

# Using Polyethylene Glycol Precipitation to Differentiate between Macroprolactinemia and Hyperprolactinemia

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## ABSTRACT

Hyperprolactinemia is a common endocrine disorder which clinically manifests in reproductive period and presents with hypogonadism, galactorrhea and symptoms related to tumor mass effects. Hyperprolactinemia is a state of excess total prolactin (PRL) concentration in serum. Three forms of PRL in sera are monomeric PRL with 23 kilo-dalton (kDa) MW which acts bioactively which is predominant in patients with prolactinomas, dimeric PRL; 45-60 kDa MW which is not clearly bioactive and macroprolactin with MW bigger than 100 kDa which is not bioactive. Macroprolactinemia is common and is associated with idiopathic hyperprolactinemia. Serum PRL is routinely screened for diagnosis of hyperprolactinemia. Nowadays, laboratory automatic analysis to measure serum PRL level using sandwich immunometric methodology is accurate and reliable. Limitations of these immunoassays are interference from prolactin/IgG auto-antibody complex termed macroprolactin. This condition may lead to misdiagnosis and mismanagement of cases because the total PRL measurement comprises macroprolactin and active PRL. Using 25% polyethylene glycol (PEG) precipitation quantitatively provides the bioactive PRL by gel filtration chromatography which remains the gold standard to fractionate the level of PRL concentration. Recommended cut point recoveries of PRL less than 40% or 50% is indicative of the predominance of macroprolactin whereas more than 50% or 60% is diagnosed as true hyperprolactinemia. PEG precipitation is the best practice and recommended as the worldwide method to divide macroprolactin and active PRL for detection of the true origin because it is reproducible, easily performed and is effective screening. This review emphasizes about pathophysiology of hormone PRL and expresses the necessary method to clarify between macroprolactinemia and truly hyperprolactinemia.

**Keywords:** Macroprolactin, hyperprolactinemia, polyethylene glycol precipitation

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## INTRODUCTION

Prolactin (PRL) is an important endocrine hormone, which is secreted from lactotroph cells of pituitary gland. This hormone secretes in a circadian manner and increases in level during pregnancy and stress. Secretion is

controlled via a negative feedback loop whereby circulating prolactin stimulates hypothalamic dopamine secretion, which inhibits prolactin release by the pituitary gland.<sup>1</sup> The hormone PRL in humans can also be generated locally in numerous tissues including the endometrium, brain, breast, skin, lymphocytes, and adipocytes. The function of PRL involves in numerous processes, such as reproduction, metabolism, immunology, and behavior as well as cancer.<sup>2</sup>

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## 1. Prolactin gene and prolactin in sera

The human PRL gene locates on the short arm of chromosome 6p22.2-p21.3 locus 21593972-22571892 and exists in a gene poor region of the genome and consists of a single gene. It contains five coding exons, transcribed directly from a pituitary specific promoter, and a non-coding exon transcribed from an alternative promoter, which drives expression in non-pituitary tissues.<sup>2</sup> The organization of the PRL gene is more complex. Its transcription is regulated by two main independent promoter regions. The human prolactin complementary DNA (cDNA) is 914 nucleotides in length. Its transcript messenger RNA (mRNA) contains 681 nucleotides, encoding for a pro-hormone (pre-prolactin) of 227 amino acids. During prolactin processing, the 28 amino acid signal peptide is proteolytically cleaved, resulting in a mature single chain of 199 amino acids. The structure has three highly conserved intra-molecular disulfide bonds between six cysteine residues. It circulates in multiple forms with difference molecular sizes, and three major species are identifiable by gel filtration chromatography (GFC). First, monomeric PRL which has an approximate molecular weight (MW) 23 kDa, which is a bioactive form. Second, big PRL of MW 45-60 kDa which is not clearly bioactive. Third is big-big PRL or macroprolactin of MW more than 100 kDa which consists of complexes of normal 23 kDa monomeric PRL and a 150 kDa IgG molecule which is a non bioactive form. The sera of both normal patients and most patients with hyperprolactinemia consists predominantly of 65-80% monomeric PRL, 10-20% is dimeric PRL or big PRL, and less than 10% is macroprolactin. The macroprolactin is a complex form, which clearly slows the clearance rate. It can result in apparent hyperprolactinemia.<sup>3-5</sup>

## 2. Hyperprolactinemia

Hyperprolactinemia is a common endocrine disorder. The clinical manifestations in reproductive period presents with hypogonadism (infertility, menstrual abnormality), galactorrhea, and symptoms related to tumor mass effects (headaches, visual disturbance). These symptoms should prompt the measurement of PRL level.

