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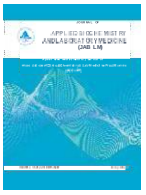

Review article

Diagnosis of Hyperprolactinemia by Single Serum Prolactin Determination: Challenges and Recommendations

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Keywords:
Prolactin, Hyperprolactinemia, Macroprolactin, Stress, Venepuncture, Variation

ABSTRACT

Secretion of prolactin follows a circadian rhythm of secretion, and several factors play an important role in the regulation of its secretion. An accurate diagnostic evaluation is essential for the proper management of the patient, which can be accomplished through a narrow observation and critical analysis of all the prolactin results which are above the standard upper limit of normal. If the circulating prolactin level exceeds beyond five times the upper normal limit, a single test is sufficient to diagnose hyperprolactinemia. However, mildly elevated (20–40 ng/ml) results should be confirmed with at least two tests to counteract the circadian fluctuation or other factors causing transitory elevation. Repeat analysis of circulating prolactin on a later date preferably with 2–3 samples collected at few min interval is also recommended to minimise the effect of pulsatility when the elevation of serum PRL is doubtful (may be due to venepuncture induced stress) or when results are inconsistent with the clinical features. This review article presents an overview of the biological and analytical aspects of prolactin along with the impact of stress on prolactin secretion, as well as the current approach employed to tackle the chances of misdiagnosis and overtreatment. However, to understand the exact mechanism of stress-induced hyperprolactinemia and its implications, further research is required.

INTRODUCTION

Prolactin (PRL) was first introduced by Riddle et al. in 1933, and its role in the proliferation and differentiation of mammary cells as well as lactation was identified [1,2]. This review focuses on the biological and analytical aspects of this fascinating hormone along with the probable causes of variations in the analytical findings with special emphasis on stress-induced variations, which are of interest to clinicians treating their patients.

PROLACTIN CHEMISTRY

Human PRL is encoded by a single gene on chromosome 6 [1,3,4]. It is a 23-kDa single-chain polypeptide of 198 amino acid residues and shares similar genetic and structural properties with growth hormone and placental lactogen [4,5].

ISOFORMS

Circulatory PRL has three molecular isoforms based on its size heterogeneity. In addition to its monomeric form, two other forms also exist, namely, a dimeric form 'big prolactin' of 50–60 kDa size and a larger polymeric form 'big-big prolactin' (macroprolactin) of greater than 150 kDa size, which is formed by binding 23 kDa prolactin with IgG autoantibodies. Both these forms exhibit minimal biological activity [1,4,6-11].

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SECRETION

PRL is secreted from the acidophilic lactotroph cells of the anterior pituitary gland, and several factors influence the regulation of PRL secretion. It follows circadian rhythm of secretion, with a nocturnal increase, showing 2–3 times higher secretion during the night. Maximum secretion occurs during rapid eye movement (REM) sleep, and secretion is 20–30% higher in the morning than in the afternoon, reaching its peak between 4 AM and 6 AM. Short-term 'pulses' are less consistent [4,5]. Although no marked changes are observed during the menstrual cycle, there may be oestrogen-induced slight mid-cycle elevation as oestrogens have a direct action on the pituitary that increases PRL secretion [5,12]. There may be ectopic secretion of PRL from ovarian dermoids or bronchogenic carcinoma [7,13].

