

# Macroprolactin; A Frequent Cause of Misdiagnosed Hyperprolactinemia in Clinical Practice

Vaishya, Richa<sup>1</sup>; Gupta, Rahul<sup>2</sup>; Arora, Sarika<sup>3\*</sup>

1- Guru Nanak Eye Center, New Delhi, India

2- Department of Neurosurgery, G.B. Pant Hospital, New Delhi, India

3- Department of Biochemistry, Lady Hardinge Medical College, New Delhi, India

## Abstract

**Introduction:** Macroprolactin is a significant cause of misdiagnosis, unnecessary investigation, and inappropriate treatment in patients with hyperprolactinemia. Its frequency has not been clearly established due to technical difficulties in identifying it. Most laboratories and clinicians are unaware of macroprolactin interferences in prolactin assays.

**Materials and Methods:** A comprehensive literature search was conducted on the websites of the National Library of Medicine (<http://www.ncbi.nlm.nih.gov>) and PubMed Central, the US National Library of Medicine's digital archive of life sciences literature (<http://www.pubmedcentral.nih.gov/>). The data were also looked for in relevant books and journal.

**Results:** Macroprolactin is a non-bioactive prolactin isoform usually composed of a prolactin monomer and an IgG molecule having a prolonged clearance rate similar to that of immunoglobulins. This isoform is clinically non-reactive but it interferes with immunological assays used for the detection of prolactin.

**Conclusion:** There is a need to understand and explore the recent progress in the diagnosis and pathophysiology of macroprolactinemia for improving patient care.

**Keywords:** Hyperprolactinemia, Macroprolactin, Polyethylene glycol assay, Prolactin antibody, Prolactin.

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\* Corresponding Author:  
Dr. Sarika Arora,  
Department of  
Biochemistry, Lady  
Hardinge Medical  
College, New Delhi-  
110001, India.  
E-mail:  
sarikaarora08@rediffmail.  
com

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## Introduction

Human prolactin (PRL) is a hormone secreted by the anterior pituitary lactotropic cells. Like any other anterior pituitary hormone, secretion of PRL also falls under hypothalamic control. PRL is unique amongst the adeno-hypophyseal hormones, in that the primary control of its secretion is inhibitory rather than stimulatory. Dopamine is believed to be the principal prolactin inhibiting factor (PIF) that regulates PRL secretion;  $\gamma$ -aminobutyric acid (GABA) can also inhibit PRL release, but thyroid releasing hormone (TRH) tends to stimulate its secretion.

PRL is synthesized as a prehormone with a molecular weight of 26 kDa. When a preprolactin molecule is cleaved, the resulting PRL polypeptide has a molecular weight of 23 kDa. This monomeric form of PRL is the major circulatory form and is also known as little PRL and it is known to be both biologically and immunologically active. Human PRL is, however, found to be heterogenous in molecular size – the other forms mainly include the big PRL with a molecular weight of approximately 50 kDa and the tetrameric big-big form with a molecular weight greater than 150 kDa (1, 2). These latter

two forms are known to have low biological activity.

**Historical Significance:** Whittaker et al. first described an interesting case of hyperprolactinemia with predominant big-big PRL on gel chromatography. The patient showed no clinical symptoms related to hyperprolactinemia, such as amenorrhea or galactorrhea. Despite high PRL levels, spontaneous pregnancy was also possible (3). Anderson et al. also demonstrated the predominance of the highest molecular weight prolactin in a woman complaining of infertility who conceived subsequently. They demonstrated the bioactivity of macroprolactin component *in vitro* and suggested that the absence of *in vivo* bioactivity might be the result of the high molecular mass of the complex preventing passage through the capillary endothelium to its target cells (4). Later in 1985, Jackson et al. (5) first used the term "macroprolactinemia" for such patients with marked hyperprolactinemia whose PRL mainly consisted of big-big PRL. Thereafter, several cases of macroprolactinemia have been reported.

This review aims to discuss the etiology of hyperprolactinemia with a special emphasis on macroprolactinemia, its diagnostic strategies, its clinical implications and the importance of its detection in clinical settings.