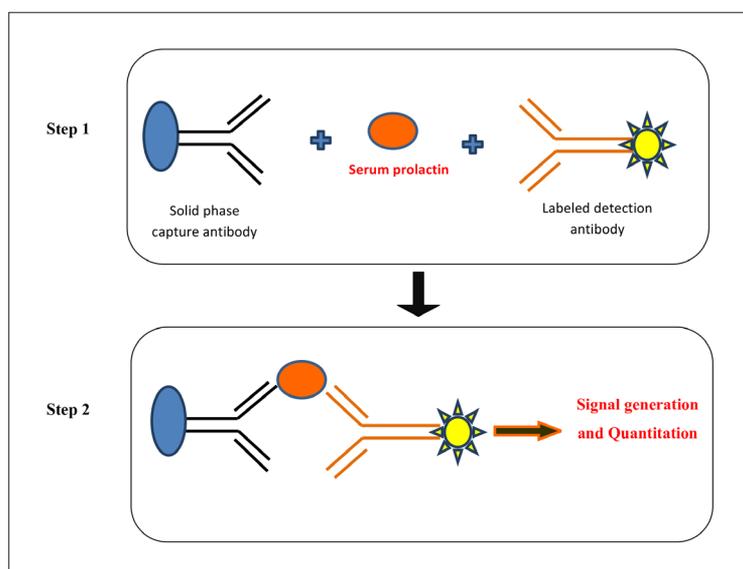
Serum PRL level is easily measured in modern clinical laboratories with automated immunoassay methodology.<sup>4</sup> Current immunoassays generally employ a two-site immunometric or sandwich principle. PRL is reacted with both captured antibody, which is often immobilized on a solid phase, and a labeled antibody for detection. After capturing of the analyze-antibody sandwich and removal of un-reacted reagents by a wash step, the signal generated is directly related to the amount of PRL present as shown in Fig 1.

The term hyperprolactinemia is based on biochemical findings rather than on a clinical state. Typically, a normal upper limit of normal PRL is 300 mIU/L (14 ng/mL) in men and 550 mIU/L (26 ng/mL) in women. The prevalence of hyperprolactinemia ranges from 0.4% in an unselected normal adult population to 9-17% in women with reproductive disorders. The guidelines for the diagnosis and treatment of hyperprolactinemia have been recommended by Biller BM in 1999.<sup>6</sup> The levels higher than 2,000 mIU/L (94.54 ng/mL) are suggestive of microprolactinoma, and levels in excess of 6,000 mIU/L (283.63 ng/mL) are mostly diagnostic of macroprolactinoma.<sup>7</sup>

In order to display a correct biochemistry diagnosis between abnormal active and inactive form of PRL in case of hyperprolactinemia, a special laboratory method is needed to differentiate these two conditions. Moreover, the presence of increased levels of macroprolactin together with normal levels of monomeric PRL which characterizes macroprolactinemia is frequently seen among patients who come to medical attention.<sup>8-10</sup>

There are three main categories which cause of hyperprolactinemia; physiologic, pharmacologic and pathologic. Furthermore, as previously mentioned, hyperprolactinemia can arise when macroprolactin is the predominant isoform as shown in table 1.<sup>9,11</sup>

Macroprolactinemia is defined by the presence of more than 50% of serum PRL as macroprolactin (big-big-PRL) which is an isoform of high molecular weight and low biological activity.<sup>12</sup> Macroprolactin causes hyperprolactinemia because of low renal PRL clearance and a decreased stimulation of dopaminergic tonus. Macroprolactinemia is responsible for hyperprolactinemia in



**Fig 1.** Schematic illustrates the principle of a two-site immunogenic assay for prolactin. Two antibodies specific for different epitopes on prolactin are used; a capture antibody attached to a solid-phase matrix, and labeled detection antibody. During the incubation period. **(Step 1)** The reagent antibodies react with prolactin in serum to form a sandwich. **(Step 2)** After a wash step to remove unbound material, the detection antibody has now bound generates a signal that directly related to the concentration of serum prolactin.

**TABLE 1.** Causes of hyperprolactinemia.

**Physiologic :** Pregnancy; lactation; stress; sleep; coitus; exercise

**Macroprolactinemia**

**Drug-induced :** Neuroleptic agents, antidepressant agents, estrogens, etc.

**Pathologic :**

Systemic diseases – Primary hypothyroidism; adrenal insufficiency; PCOS; renal insufficiency; cirrhosis; pseudocyesis; epileptic seizures

Hypothalamic diseases – tumors (craniopharyngiomas, dysgerminomas, meningiomas, etc.); infiltrative disorders (histiocytosis, sarcoidosis, etc.), metastasis; cranial radiation; Rathke’s cleft cysts, etc.