REGULATION OF PROLACTIN SECRETION

Several factors play an important role in the regulation of PRL secretion. It is primarily under the inhibitory control of dopamine, wherein the secretion is suppressed through pituitary dopamine type 2 (D2) receptors [4,5]. Factors that have been identified to inhibit actions of dopamine neurons and thus indirectly upsurge PRL secretion include cholecystokinin, ɣ amino butyric acid (GABA), galanin, histamine, nitric oxide, noradrenaline, estrogen, opioids, and serotonin [1,5,14]. On the contrary, several factors, such as acetylcholine, angiotensin II, atrial natriuretic peptide, bombesin-like peptides, calcitonin, neuropeptide Y, neurotensin, oxytocin, pituitary adenylate cyclase-activating peptide, thyrotropin-releasing hormone, vasoactive intestinal peptide, and vasopressin inhibit PRL release (Figure 1) by stimulating dopamine neurons [1,15]. PRL secretion is also regulated through a short-loop negative feedback mechanism by PRL itself, increasing dopamine secretion and suppressing PRL secretion [1,16]. In addition, some cytokines are also involved in PRL secretion; for example, interleukin-1 (IL-1), IL-2, and IL-6 stimulate PRL secretion, whereas, interferon-gamma (INF-γ) and endothelin-3 are inhibitory cytokines [4,17]. Drugs, such as phenothiazines, increase PRL secretion [5]. Major stress such as that associated with general anesthesia and major surgery raises PRL secretion by several-fold. By contrast, no evidence can objectively clarify whether minor stress, such as psychological trauma, outpatient attendance, and venepuncture, raises PRL levels [5,18]. Some physiological factors, including pregnancy and sucking, stimulate PRL secretion.

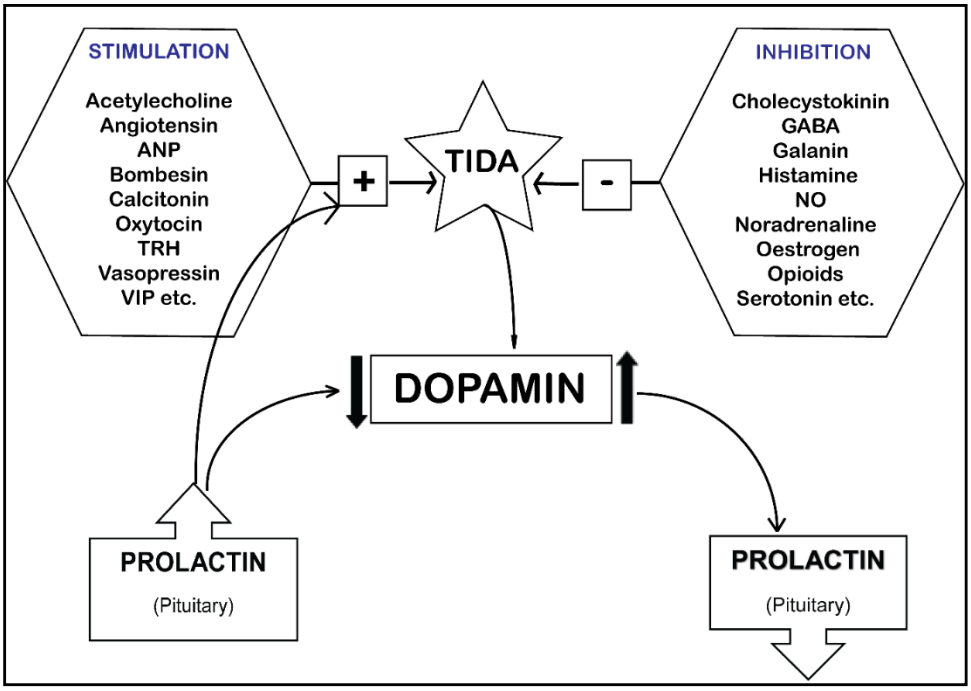

Figure 1. Regulation of prolactin secretion through dopamine and short loop feedback mechanism.

TIDA represents Tuberoinfundibular dopaminergic neurons, ANP = atrial natriuretic peptide, GABA = ɣ amino butyric acid, NO = nitric oxide, TRH = thyrotropin releasing hormone and VIP = vasoactive intestinal polypeptide.

METABOLISM

The biological half-life of PRL is 20–50 min. It is metabolized in the liver and eliminated by both liver and kidney. The normal adult circulating prolactin levels in the case of females and males are 10–25 µg/L (10–25 ng/mL) and 10–20 µg/L (10–20 ng/mL), respectively [4].

