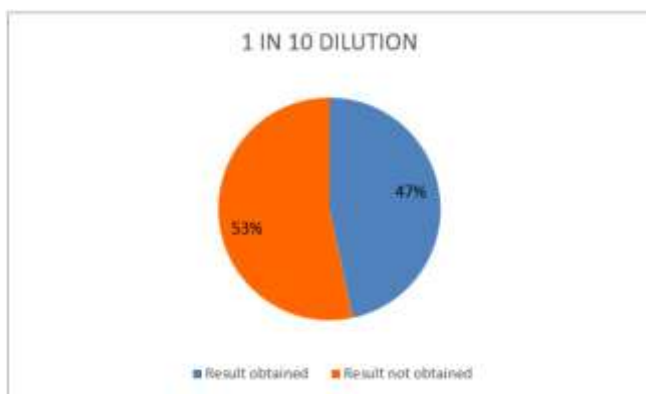
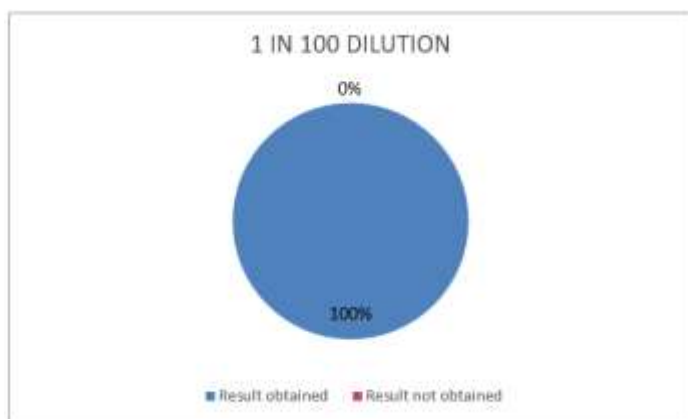
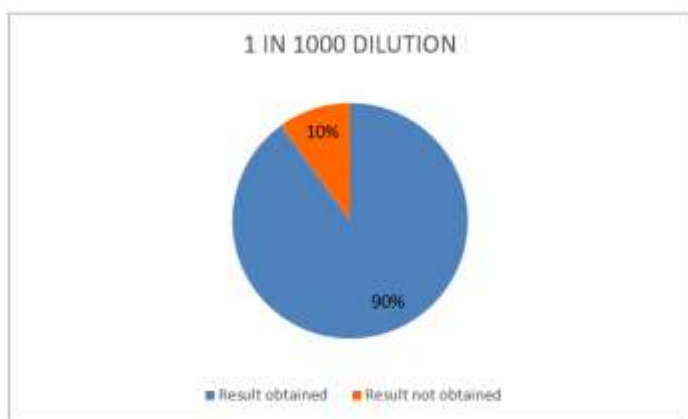


Figure 2

**Figure 3****Figure 4**

The study revealed that a 1:10 dilution did not impart a statistically significant change to the Prolactin estimate. Subsequent exploration of higher dilutions, specifically 1:100 and 1:1000, was undertaken in an endeavour to attain more precise values; nevertheless, no statistically significant difference was discerned between the two dilution levels.

Noteworthy was the observation that optimal results, amounting to 100%, were consistently obtained when the assay was performed at a 1:100 dilution. This finding underscores the efficacy of the 1:100 dilution in ensuring accurate and reliable Prolactin measurements, emphasizing its utility in mitigating potential discrepancies introduced by higher dilutions. The robustness of the 1:100 dilution in yielding consistent outcomes substantiates its role as a preferred dilution protocol for serum prolactin assessment in the context of suspected hyperprolactinemia.

Discussion

The diagnosis of hyperprolactinemia is made when serum PRL levels are found on two separate occasions to be above the standard upper limit of normal range (usually 20 to 25 ng/mL or 400 to 500 mIU/liter).^{20,21} Nevertheless, a single determination may be sufficient if PRL levels are clearly elevated (e.g., > 100 ng/mL).²²

This study on establishing and validating a dilution protocol for high serum prolactin levels addresses a critical aspect in the accurate assessment of hyperprolactinemia, a prevalent endocrine disorder affecting the hypothalamic-pituitary axis. Immunological assays, while widely utilized, present challenges, notably the high dose hook effect, leading to false-low results in hyperprolactinemia. Dilutions are commonly employed to counteract this effect;

however, the potential errors introduced by the dilution process necessitate a thorough validation of the protocol to ensure result accuracy.

The pilot study involved the analysis of serum prolactin levels directly and after 1:10, 1:100, and 1:1000 serial dilutions in 30 patient samples with suspected hyperprolactinemia. The results revealed that a 1:10 dilution did not significantly alter the prolactin estimate. Higher dilutions of 1:100 and 1:1000 were explored, but intriguingly, no significant difference was observed between the two. Remarkably, a 1:100 dilution yielded 100% of results, establishing it as particularly effective in mitigating the high dose hook effect. This reinforces the practical significance of sample dilution as a straightforward method for identifying falsely low concentrations, a crucial consideration in hyperprolactinemia cases.

These findings gain significance within the larger context of diagnosing and managing hyperprolactinemia. Hyperprolactinemia, characterized by abnormally high levels of prolactin in the blood, presents diagnostic challenges due to varied etiologies. Clinicians face the complexities of interpreting prolactin levels, considering conditions that can lead to misdiagnosis, such as prolactinomas or other causes that can result in either falsely high or low levels. The high dose hook effect, a documented phenomenon for decades, continues to pose challenges, particularly in immunometric assays.³⁻¹²

Various approaches to eliminate the high dose hook effect have been discussed in the literature, including testing undiluted and after dilution, pooling patient samples, and using two-step immunoassays with wash steps or neural network classifier systems.¹³⁻¹⁸

The study aligns with recommendations from the Endocrine Society Clinical practice Guideline²³, emphasizing the need for sample dilution, especially in cases of large pituitary tumors where the high dose hook effect is more likely.

The discussion underscores the intricacies associated with immunoassays in modern endocrinology. The specificity of diagnostic antibodies depends on meticulous reagent selection by manufacturers, and challenges such as limited standards and antibody interference are recognized concerns. The historical problem of Hook's effect, particularly in old 2-site immunometric assays, is acknowledged, but the study demonstrates the rarity of this occurrence in modern assays, especially for prolactin concentrations below 20,000 ng/mL.³

In conclusion, the study advocates for the routine use of sample dilution in the Prolactin immunoassay, specifically recommending a 1:100 dilution to avoid the high dose hook effect. Despite advancements in assay reliability, the awareness of the high-dose hook effect remains paramount, prompting physicians to judiciously employ sample dilution techniques when necessary. The findings from this pilot study contribute valuable insights to the ongoing discourse on optimizing prolactin assessment in suspected hyperprolactinemia cases.

Conflict of Interest: Nil

Acknowledgment: Nil

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