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Prevalence of Macroprolactinemia in Indian Females with Hyperprolactinemia: A Pilot Study

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Abstract

Background and Aim: Post-translational modifications of prolactin (PrL) generate various isoforms that can interfere with the accurate measurement of monomeric PrL concentrations. Macroprolactinemia (MacroPRLemia) is defined as hyperprolactinemia caused by elevated levels of macro-PrL. Specifically, the term 'macroprolactinemia' is applied when macro-PrL levels exceed 60% of the total serum PrL concentration, as estimated by polyethylene glycol (PEG) precipitation. Clinically distinguishing MacroPRLemia from true hyperprolactinemia is essential, as the former is generally considered a benign condition that requires no medical intervention. Due to its technical simplicity and cost-effectiveness, PEG precipitation is widely utilized for macro-PrL screening in routine clinical laboratories. However, data regarding the prevalence and burden of MacroPRLemia in Indian females remains unavailable. Therefore, the present study was designed to address this gap in the literature.

Methods: This study was conducted at the Central Clinical Laboratory of the Adesh Institute of Medical Sciences and Research, a tertiary care teaching hospital in Bathinda, Punjab, India. Females aged 18 to 40 years attending the Gynecology Outpatient Department (OPD) for infertility check-ups, whose PrL levels were above the laboratory's upper reference limit (24.8 ng/ml), and who had started treatment, were included. Subjects with a history of prolactinomas or other pituitary diseases (excluded via imaging or clinical history), thyroid disorders, or those taking medications that can influence prolactin levels, such as antipsychotics and antidepressants, were excluded.

Results: Among the 900 females who underwent PrL testing, 98 were found to have hyperprolactinemia. Of these, twelve females showed the presence of macroprolactin.

Conclusion: The occurrence of macroprolactinemia in hyperprolactinemic females is not a rare phenomenon, with a prevalence of 12.2%.

Keywords: Adipose stem cells, Osteogenic Differentiation, Betaine, Electromagnetic Field

1. Introduction

Hyperprolactinemia (HyperPRLemia) is the most frequent cause of oligo-amenorrhea, sterility, impotence, osteoporosis/osteopenia in males as well as females. (1) Prolactin (PrL), post-translational modifications generate isoforms that can impede the measurement of monomeric PrL concentration. (2,3) There are three key isoforms of prolactin (PrL) that are most medically relevant: I) Glycosylated Prolactin (25 kDa) – This form tends to aggregate into dimers due to its structure. II) Intermediate Prolactin (50–60 kDa) – Making up about 20–25% of total serum

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prolactin, this isoform has reduced physiological activity and shows inconsistent detection across different immunoassays. III) Macroprolactin (150 kDa) – also known as "big-big prolactin," this high-molecular-weight complex results from glycosylation, aggregation, and bonding (covalently or non-covalently) to itself or to immunoglobulins (typically IgG, infrequently IgA). In most cases, macroprolactin accounts for fewer than 10% of total serum prolactin. (4,5)

Gel filtration chromatography (GFC) is considered the gold-standard technique for differentiating the aforementioned PrL forms from one another. However, the GFC technique is costly, labour-intensive, and not routinely performed in most laboratory medicine units. 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 for performance in routine clinical laboratories (6,7).

Macroprolactinemia (MacroPRLemia) is defined as HyperPRLemia due to high levels of macro-PrL. The term 'macroprolactinemia' is used when the levels of macro-PrL exceed 60% of the total serum PrL concentration estimated by polyethylene glycol (PEG) precipitation (8,9). 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 (2,10).

No data is available on the burden of MacroPRLemia in Indian females. With this in mind, we planned this pilot study with the following objectives:

Objectives of the study

1. To determine PrL levels following PEG precipitation in subjects with HyperPRLemia.
2. To assess macro-PrL levels in patients with HyperPRLemia.
3. To identify the prevalence of MacroPRLemia in females with HyperPRLemia who underwent treatment to reduce PrL levels.

2. Methods

Study design

This study was designed as **a** hospital-based prospective cross-sectional pilot study.

Study setting

This study was conducted in the Central Clinical Lab of the Adesh Institute of Medical Sciences and Research, Bathinda, a tertiary care teaching hospital in Punjab, India.

Being a pilot study, the samples were collected based on the availability and consent of the subjects, and due to resource constraints, we opted for a sample size of 900 (11).

Inclusion criteria

Participants included females aged 18 to 40 years attending the Gynecology Outpatient Department (OPD) for infertility check-ups, whose PrL levels were above the upper limit of the laboratory reference range (24.8 ng/mL), and who were started on treatment.

Exclusion criteria

Exclusion criteria included subjects with a history of prolactinomas or other pituitary diseases (identified via imaging or clinical history), thyroid disorders, and those taking medications that influence prolactin levels, such as antipsychotics and antidepressants.