FUNCTION

In addition to its key role in breast development and induction, as well as the maintenance of lactation, PRL has several other functions. It inhibits the secretion of gonadotropins through suppression of gonadotropin-releasing hormone (GnRH) and thus impedes reproductive function [4]. It also plays an important role in water and electrolyte balance, immunoregulation [4,19,20], hepatocyte turnover [4,21], proliferation of vascular smooth muscle and intestinal mucosa [4,22,23]; it stimulates adipogenesis, inhibits lipolysis, and promotes insulin sensitivity [4,24].

HYPERPROLACTINEMIA (HYPER-PRL)

Hyper-PRL is an expression of an abnormal biochemical state rather than a medical condition. It implies to elevation of serum PRL levels above the standard upper limit of normal, presenting reproductive problems, mostly infertility in females. The prevalence of hyper-PRL is 0.4%, affecting approximately 10 per 100,000 males and 30 per 100,000 females [4,5,7,9,25-29]. However, the estimated prevalence has been reported to be 5% in the family planning clinic population and 17% in women with reproductive disorders [1,14].

Incidence is four times more in females aged 25–34 years than

in males [4,25,26].

Hyper-PRL may be functional, physiological, analytical, Common physiological causes of hyper-PRL are pregnancy and lactation. It may also increase following ingestion of high-protein diets, stress (including venepuncture), sleep, physical exertion, hypoglycemia, or sexual intercourse [1,7,30].

ANALYTICAL HYPER-PRL

Analytical causes of hyper-PRL are also very common, and the presence of circulating big-big PRL or macroprolactin (Macro-PRL) is the most likely cause. Approximately 4% of the general population is diagnosed with circulating Macro-PRL, which presents a prevalence of 12.5–40% in patients with raised PRL results [1,7,31-33]. As Macro-PRL is biologically inactive, it does not produce any clinical symptoms of hyper-PRL and has no clinical significance. However, it may lead to misdiagnosis and imprudent treatment [7,34,35]. Sometimes macro-PRL may also co-exist with hyper-PRL. Macro-PRL reportedly increases with advancing age, and it responds to dopamine antagonists and other physiological stimuli like monomeric PRL [6,7].

PATHOLOGICAL HYPER-PRL

The most alarming cause of pathological hyper-PRL is sellar and parasellar lesions, including PRL secreting adenomas (prolactinoma, growth hormone/prolactin-secreting, adrenocorticotropic hormone/prolactin-secreting adenoma), hypothalamic/pituitary stalk disorders (granuloma, radiation, injury), non-pituitary tumors, sarcoidosis, craniopharyngioma, empty sella syndrome, vascular malformations, and pituitary metastases [1,7,36]. Hyper-PRL may also be associated with pathological, idiopathic, or pharmacological.

FUNCTIONAL/PHYSIOLOGIC HYPER-PRL

primary hypothyroidism caused by thyrotroph and/or lactotroph hyperplasia, polycystic ovary syndrome, hepatic cirrhosis, epilepsy, chest injury, pseudopregnancy, Cushing's disease, and Addison's disease. Furthermore, hyper-PRL is observed in chronic renal failure owing to decreased renal clearance [1,7,37-40].

Prolactinoma is the most common organic cause of hyper-PRL and represents approximately 50% of the cases, showing a prevalence of 100 per 1 million [1,7,41-43]. It is more common in women and is usually benign; however, it may be malignant in some rare cases [7,44,45].

IDIOPATHIC HYPER-PRL

Idiopathic hyper-PRL is considered when no cause of hyper-PRL can be identified and no visible pituitary adenomas are sighted on imaging. Typically, idiopathic hyper-PRL presents a small microadenoma (<2 mm) which is too small for detection by imaging. Familial cases are also reported, caused by a genetic mutation in the PRL receptor located on chromosome 5, resulting in the formation of inactive PRL isoforms and PRL insensitivity [1,7,46-49].

