Original Article

Evaluation of Macroprolactin Levels in Females with Hyperprolactinemia

Premjeet Kaur Bhuee[1,*], Amandeep Birdi[2]

[1]Department of Biochemistry, Adesh Institute Of Medical Sciences and Research, Bathinda, Punjab, India
[2]Department of Biochemistry, GGS Medical College, Faridkot, Punjab, India

***Correspondence:** premjeet9@gmail.com

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Abstract

Background: Macroprolactinemia is defined as hyperprolactinemia due to high levels of macroprolactin. The term 'macroprolactinemia' is used when the levels of macroprolactin exceed 60% of the total serum prolactin concentration estimated by polyethylene glycol (PEG) precipitation. Limited data is available on the burden of macroprolactinemia in Indian females.
Aim: Find the prevalence of macroprolactin levels in females with hyperprolactinemia.
Methods: The study was a hospital-based cross-sectional study with 98 subjects conducted in Central Clinical Lab of Adesh Institute of Medical Sciences and Research, Bathinda, a tertiary care teaching hospital in Punjab, India from July 2024 to April 2025. Subjects included females aged 18 years to 40 years attending the gynaecology OPD for infertility check up whose PrL levels were above the upper limits of laboratory reference range (25 ng/ml in females).
Results: A total of 98 patients having a mean age of 29.2 ± 9.6 years were detected to have serum prolactin > 25 ng/mL over a period of July 2024 to April 2025. Further, 12 (12.2%) were detected to have macroprolactinemia. A total of 12 out of 98 patients detected to have macroprolactinemia had serum prolactin in the normal range post-PEG precipitation. Hence, 86 patients (87.75%) continued to have raised serum prolactin levels, even after post-PEG precipitation. Twelve patients were found to have macroprolactinaemia, and the prevalence of macroprolactinemia in hyperprolactinemia females came out to be 12.2%.
Conclusion: The occurrence of macroprolactinemia in hyperprolactinemic females is not a rare phenomenon, with a prevalence of 12.2%. Differentiation of subjects with hyperprolactinemia secondary to macroprolactinemia from those with true hyperprolactinemia is clinically essential because the former condition is considered benign and generally requires no further investigation or medication. Further investigations like X-ray and MRI were dropped, which saved time and money of the patient.

Keywords: Prolactin isoforms; Polyethylene Glycol; Gel filtration chromatography; Infertility

Introduction

Prolactin hormone is synthesised by the pituitary gland and is involved in many reproductive functions in both males and females. The pituitary is an endocrine gland formed by the protrusion of the hypothalamus at the base of the brain. It rests upon the sella turcica in the middle cranial fossa covered by diaphragma sellae. Prolactin is a single polypeptide chain of 199 Amino acids. It is encoded by a single gene located on the short arm of chromosome 6 [1]. The molecule of prolactin shows a lot of heterogeneity due to the activity of three disulphide bonds. There are three isoforms which are most relevant: first is the glycosylated PrL (25 kDa) whose structure facilitates aggregation into dimers; second is the 50–60 kDa PRL isoform (20–25% of total serum PrL) which exhibits diminished physiological activity and varied reactivity in various immunoassays; and lastly, the higher molecular weight complexes (150 kDa), formed by glycosylation, aggregation

and covalent or non-covalent bonding to each other and to immunoglobulins (generally IgG and less frequently with IgA) to form the so-called macroprolactin (or big big PrL, macro-PrL), which represents less than ten percent of total PrL in most individuals. Prolactin (PrL), after post-translational modifications, generates modified forms that can impede the measurement of monomeric PrL concentration. Prolactin secretion shows a circadian rhythm. Its concentration is higher during the night and lower during the day. Prolactin helps in the development of the mammary gland. It also stimulates the growth of alveoli in the mammary gland and stimulates milk production by alveolar epithelial cells [2, 3, 4, 5].

There are various physiological conditions like pregnancy, breast feeding, nipple stimulation, and stress in which serum prolactin levels increase. The pathological causes of hyperprolactinemia are lactotroph adenomas, decreased dopaminergic inhibition, and diseases of the hypothalamus affecting the secretion or delivery of dopamine. Other causes can be idiopathic hyperprolactinemia, familial hyperprolactinemia, and hypothyroidism. Signs and symptoms of hyperprolactinemia in premenopausal women are menstrual cycle irregularities, effect on bone mineral density, and galactorrhea. In postmenopausal women, it can cause galactorrhea. In men, it may manifest as erectile dysfunction, infertility, and galactorrhea [6].

Hyperprolactinemia is usually defined as fasting serum prolactin levels at least 2 hrs after waking up above 25 ng/ml. Pitfalls in diagnosis are Hook effect and macroprolactin [7].