Methodology

Serum samples were collected from the enrolled patients and stored at -20 °C until processing. Samples were brought to room temperature before analysis. Samples were processed using polyethylene glycol (PEG) sedimentation. After centrifugation, the supernatant was collected and analyzed for prolactin (PrL) levels using the SNIBE Maglumi analyzer (with a linearity limit of 250 ng/mL), which operates via chemiluminescent microparticle immunoassay, following the manufacturer's guidelines.

The study included only those patients whose initial serum prolactin levels exceeded the laboratory's reference range for pre-PEG prolactin (24.8 ng/mL for females). Following this initial measurement, the samples underwent PEG treatment using the following protocol: equal volumes of serum and a 25% PEG 6000 solution (prepared by dissolving 25 g of PEG 6000 in 100 mL of 0.9% NaCl or distilled water and stored at 4 °C) were mixed for 30 seconds using a vortex mixer. The mixture was then centrifuged at 3,500 rpm for 30 minutes using an ELECTRA BL centrifuge from EURO LAB, which operates within a 0–4,000 RPM range with 10 RPM accuracy.

The prolactin concentration in the resulting supernatant—representing monomeric prolactin—was measured and adjusted for the 1:2 dilution factor. Macroprolactin (macro-PrL) levels were determined as the percentage of PEG-precipitated prolactin using the following formula:

(Total PrL – Free PrL) / Total PrL × 100.

Statistical Analysis

Descriptive statistics, such as frequencies and percentages, were used to present the data. Data

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analysis was performed using Microsoft Excel. Data were presented as mean ± SD.

3. Results

A total of 900 females aged 18 to 40 years were included in the study, who underwent PrL testing for various clinical reasons. Of these, 98 females presented with hyperprolactinemia and underwent PEG precipitation. Serum pre-PEG prolactin concentrations ranged from 34.8 to 139 ng/mL, and post-PEG concentrations ranged from 3.6 to 101 ng/mL. The percentage of PEG-precipitated PrL, representing macro-PrL, ranged from 1.9% to 88.3%. Twelve patients were found to have macroprolactinemia, with a prevalence of 12.2% among the hyperprolactinemic female patients.

Table 1. Shows the Pre PEG PrL, Macro-PrL and Post PEG PrL in the cases of MacroPRLemia 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

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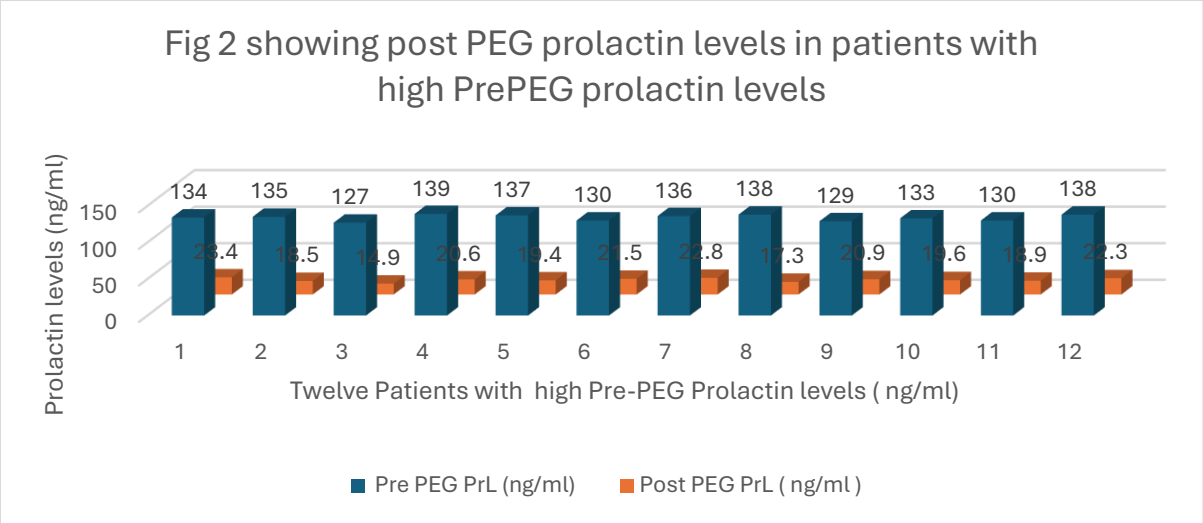


Figure 1. Shows the Pre PEG PrL and Post PEG PrL in the cases of MacroPRLemia.