PHARMACOLOGICAL HYPER-PRL

Hyper-PRL associated with the use of various medications (listed in Table 1) has been reported to increase serum PRL by ten-fold above the baseline [1,7,9,50-53].

Table 1. List of pharmacological agents with the ability to induce hyperprolactinemia.

Sl. No.	Classes of Drugs	Examples
1	Antipsychotics (neuroleptics)	
	First generation antipsychotics	Chlorpromazine, Fluphenazine Haloperidol
	B. Second generation antipsychotics	Paliperidone, Risperidone, Quetiapine, Amisulpride
2	Antidepressants	
	Tricyclic antidepressants	Amitriptyline, Desipramine Clomipramine, Amoxapine
	Mono amine oxidase inhibitors	Pargyline, Clorgyline
	Selective serotonin reuptake inhibitors	Sertraline, Fluoxetine, Paroxetine
	Serotonin, noradrenaline reuptake inhibitors	Venlafaxine, Duloxetine, Reboxetine
3	Other Psychotropics	Buspirone, Alprazolam
4	Antihypertensives	Methyldopa, Verapamil, Reserpine
5	Opioid analgesics	Morphine, Methadone
6	H_2 antagonists	Cimetidine, Ranitidine
7	Estrogens	Oral contraceptives
8	Prokinetics	Amoxapine, Metoclopramide Domperidone
9	Others	Fenfluramine, Physostigmine Chemotherapics

DIAGNOSTIC STRATEGIES

For diagnostic evaluation, a thorough medical history, including symptoms, medications, comorbidities, excessive breast stimulation, and lifestyle factors, along with physical examinations for galactorrhea, gynecomastia, goiter, spider angiomas, ascites, facial edema, chest wall lesions, nipple piercings, and visual field defects are crucial. Further, hormonal indicated to detect any lesion compatible with a pituitary tumor [9,56,57].

PROLACTIN ASSAYS

Ideally, the best time of sample collection for blood testing is 2–3 hours after waking [4]. The current methodology used to measure circulating PRL levels is based on the two-site immunometric or sandwich assay principle, wherein the PRL present in the sample reacts with an immobilized capture antibody followed by a labeled detection antibody. The signal generated by the PRL-antibody complex is directly proportional to the amount of PRL present in the sample. Cross-reactivity or interference arising from circulating growth hormone, human placental lactogen, and heterophilic antibodies is rarely encountered with these types of sandwich assays [1,7,58].

PRL ASSAY PITFALLS

The contemporary automated immunoassay systems employing assays and imaging studies can aid in the diagnostic evaluation of hyper-PRL [1,9,27]. Estimation of circulating TSH, free T4, creatinine, IGF-1 levels, and β-hCG is recommended along with the measurement of serum PRL level to rule out secondary causes of hyper-PRL [9,27,28,39,54-56]. After the exclusion of all other probabilities of hyper-PRL, magnetic resonance imaging or computerized tomography is

the sandwich assay principle are unable to differentiate between macro-PRL and monomeric PRL. Therefore, along with the monomeric PRL, macro-PRL can also bind with the capture and labeled antibody, which are used for the measurement of PRL and generate a high-intensity signal for PRL, giving an erroneous result [6,7]. Such type of erroneous findings can be prevented by treating the serum with an equal volume of 25% (w/v) polyethylene glycol (PEG) before processing the sample in immunoassay to precipitate the macro-PRL. Although the gold standard for the diagnosis of macro-PRL is gel filtration chromatography, PEG precipitation of serum is preferred as gel filtration chromatography is expensive and time-consuming [9,11,28,46,59-62]. PEG helps to precipitate immunoglobulin and immunoglobulin complexes and precipitates the macro-PRL that contains IgG. However, it is observed that approximately 20% of the monomeric PRL is also co-precipitated with IgG [1,7,46,63].