Pituitary diseases – Prolactinomas; acromegaly; thyrotropinomas; Cushing’s disease; infiltrative disorders; metastasis; lymphocytic hypophysitis; empty sella syndrome, etc.

Stalk disorders – Hypophysitis; traumatic brain injury (TBI)

Neurogenic – Chest wall lesions (burns; breast surgery; thoracotomy; nipple rings; herpes zoster; etc.); spinal cord injury (cervical ependymoma; tabes dorsalis; extrinsic tumors; etc.), breast stimulation, etc.

**Idiopathic**

Ectopic prolactin production – Renal cell carcinoma; ovarian teratomas; gonadoblastoma; non-Hodgkin lymphoma, uterine cervical carcinoma; colorectal adenocarcinoma, etc.)

10-46% of the cases.<sup>13</sup> It should be screened in the following situations: individuals with high PRL levels, but no indications of clinical symptoms, atypical clinical picture, conflicting PRL results in distinct assays, and delayed decline of serum PRL levels with the usual doses of dopamine antagonists.<sup>14</sup> If monomeric PRL level is also

high, the clinical picture can be present, despite the presence of macroprolactinemia.<sup>9</sup>

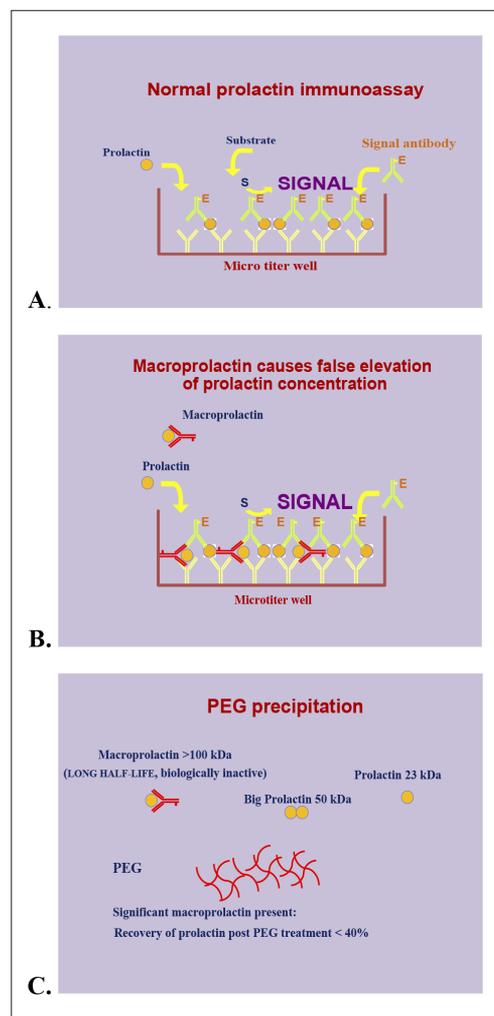
The clinical significance of macroprolactin might be mimicking with hyperprolactinemia. This condition leads to clinical confusion and inappropriate management. It is noteworthy that hyperprolactinemia due to macroprolactin has

been found in 15-20% of people classified as idiopathic hyperprolactinemia.<sup>15</sup> The differentiation between high levels of active and inactive PRL, is a necessary process to avoid misdiagnosis and mismanagement, especially unnecessary pituitary exploration.<sup>8,16</sup>

Unfortunately, macroprolactin is detected to varying degrees by all PRL immunoassays and leads to cause confusion for diagnosis. Most manufacturers commonly use auto-analyzers for serum PRL. Besides, these analyze by reference to international standard and relative reactivity towards macroprolactin.<sup>5,17</sup> Previous study by Smith TP et al., examined the ability of nine common immunoassays to measure PRL in 10 sera containing predominantly macroprolactin. The results demonstrated with 2.3-7.8 fold difference in measured prolactin levels, depending on which of those nine assay systems were used.<sup>18</sup> The objective of this review article is to point out the need to evaluate active prolactin for differential diagnosis of true hyperprolactinemia or macroprolactinemia.