### Materials and Methods

A comprehensive literature search was conducted on the websites of the National Library of Medicine (<http://www.ncbi.nlm.nih.gov>) and PubMed Central, the US National Library of Medicine's digital archive of life sciences literature (<http://www.pubmedcentral.nih.gov/>). Relevant books and journal articles were also searched.

### Results

**Etiology of Hyperprolactinemia:** There are several known causes of hyperprolactinemia – both physiological and pathological. However, in some cases the high levels of PRL cannot be explained even after an extensive clinical, hormonal and neuro-radiological work-up (6). Such patients may be categorized as cases of idiopathic hyperprolactinemia. Some of these patients may have radiologically undetected microprolactinoma,

however, some may present with macroprolactinemia. Macroprolactinemia can be a significant cause of hyperprolactinemia and should not be overlooked while making a differential diagnosis for hyperprolactinemia.

**Causes of Hyperprolactinemia:** The common causes of hyperprolactinemia may be broadly grouped into physiological and pathological causes as described below:

- Physiological causes include
- Pregnancy
- Stress
- Pain states
- Excessive physical training

#### Pathological causes:

- Repetitive mechanical stimulation of breast
- Chest wall trauma
- Hepatorenal disease
- Primary hypothyroidism
- Pituitary adenoma
- Intracranial tumors compressing the pituitary stalk or hypothalamus
- Empty sella syndrome
- PRL stimulating drugs:
  - Dopaminergic blocking agents
  - Dopaminergic depleting agents
  - Non-catecholamine dependent agents
  - H<sub>2</sub> receptor blocking agents
  - Tricyclic antidepressants
- Idiopathic: (unknown causes) which may be due to macroprolactin

**Pathophysiology of Macroprolactinemia:** The condition is characterized by the predominance of circulating high molecular mass PRL forms which have coupled with anti-PRL immunoglobulins. These autoantibodies have been found to be immunoglobulin G (IgG) isotypes with low receptor affinity (7 - 11). The other evidence supporting the IgG nature of the autoantibodies is the presence of macroprolactin in the fetal cord blood from a mother with macroprolactinemia (12), suggesting the passive transfer of IgG-bound prolactin from mother to fetus.

A positive correlation has been demonstrated with anti-PRL antibody titers and the serum PRL concentrations indicating autoantibodies as a possible cause of hyperprolactinemia in such cases (10). Macroprolactinemia occurs when more than 30 - 60% of the patients' prolactin is in the form of macroprolactin (13). Despite the high

prevalence of macroprolactinemia, the pathogenesis and the source of these antibodies still remain unclear.

A speculation on the source of these antibodies suggests that posttranslational modifications (glycosylation and phosphorylation) of some proteins may create neo-epitopes for the production of autoantibodies (14, 15). Posttranslational modifications of PRL have also been reported: glycosylation in most species; phosphorylation in rats, bovines and birds; deamidation in rats, mice, sheep and humans and sulfation in ovine or sheep and buffalo (16). Furthermore, Hattori et al. recently showed that human pituitary PRL was phosphorylated at serine residues and it existed in partially dephosphorylated form in the blood (17). If pituitary phosphorylated forms of PRL are intolerant to the immune system, leakage of such forms of PRL upon hypophysitis or lack of dephosphorylation may cause an autoimmune response leading to the presence of autoantibodies associated with macroprolactin.

Monomeric prolactin is bioactive, whereas macroprolactin is considered biologically inactive, although it retains its immunoreactivity properties (7, 10, 18, 19). A recent study on the characterization of macroprolactin confirmed that it mainly contained an IgG molecule/ fragment with a PRL molecule (20). Kavanaqh et al. evaluated the relevant autoimmune markers in such cases\_ sera were assayed for antithyroid antibodies, antinuclear antibodies, C-reactive protein (CRP) and CD5 positive B cells. Normal, hyperprolactinemic and macroprolactinemic seras were compared and the macroprolactinemic sera did not yield any evidence related to the increase of autoimmunity markers when compared with the other two, suggesting no probable association between macroprolactin autoimmunity (20).