Another common pitfall of the PRL assay is the hook effect. This is usually encountered when a significantly high circulating PRL saturates the antibodies employed in the two-site immunometric method. Consequently, labeled detection antibodies bind directly with the excess PRL without capturing antibodies, thus giving erroneous results, which are lesser than the actual values [7,64]. This can happen in giant prolactinomas where the actual PRL levels can be several folds higher than the standard upper limit of normal but usually reported as normal [7,65]. This type of incongruity can be prevented by diluting the sera and serial dilution up to 1:100 [7,27].

In addition, high circulating biotin can also affect the PRL result by preventing the formation of an antigen-antibody complex, thus yielding a deceptively low PRL result. Similarly, heterophilic or human anti-mouse antibodies may also interfere with PRL readings in patients with autoimmune diseases receiving antibody treatment [7,66,67]. In such cases, antibody precipitation, the use of antibody blocking tubes, serial dilution of the sample, or the application of a different methodology for processing the sample may help to obtain a more accurate result [7].

CIRCULATING PRL IN RESPONSE TO STRESS

The biological response to stress is a very complex phenomenon, which causes secretion of adrenaline and noradrenaline, as well as the release of PRL. Ample pieces of evidence are supporting the role of PRL in numerous stress-induced systemic disease pathologies, developed secondary to hyper-PRL [4,68-71]. Therefore, it is of paramount importance to determine the mechanism by which PRL regulates and responds to emotional stress.

MECHANISMS OF STRESS-INDUCED HYPER-PRL

As reported earlier, dopamine is not responsible for stress-induced hyper-PRL; PRL-releasing factors such as prolactin-releasing peptide (PrRP) are considered to be responsible for stress-induced hyper-PRL. Studies have shown that PrRP in animal models can stimulate corticotrophin-releasing hormone (CRH), mediate the release of adrenocorticotropic hormone (ACTH), and alter the hypothalamic-pituitary-adrenal (HPA) axis in conjunction with noradrenaline. It has also been reported that irrespective of the gender bias, a highly significant correlation exists between the magnitude of PRL secretion and the magnitude of ACTH secretion in response to stress as both hormones are secreted from the anterior pituitary under the influence of the same releasing factor [72]. Further, the nocturnal rise of PRL secretion is considered to be mediated by serotonin, which is presented as a multifaceted response to stress. However, it is too early to comment on the above observation as it requires further investigation and is therefore beyond the scope of this review [4].

THE BIOLOGICAL SIGNIFICANCE OF STRESS-INDUCED HYPER-PRL

The biological significance of stress-induced hyper-PRL is still under experimentation. There is evidence indicating the regulative role of PRL in response to stress. Experiments on animal models have revealed that PRL results in corticosterone release through its action on the adrenal gland [72,73]. It was also reported that the HPA axis reactivity could be inhibited by intra-cerebral infusion of PRL in animal models and thus could be considered as a regulator of stress response. Moreover, the level of the PRL release is correlated with the magnitude of the HPA-axis responses, though the mechanism is not fully understood. Prolactin has been reported to play a protective role against the damage caused by stress. It has been reported that stress-induced hyper-PRL acts as a buffer towards the immunosuppressive effects of stress and thus plays the role of an immune-enhancing hormone. Reports also state that stress-induced hyper-PRL can prevent the development of gastric ulcers secondary to stress response [72].

THE DISCREPANCY OF PRL RESPONSES IN LABORATORY STRESS STUDIES

Several studies have revealed that serum PRL level is elevated in response to acute

psychological stress [72,74-77]. However, some studies have revealed completely contradictory results [72,78-83]. Several justifications related to these contradictory outcomes have been noticed. One narration is that the reduced or unaltered serum PRL level in response to acute stress may be attributed to the study design, wherein the baseline level of PRL is increased before the stress test owing to anticipatory stress, which masks the actual variation during the test [72,80]. Secretion of PRL follows a diurnal rhythm, and a nocturnal elevation is observed followed by a continuous and abrupt decline for a few hours after awakening. If sampling is performed within this period, this functional decrease may have a significant influence on the stress test, which explains the unaltered or decreased status of PRL following the stress test [72,83].