### 3. Methodology for laboratory detection and quantitation of macroprolactin

First, the gold standard for the diagnosis of macroprolactinemia is gel-filtration chromatography (GFC), but this method is expensive and time consuming. Thus, it is not ideal for routine laboratory use. Second, immunoprecipitation and adsorption examine the ability of both protein A and protein G to remove macroprolactin from sera containing pre-dominantly macroprolactin prior to immunoassay. The results have indicated that both of these reagents are effective in removing macroprolactin from serum, although, monomeric PRL levels obtained in treated sera were approximately 30% higher than those obtained by GFC. Third, ultrafiltration is applied to precipitate sera containing macroprolactin. This procedure seems to be useful. However, when macroprolactin levels determined by ultrafiltration and GFC were compared, they observed that the data were widely discrepant in a significant number of cases. These findings have been reported by this group.<sup>19-20</sup> Forth, precipitation with polyethylene glycol (PEG) at a concentration of 25% (weight/



**Fig 2.** Illustrating of serum PRL immunoassay and the effect of PEG precipitation on the major forms of circulating serum PRL. **(A)** Immunoassay of normal PRL **(B)** Immunoassay has shown false elevated PRL from macroprolactin **(C)** Effect on monomeric PRL, big PRL, and macroprolactin after PEG precipitation.

**Note.** S = Substrate, E = Enzyme label antibody

volume) has the ability to precipitate immune complexes. This method has been widely used to screen for the presence of macroprolactin in hyperprolactinaemic serum.<sup>21</sup> The PEG precipitation is a relatively crude technique, which separates proteins according to their solubility. When applied to serum, the PEG precipitation is relatively specific for precipitation of immunoglobulin and immunoglobulin complexes. Hence, most common forms of precipitation of macroprolactin contain IgG as illustrated in Fig 2. In addition, PEG has added advantage of precipitating big PRL.<sup>22-23</sup> This method has been validated against GFC by a number of groups with PRL recoveries of <40%

following treatment of sera with PEG. These methods have been proposed for the detection of macroprolactinaemia.<sup>24-28</sup> The recovery by <40% is indicative of predominance of macroprolactin whereas recoveries >60% point to the diagnosis of monomeric hyperprolactinemia.<sup>25,28-29</sup> The PEG precipitation method is reproducible, easily performed and the most widely applicable effective screening test for the detection of hyperprolactinemia due to macroprolactin of most laboratories.<sup>30-34</sup> The PRL recovery ratio <50% is used as a cut point for the PEG precipitation protocol of Mayo medical laboratory. It is the method for detection of macroprolactin which is a routinely available measurement. This technique is able to accurately assess the patient for true hyperprolactinemia.<sup>35</sup> Similarly Fahie-Wilson M et al., used PEG precipitation with 50% of PRL recovery ratio to detect macroprolactin. That was a common cause of interference in prolactin immunoassays for further investigation of hyperprolactinaemia.<sup>33-34</sup> The validated reference intervals for total prolactin and post-PEG prolactin were established in 2008 by Beltran L, et al. It was based on 6 widely used immunoassay platforms for accurate diagnosis of true hyperprolactinemia.<sup>21</sup> It is essential that the macroprolactin and prolactin concentration be quantified on the same system due to the variability of different testing platforms. A clear understanding of PRL test results can help clinicians to avoid misdiagnosis and mismanagement of hyperprolactinemic patients, as well as unnecessary further investigation. When the laboratory receives a request for measurement of serum PRL, clinicians should attempt to choose the proper test to classify the true bioactivity level of circulating PRL. Accordingly, the objective of the specific laboratory should be to answer the clinical question by providing a measurement of monomeric PRL.

## CONCLUSION

Serum PRL measurements are frequently requested for investigation in patients with reproductive disorders and hyperprolactinemia. Once hyperprolactinemia has been diagnosed, it is essential to identify the underlying cause before

any form of treatment is considered. The physiologic, pharmacologic and secondary causes are readily identifiable. The reports of biochemical hyperprolactinemia might be misleading. The physicians should have awareness about mimicking conditions of hyperprolactinemia. This complexity stems from the presence of a biologically inactive form of prolactin termed macroprolactin (big-big prolactin) that is detected by all PRL immunoassays. Moreover, it commonly results in misdiagnosis and subsequent mismanagement. Laboratories can achieve this objective by using PEG precipitation. This method can separate the high molecular mass forms of PRL and report the residual PRL value along with an appropriate reference range. The recovery ratios of PRL cut points at <40% or <50% are indicative of predominance of macroprolactin whereas recovery ratios >50% or >60% point to the diagnosis of monomeric hyperprolactinemia. The use of PGP precipitation assay could improve the accurate diagnosis of macroprolactinemia by identifying interference from macroprolactin in PRL assays. Beyond that, understand the results of serum PRL measurements might be a mimicking condition. If monomeric PRL was within the reference range, the elevated total PRL would be due to high molecular mass forms of PRL (macroprolactin). Therefore, it is not a cause of pathological significance. On the other hand, if the monomeric PRL was elevated, this might actually be the pathological condition. The clear understanding of PRL biology and suitable laboratory technique lead to provide proper diagnosis and management in case of hyperprolactinemia.

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