The bound form of the molecule is unable to bind to its receptors resulting in the failure of hypothalamic negative feed-back mechanism leading to hyperprolactinemia. At the same time, macroprolactin is not readily cleared by the kidneys which lead to its increased concentration in the body.

The clearance study done in rats has revealed that IgG-bound PRL is cleared from the circulation more slowly as compared to free PRL (10). A recent study has also demonstrated that anti-PRL

autoantibodies are stable for at least 5 weeks, suggesting that macroprolactinemia is a chronic condition in humans (21).

**Laboratory Investigation for Macroprolactin:** A great cause of concern lies in the fact that macroprolactinemia is often neglected in the differential diagnosis of hyperprolactinemia. Consequently, patients may need to undergo unnecessary and costly diagnostic investigations, inappropriate treatments and unnecessary follow-ups. The most common reasons for this could be the lack of awareness amongst specialists and also partly due to the lack of proper and cost-effective diagnostic methods.

Macroprolactin is found to interfere with most commercially available immunoassays used for prolactin. As a result, false high prolactin values (apparent hyperprolactinemia) are obtained and these values depend on the assay method employed (11).

Immunoassays used for the measurement of PRL have been subdivided into three classes according to their reactivity with macroprolactin: low-, medium-, and high-reading methods (22).

In the past, no laboratory method was available for the simple diagnosis of macroprolactin. There are a few studies indicating the use of prolactin suppressive and stimulatory tests (using bromocriptine, dopamine and TRH) in patients with macroprolactinemia but with conflicting results (10, 23).

The gel filtration chromatography (GFC) is known to be the gold standard or the reference assay for detecting macroprolactin (24), but it is a time-consuming and labor intensive method which discourages clinicians for the work-up of an unclear finding. However, in recent years polyethylene-glycol (PEG) precipitation method has offered a simple, cheap and a rapid method for the detection of macroprolactin (25). It is a highly suitable method for screening macroprolactin and is 27 times cheaper compared to gel filtration chromatography (26). A concentration of 25% PEG is added to an aliquot of serum specimen and the PEG-treated sample is incubated for a short period and then centrifuged to precipitate out macroprolactin. After centrifugation, the supernatant containing the unprecipitated prolactin is tested along with an unprecipitated aliquot of the serum specimen. Recovery of less than 40% after

PEG is considered a reliable diagnostic criteria for macroprolactin; recovery values around 40-50% should ideally be taken for chromatographic work-up and values >50% rule out macroprolactinemia (25 - 27). More recent studies have also confirmed PEG precipitation method as a reliable and cost effective method for diagnosing macroprolactin (28, 29).

Ram et al. (30) have found that monomeric PRL is co-precipitated with serum globulins by PEG and the increased serum globulin concentrations can increase the amount of monomeric PRL precipitated by PEG giving a false estimate of the monomeric PRL and a false impression of macroprolactin presence. The results of PEG precipitation test should, therefore, be interpreted with caution in patients with elevated serum globulin concentrations, such as in patients with IgG myeloma and polyclonal hypergammaglobulinaemia due to human immunodeficiency virus [HIV] infection.

**Radiological Evaluations in Macroprolactinemia:** Pituitary imaging by computerized axial tomography (CT) or by magnetic resonance imaging (MRI) are usually negative in patients with macroprolactinemia. However, in some cases, radiographic abnormalities appear, but the frequency is much less as compared to that seen in patients with hyperprolactinemia due to other causes. Due to the unexpected high frequency of pituitary abnormalities observed in one of the studies, the author suggested that the diagnostic algorithm of hyperprolactinemic states should include both polyethylene glycol (PEG) precipitation test and MRI imaging (31).

### Discussion

Macroprolactin is not age-related. Both sexes are affected, although the majority of patients are female. Patients with macroprolactinemia usually have normal menstrual cycles, minimal galactorrhea and spontaneous conception. However, some patients may present with clinical symptoms of hyperprolactinemia, if high levels of both macroprolactin and little PRL are present (10). In the study by Hittori N. et al., 15 women with macroprolactinemia were evaluated and it was found that 11 had normal menses, two had oligomenorrhea and two were in postmenopausal state (10). Two of these patients also complained of galactorrhea.