Hyperprolactinemia (HyperPRLemia) is the most frequent cause of oligo-amenorrhea, sterility, impotence, and osteoporosis/osteopenia in males as well as females. Macroprolactinemia (MacroPRLemia) is defined as HyperPRLemia due to high levels of macro-PrL. The term 'macroprolactinemia' (MacroPRLemia) is used when the levels of macro-PrL exceed 60% of the total serum PrL concentration estimated by polyethylene glycol (PEG) precipitation [6, 7]. Differentiation of subjects with HyperPRLemia secondary to MacroPRLemia from those with true HyperPRLemia is clinically essential because the former condition is considered benign and generally requires no further investigation or medication [2, 8].

Gel filtration chromatography (GFC) is considered the gold standard method for differentiating the above-mentioned PRL forms from one another. However, the GFC method is costly, labour-intensive, and not routinely done in most clinical laboratories [9]. PEG precipitation is commonly used as an alternative for the screening of big big PrL in view of its technical simplicity and relatively low cost to perform in routine clinical laboratories [10]. A number of studies have validated the PEG method against GFC [2, 10, 15]. In spite of some limitations, PEG PrL precipitation is still the most widely used technique for macro-PrL screening. The limitations are: a) PEG can also partially sediment monomeric PrL (20–25%) and, therefore, falsely lower the PrL concentration [2]; b) the presence of PEG in the sample can interfere with some immunoassays; c) presence of very high concentrations of gamma globulin, false positives may occur for the presence of macro-PrL [11]; d) False negatives can occur with IgA, since PEG precipitation of IgA is partial.

Keeping this in mind, this study was planned with the following objectives: To estimate PrL after PEG precipitation in HyperPRLemia subjects. To estimate macro-PrL in HyperPRLemia patients. To identify the prevalence of MacroPRLemia in HyperPRLemia females who underwent treatment for reduction of PrL levels.

Materials and Methods

Study design and Study setting: This study was designed as a hospital-based prospective cross-sectional study conducted in the Central Clinical Lab of Adesh Institute of Medical Sciences and Research, Bathinda, a tertiary care teaching hospital in Punjab, India from July 2024 to April 2025. IEC gave its approval (Ref. No. AU/DAA/06/2024/FA/489 on 28/6/24).

Sample size: The sample size was calculated using Cochran's formula, based on an estimated macroprolactinemia prevalence of 13.7% [3]. The formula used was Z^2PQ/e^2, where Z represents the Z-score. The calculated minimum sample size was 78, but this was increased to 98.

Inclusion criteria: Females aged 18 years to 40 years attending the gynaecology OPD for infertility check up whose PrL levels were above the upper limits of laboratory reference range (25 ng/ml (or ug/L) in females) [12].

Exclusion criteria: Subjects having a history of prolactinomas or other pituitary disease.

Methodology: Serum samples (3 ml) were collected from the enrolled patients and were stored at -20°C till processing. Samples were processed with PEG sedimentation and the supernatant was taken after centrifugation and was run for PrL estimation on a Maglumi analyser of SNIBE company by using a chemiluminescent microparticle immunoassay method according to the manufacturer's protocol. This study included only those patients whose initial serum PrL were above the upper limits of the laboratory's reference range for pre-PEG PrL (25 ng/ml in females). After the initial measurement of serum PrL, samples were treated with PEG solution according to the following PEG separation protocol: an equal volume of serum and PEG 6000 solution at 25% (weight/volume, for example, dissolving 25 g of PEG 6000 in 100 ml of NaCl 0.9% or distilled water, stored at 4°C) is mixed for 30 s with a vortex mixer and centrifuged at 3500 rpm for 30 min. The PRL concentration in the supernatant is measured and corrected for the dilution factor (1:2). The post-PEG PrL concentration (corrected by the dilution factor) is the patient's monomeric PrL [7]. The percent PEG-precipitated PrL, which represents

macro-PrL, is calculated as (total PrL-post PEG precipitation PrL)/total PrL × 100 [13].

Statistical Analysis: Descriptive statistics such as number and percentages were used to present the data. Data analysis was performed using excel software. The data is presented as mean ±SD.

Results

A total of 98 patients having a mean age of 29.2 ± 9.6 years were detected to have serum prolactin > 25 ng/mL over a period of July 2024 to April 2025. Age quartiles-based analysis revealed no significant difference in the occurrence of macroprolactinemia across any of the age groups (quartile-1 [18–24 years], 2/25 [8%]; quartile-2 [24–30 years], 4/26 [15.38%]; quartile-3 [30–40 years], 3/24 [12.5%]; quartile-4 [40–80 years], 3/23 [13.0%]; p = 0.35). Further, 12 (12.2%) were detected to have macroprolactinemia. A total of 12 out of 98 patients detected to have macroprolactinemia had serum prolactin in the normal range post-PEG precipitation. Hence, 86 patients (87.75%) continued to have raised serum prolactin levels, even after post-PEG precipitation. Twelve patients were found to have macroprolactinaemia, and the prevalence of macroprolactinemia in hyperprolactinemia females came out to be 12.2%. Table 1 depicts the prevalence of macroprolactin based on the underlying etiology or reason for testing. Table 2 and Figure 1 show the Pre-PEG PrL, Macro-PrL, and Post-PEG PrL in the cases of MacroPRLemia. Table 1 also depicts the reason for test requisition. It was found that patients with high pre-PEG PrL (usually above 100 ng/ml) showed the presence of MacroPRLemia, i.e., percent PEG-precipitated PrL (or macroprolactin) above 60%. The common reasons for prolactin test requisition were irregular periods, polycystic ovarian syndrome, infertility, and hypothyroidism.