RECOMMENDATION/APPROACH

Hyper-PRL is diagnosed when the circulating PRL level reaches beyond 25 ng/mL (25 µg/L). However, in case of mildly elevated (20–40 ng/ml (20–40 µg/L) circulating PRL levels, the diagnosis should be confirmed with at least two tests to counteract the circadian fluctuation or other factors causing transitory elevation. If the circulating PRL level exceeds five times the upper normal limit, a single test is sufficient to diagnose hyper-PRL [4,25]. Regarding the ideal practice of sample collection for estimating circulating PRL, various recommendations and controversies have been reported [84,85]. According to the Endocrine Society Clinical Practice Guideline, a single measurement of circulating PRL is sufficient to confirm the diagnosis of hyper-PRL provided sample collection is conducted without excessive venepuncture stress. Therefore, the probability of venepuncture-induced stress-mediated variation of circulating PRL cannot be ruled out and must be taken care of to minimize overdiagnosis and treatment. Repeat analysis of circulating PRL on a later date preferably with 2–3 samples collected at an interval of 15–20 min is also recommended to minimize the effect of pulsatility when the elevation of serum PRL is doubtful (may be due to venepuncture-induced stress) or when results are inconsistent with the clinical features [27,86,87]. It is also suggested that the elevated circulating PRL level must be re-evaluated before reporting unless it is elevated

(>80–100 ng/mL) [28,84,88]. Several studies have observed a significant increase in the circulating PRL because of venepuncture-induced stress and suggested serial sampling for analysis at intervals of a few minutes with a rest period [4,84,89-94]. To reduce pain and fear of multiple venepuncture pricks, it was also advocated to use an intravenous catheter or cannula for sample collection [84,91,94]. However, preparing a pool from the samples collected at different time intervals with rest, followed by measurement of the analyte from the pooled sample was considered a better option as it could conserve time and resources [84,93,95,96].

Further, in the case of asymptomatic hyper-PRL, it is advisable

to rule out macro-PRL as macro-PRL is a common cause of hyper-PRL. Therefore, the exclusion of macro-PRL in all cases of hyper-PRL or otherwise routine screening for macro-PRL may help to exterminate unnecessary testing and treatment. In addition, the possibility of hook effect must be considered in regards to normal or moderate elevation of circulating PRL with a clinical presentation of large pituitary adenomas. The confirmation should be accomplished with a dilution of the sample to prevent misdiagnosis and treatment [27,56,97,98].

FUTURE DIRECTIONS

The mechanism of action, as well as the regulation of secretion of PRL, is a very complex phenomenon. Hyper-PRL is regarded as a serious endocrine disorder; however, it is considered to be a natural and favorable condition during pregnancy and lactation. Experimental findings on animal models have revealed that stress exhibits a biphasic effect on circulating PRL levels, that is, an early brief phase of stimulation followed by an extended period of inhibition [99,100]. It was also evident that sudden exposure to stress resulted in an upsurge of circulating PRL. In contrast, repeated exposure to the same stressor failed to display any change in the serum PRL level. It appears that exposure to the same stressor causes adaptation to the stimulus, resulting in a lower physiological response [99,101]. Similar biphasic or "two faces" nature of PRL has also been observed in the immune system. At physiological concentrations, PRL stimulates NK cell activity, whereas at higher concentrations, it inhibits the NK cells [99,102]. PRL influences many physiological processes and plays a crucial role in various diseases. However, its mechanisms of action are not clearly understood and are the subject of research. Moreover, the role of PRL in metabolic homeostasis, immune regulation, and sex-dependent stress response also needs further elucidation [4,20].

CONCLUSIVE REMARKS

Secretion of PRL is not only influenced by numerous environmental factors but is also strongly regulated by stress. Because of the significant role of this hormone in stress responses, it is often called the stress hormone. This review aims to present an overview of the biological and analytical aspects of PRL and the impact of stress on PRL secretion as well as the current approach employed to address the chances of misdiagnosis and overtreatment.

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