In another study by Vallette-Kasic et al., out of 106 patients with macroprolactinemia, 61% had normal menstruation and 54% did not have galactorrhea (24). In another study undertaken in 14- to 40-year-old hyperandrogenic women with hyperprolactinemia, presence of macroprolactin was demonstrated in 55% of the patients (32).

Taghavi M. et al. investigated 17 infertile women with hyperprolactinemia for macroprolactin using PEG. About 35% of these women were found to have macroprolactinemia. Galactorrhea was present in 81.8% of women with true hyperprolactinemia and 33.3% of women with macroprolactinemia, whereas oligomenorrhea was found to be present in 90.9% and 16.6% of the women in the respective groups (33). However, the pituitary images were normal in 45.5% of the women with true hyperprolactinemia and 100% of women with macroprolactinemia.

The possible reasons for asymptomatic presentation of macroprolactinemia include binding of antibodies to the epitopes for PRL receptors, thus, reducing the bioactivity; while others may bind to the epitopes unrelated to receptor binding. Hattori et al. recently reported that epitopes of anti-PRL autoantibodies in patients with macroprolactinemia were located near the binding site 1 of human PRL receptors (34), suggesting a possibility that anti-PRL autoantibodies may compete with the PRL molecule for binding to its receptors, resulting in reduced *in vivo* bioactivity. The second reason for the absence of symptoms can probably be related to the limited activity of macroprolactin. There have been studies indicating lack of macroprolactin in the pituitary tissue and in the extravascular space (cerebrospinal fluid) (35). The culture medium of pituitary tissue also has shown no macroprolactin in cases of hyperprolactinemia attributable to macroprolactin (24). This absence of macroprolactin in extravascular spaces and pituitary might be explained by the large molecular size/ changes in the net charges of the macroprolactin molecules, which cannot cross the endothelium and thus remain in the intravascular compartment preventing its access to PRL receptors at all.

On the other hand the rise in the levels of little PRL leads to the onset of clinical symptoms of hyperprolactinemia, suggesting that the binding of PRL to the receptor and the post-receptor

mechanisms are intact. However, recent studies have debated that macroprolactin may have some *in vivo* bioactivity; therefore, it could be regarded as a circulating store of potentially bioactive PRL. It is possible that intermittent dissociation from the low affinity, high capacity IgG antibody in macroprolactin may lead to increased bioavailability of monomeric PRL (36).

Thus, there arises the need to differentiate between the apparent benign clinical condition of macroprolactinemia, that hyperprolactinemia is entirely explained by the presence of macroprolactin and the true hyperprolactinemia, which is due to increased levels of monomeric PRL and requires therapy. Macroprolactinemia is not known to require specific treatment (10, 25) and no response to the antiprolactinemic therapy has been discovered in the studies carried out for this purpose (10, 19). However, one study showed that treatment with dopamine agonists lowers the serum macroprolactin levels (37).

### Conclusion

The question on the clinical importance of high-molecular prolactin isoforms is stressed upon in modern scientific literature. For several years, macroprolactinaemia did not attract much attention as the identity of the large forms was unknown and their identification by gel filtration was difficult and expensive. However, over the last few years, the subject has been studied, especially the introduction of PEG method for the diagnosis of macroprolactin. Literature reveals that failure to identify macroprolactinaemia leads to unnecessary investigation, incorrect diagnosis and inappropriate treatment.

Clinicians should include macroprolactinemia in the differential diagnosis of hyperprolactinemia. The presence of macroprolactin should always be suspected when a patient's clinical history and/ or radiographic data are incompatible with his/ her PRL values. Awareness amongst the medical laboratories is very important as not many laboratories take into account the interference of macroprolactin in PRL assays. Routine screening of patients with high PRL values is strongly recommended so as to reduce the use of imaging and dopamine agonist treatment and to save time and money. However, it is recommended that laboratories should validate their method by

comparison with GFC (using their own prolactin assay) before using the cost effective PEG method. More studies characterizing the molecular aspects and the pathophysiology of macroprolactin are required to understand the clinical relevance and significance of macroprolactinemia.

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