Table 1: Prevalence of macroprolactinemia based on the underlying etiology or reason for testing.

Diagnosis	Macroprolactinemia absolute number (percentage)
PCOS (n = 16)	2 (12.5%)
Infertility (n = 28)	3 (10.7%)
Drug-induced hyperprolactinemia (n = 12)	-
On psychiatry medications (n = 2)	-
Hypothyroidism (n = 15)	2 (13.3%)
Menstrual irregularities (n = 25)	4 (16.0%)

Table 2: Shows the Pre-PEG PrL, Macro-PrL, and Post-PEG PrL in the cases of Macroprolactinemia and the reason for test requisition.

Pre-PEG precipitation PrL (ng/ml)	Percent PEG-precipitated PrL (Macro-PrL) (%)	Post-PEG precipitation PrL (ng/ml)	Reason for test
134	82.5	23.4	Irregular periods
135	86.2	18.5	Polycystic ovarian syndrome
127	88.3	14.9	Infertility
139	85.1	20.6	Hypothyroidism
137	78.1	19.4	Irregular periods
130	84.9	21.5	Hypothyroidism
136	86.3	22.8	Infertility
138	87.2	17.3	Polycystic ovarian syndrome
129	83.6	20.9	Menstrual irregularities
133	85.4	19.6	Menstrual irregularities
130	79.3	18.9	Infertility
138	84.6	22.3	Hypothyroidism

Discussion

In the present study, true HyperPRLemia was defined as HyperPRLemia identified in patients whose post-PEG PrL levels remained above the post-PEG reference intervals [13, 14, 15], while MacroPRLemia was defined as a concentration of macro-PrL exceeding 60% of the total serum PrL concentration determined by PEG precipitation [6, 13].

All commercially available immunoassays detect total PrL. MacroPRLemia must be ruled out to confirm whether PrL levels are actually indicating HyperPRLemia. Macro-PrL can be removed by PEG. Macro-PrL is precipitated with PEG, and PrL remains measurable as supernatant [5, 14]. A number of studies have validated the PEG method against GFC [10]. In spite of some limitations, PEG PrL precipitation is still the most widely used technique for macro-PrL screening. The limitations are: a) PEG can also partially sediment monomeric PrL (20–25%) and, therefore, falsely lower the PrL concentration [2]; b) the presence of PEG in the sample can interfere with some immunoassays; c) presence of very high concentrations of

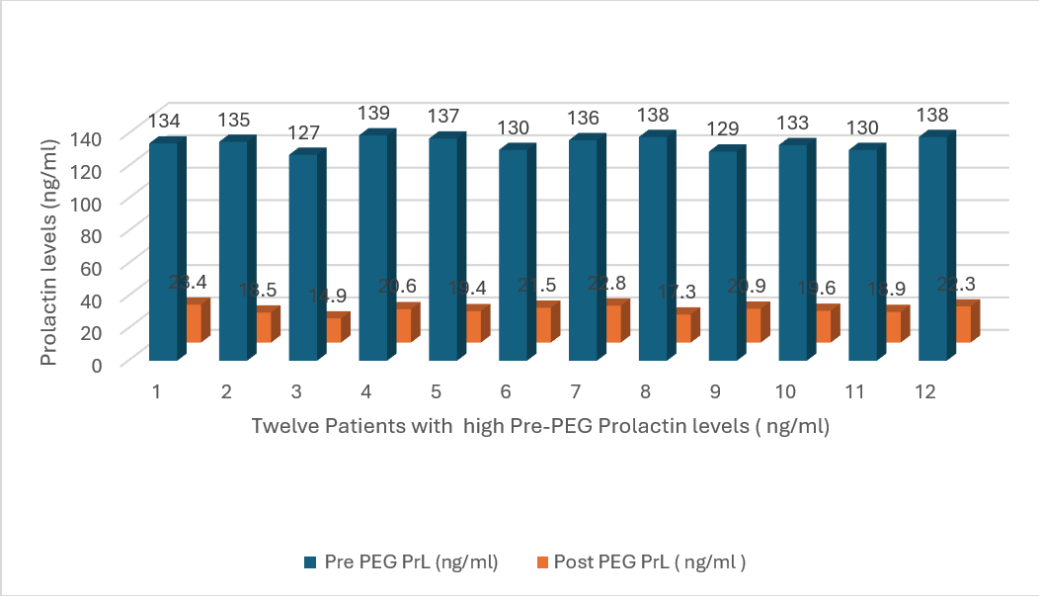

Figure 1: Shows the Pre-PEG PrL and Post-PEG PrL in the cases of MacroPRLemia.

gamma globulin, false positives may occur for the presence of macro-PrL [15]; d) False negatives can occur with IgA, since PEG precipitation of IgA is partial.