4. Discussion

In the present study, true hyperprolactinemia was defined as hyperprolactinemia identified in patients whose post-PEG PrL levels remained above the post-PEG reference intervals (12), while macroprolactinemia 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. Macroprolactinemia must be ruled out to confirm whether PrL levels actually indicate hyperprolactinemia. Macro-PrL can be removed by PEG; it is precipitated with PEG, and the remaining PrL is measurable in the supernatant (5, 14). A number of studies have validated the PEG method against GFC (9, 10). Despite certain limitations, PEG remains the most frequently used method for screening macroprolactin. These limitations include: a) PEG may partially precipitate monomeric prolactin (approximately 20–25%), potentially leading to an underestimation of prolactin levels; b) residual PEG in the sample can affect some immunoassay results; c) in cases of very high gamma globulin concentrations, false positives for macroprolactin may occur; and d) false negatives can occur with IgA, as PEG precipitation of IgA is only partial (15). There are no agreeing criteria for screening macro-PrL in patients with hyperprolactinemia. At some institutes (including ours), a clinically based approach is used, meaning the clinician requests macro-PrL screening in patients presenting without medical indications. At other institutions, macro-PrL testing by PEG precipitation is routinely performed in all patients with hyperprolactinemia. The advantage of the latter is that it avoids misdiagnosis and unnecessary treatments, while the disadvantage is the increased workload and cost for the laboratory. A third approach is the laboratory-guided macro-PrL study, where the laboratory takes the initiative. Along with hyperprolactinemia results, the laboratory issues an alert recommending macro-PrL screening in cases of clinical disparity (16). An important issue that needs attention is the dispute regarding how to express macro-PrL results after PEG, which may lead to erroneous interpretation (7). The classic symptoms of hyperprolactinemia, such as galactorrhea, infertility, decreased libido, and menstrual irregularity, are the consequences of high levels of monomeric PrL that suppress the gonadotropic axis. The physiological activity of macro-PrL in vivo is low, possibly due to its inability to bind to PrL receptors. The high molecular weight of the complex formed between the monomeric form and IgG hinders it from passing the capillary membrane. Therefore, asymptomatic hyperprolactinemia and clinical-biochemical discrepancies should alert the clinician to the possible coexistence of macro-PrL (6).

How anti-PrL autoantibodies are formed is not clear. Post-translational modifications, such as the phosphorylation of PrL, might be responsible for altered antigenicity, leading to the production of anti-PrL autoantibodies (13). Macroprolactinemia is present in 3.68% of the general population. The prevalence does not differ between women and men and tends to increase in elderly people. In patients with hyperprolactinemia, the prevalence of macroprolactinemia is reportedly 10–25% (13). In the present study, we found a prevalence of 12.2% of macroprolactinemia in Indian females with hyperprolactinemia, which is similar to the results of another study by Lim M. H. et al. in Singapore (11.3%)

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(11, 13). In contrast, a different study found a prevalence of 60.7% in hyperprolactinemic patients from Karachi (17). This variation could be due to differences in assay methods, population genetics, and healthcare practices.

Hyperprolactinemia tends to develop in patients with macroprolactinemia (17) due to the delayed clearance of macro-PrL. Patients with macroprolactinemia often lack medical indications for hyperprolactinemia. Women with macroprolactinemia can become pregnant and deliver normal babies without treatment. Long-term follow-up studies show that while macroprolactinemia persists, no symptomatic progression is noted. Thus, it is strongly recommended that all patients with hyperprolactinemia undergo macro-PrL screening to prevent needless examinations and medications.

In our study, twelve patients whose PrL levels were high initially but returned to normal after PEG treatment were able to avoid further investigations, such as CT and MRI of the brain, which saved time and costs. Differentiation of subjects with hyperprolactinemia secondary to macroprolactinemia from those with true hyperprolactinemia is clinically essential because the former is considered benign and generally requires no further investigation or medication.

Study Limitations

Since this was a pilot study, a large-scale study may be required to provide the exact prevalence of macroprolactinemia in Indian females with hyperprolactinemia.

5. Conclusion

Macroprolactinemia is not a rare phenomenon in hyperprolactinemic females, with a recorded prevalence of 12.2%. It is clinically essential to differentiate patients with macroprolactinemia from those with true hyperprolactinemia, as the former is a benign condition. If free PrL levels are within normal limits after PEG precipitation, medications and further examinations are not recommended. Moreover, because macro-PrL has low physiological activity, pregnancy is possible without treatment for hyperprolactinemia.

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Ethical Considerations and Compliance with Ethical Guidelines

This study was approved by the Ethics Committee of the Adesh University, Bathinda (Ref No: AU/EC_BHR/2K24/ 602)

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Conflict of interest

The authors declare no competing interests.

AI Using Declaration

No artificial intelligent chatbots has been used in any section of work.

Author's contributions

The authors equally contributed to preparing this article.

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