There is no agreeing criteria of screening macro-PrL in patients with HyperPRLemia. At some institutes, a clinically based approach is used, which means that it is the clinician who requests the screening of Macro-PrL in patients with HyperPRLemia presenting without medical indications. At other institutions, macro-PrL testing by PEG precipitation is routinely performed in all patients with HyperPRLemia. The advantage is that it would avoid misdiagnosis and unnecessary tests and treatments, but the disadvantage is that it increases work and costs for the laboratory. A third approach is Laboratory-guided macro-PrL study. In this case, it is the laboratory which takes the initiative. Along with the results of HyperPRLemia, the laboratory issues an alert recommending macro-PrL screening in cases of clinical disparity [16]. An important issue which needs attention is that there is dispute on how to express the results of macro-PrL after PEG, which may lead to an erroneous interpretation of the results [7].

The classic symptoms of HyperPRLemia, such as galactorrhoea, infertility, decreased libido, and menstrual irregularity, are the consequence of high levels of monomeric PrL that suppress the gonadotropic axis. Macroprolactin is not an active form, may be due to its inability to bind to PrL receptors. But because of binding with IgG, there is incomplete clearance of serum PrL, which may lead to a falsely elevated serum PrL level in lab reports. So, there can be a misdiagnosis of HyperPRLemia in these patients. This misdiagnosis can be avoided by pretreatment of serum with PEG during lab testing. Therefore, the presence of asymptomatic HyperPRLemia and clinical-biochemical discrepancies are what should alert the clinician to the possible coexistence of macro-PRL [6].

How anti-PrL autoantibodies are formed is not clear. Posttranslational modifications such as phosphorylation of PrL might be responsible for the altered antigenicity, hence, leading to the production of anti-PrL autoantibodies [13]. MacroPRLemia is present in 3.68% in the general population. The prevalence is not different between women and men, and it tends to increase in elderly people. In patients with HyperPRLemia, the prevalence of MacroPRLemia is reportedly 10–25% [13]. In the present study, we found a prevalence of 12.2% of MacroPRLemia in Indian females with HyperPRLemia which is similar to results of another study by Lim M. H. et al of Singapore (11.3%) [15]. In contrast, a different study found a prevalence of 60.7% of MacroPRLemia in HyperPRLemia from Karachi [17]. This difference could be due to differences in assay methods, population genetics, and healthcare practices.

HyperPRLemia tends to develop in patients with MacroPRLemia [17] because of the delayed clearance of macro-PrL. Patients with MacroPRLemia often lack medical indications of HyperPRLemia. Women with MacroPRLemia can get pregnant and deliver normal babies without any treatment for HyperPRLemia. Long-term follow-up studies of MacroPRLemia show that MacroPRLemia persisted but no symptomatic progression was noted. Thus, it is strongly recommended that all patients with HyperPRLemia should take the screening test of MacroPRLemia in order to prevent needless examinations and medications. Those tested for prolactin due to psychiatric medications (Table 1) did not show MacroPRLemia as not all psychiatric medications cause significant hyperprolactinemia.

The twelve patients for whom the prolactin levels came out to be normal on PEG treatment which was high earlier, further investigations like CT and MRI were dropped which saved time and money of the patient. The confirmation with CT scan

and MRI brain were not required due to confirmed low prolactin levels. Differentiation of subjects with hyperprolactinemia secondary to macroprolactinemia from those with true hyperprolactinemia is thus clinically essential, because the former condition is considered benign and generally requires no further investigation or medication.

Study Limitations: Since this was a study, a large scale study may be required to give the exact figure of the prevalence of MacroPRLemia in Indian females with HyperPRLemia.

Conclusion

The occurrence of macroprolactinemia in hyperprolactinemic females is not a rare phenomenon, with a prevalence of 12.2%. Differentiation of subjects with hyperprolactinemia secondary to macroprolactinemia from those with true hyperprolactinemia is clinically essential because the former condition is considered benign. Medications or any further examinations are not recommended if free PrL levels after precipitating macro-PrL with PEG are within normal limits. Because the physiological activity of macro-PrL is low, hence, pregnancy is possible without any treatment for HyperPRLemia.

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