Prevalence of Macroprolactinemia in People Detected to Have Hyperprolactinemia

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Abstract

Background Macroprolactinemia is an analytic laboma encountered as a part of prolactin assay. No data are available on the burden of macroprolactinemia in Indians. This study aimed to determine the prevalence and predictors of macroprolactinemia among people with hyperprolactinemia.

Methods Consecutive patients detected to have serum prolactin > 18 ng/mL as per the upper reference limit were further screened for macroprolactin by post-polyethylene-glycol (PEG)-precipitation test. Macroprolactinemia was defined as post-PEG recovery of prolactin < 40%.

Results The four most common underlying etiologies for the testing of hyperprolactinemia were polycystic ovary syndrome (n = 402; 32.71%), pituitary adenomas (n = 318; 25.87%), drug-induced hyperprolactinemia (n = 224; 18.23%), and infertility (n = 126; 10.25%). A total of 1,229 patients (male:female = 191:1038) having mean age 30.46 ± 10.14 years had hyperprolactinemia, of which 168 (13.7%) were diagnosed to have macroprolactinemia. Macroprolactinemia was significantly higher in females than males (15.03 vs. 6.28%; p < 0.001). Age quartile-based analysis revealed no difference in occurrence of macroprolactinemia. Only 34 patients (2.76%) with macroprolactinemia (< 40% recovery of prolactin post-PEG precipitation) had raised prolactin levels after recovery. These patients primarily had underlying pituitary pathology.

Conclusion Macroprolactinemia is not uncommon in people being tested for hyperprolactinemia. We should not hesitate to screen for macroprolactinemia in patients who have incidentally been detected to have hyperprolactinemia.

Keywords
- macroprolactin
- prolactin
- macroprolactinemia
- pituitary

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Introduction

Macroprolactinemia is an analytic issue/laboma encountered as a part of prolactin assay.[1,2] Macroprolactins are prolactin-immunoglobulin G circulating autoantibodies complexes that are six- to seven times higher in molecular weight than the native prolactin molecule.[3] However, the cause of the generation of prolactin autoantibodies in people with macroprolactinemia has not been well established. It is believed that there is a genetic predisposition as well as posttranslational modifications (glycosylation, phosphorylation, deamination) of the prolactin molecule, which triggers generation of autoantibodies.[3] Macroprolactins are believed to have no biological activity, as their huge size prevents it from binding to the prolactin receptors. However, they remain in the circulation for prolonged periods, due to the inability of the glomeruli in the kidneys to clear them from blood, and are picked up variably in routine prolactin assays (which cannot differentiate monomeric prolactin from macroprolactin) leading to false diagnosis of hyperprolactinemia, in people who otherwise would not have any clinical consequence related to raised native prolactin levels. Hence, macroprolactinemia represents a classical endocrine labomas, which can lead to avoidable over treatment and iatrogenic complications. Polyethylene glycol (PEG) precipitation is the most popular and easy-to-use method to precipitate out macroprolactin in a serum sample. However, some studies have suggested that up to 25% of monomer prolactin may be coprecipitated, leading to the false impression of macroprolactinemia.[4-6] Hence, macroprolactinemia is said to be present when the percent recovery of prolactin is < 40%.[7,8]

Data on the burden of macroprolactinemia in Indians are scant. Hence, the aim of this study was to determine the prevalence and predictors of macroprolactinemia among people being tested for serum prolactin levels at a tertiary care center in northern India.

Methods

Consecutive patients attending the endocrinology laboratory of the department of biochemistry referred for testing for serum prolactin were considered for the study. The study duration was from January 2019 to February 2020. The study was approved by the institute ethics committee. Patient age, sex, and referral diagnosis were noted. Clinical complaints of the patients were noted from the outpatient department assessment sheet of the department of endocrinology. In females, symptoms of menstrual disturbances, galactorrhea, and infertility were noted. In men, history of erectile dysfunction, gynecomastia, and reduced libido if present was noted. Blood sample for serum prolactin estimation was collected using a single venepuncture in the morning fasting state.

Serum samples detected to have serum prolactin > 18 ng/mL as per the upper limit of reference in our laboratory were further screened for macroprolactin by the PEG-precipitation test. Serum thyroid profile (free T3, free T4, and thyroid-stimulating hormone [TSH]) was done in all patient samples by using immunometric immunoassay technique using VITROS immunodiagnostic products reagent pack and calibrators on VITROS Eci (Orthoclinical Diagnostics, United States). The reportable range for free T3, free T4, and TSH were 0.5 to 22.8 pg/mL, 0.07 to 6.99 ng/dL, and 0.015 to 100 µIU/mL, respectively. The euthyroid reference range for free T3, free T4, and TSH was 2.77 to 5.27 pg/mL, 0.78 to 2.2 ng/dL, and 0.46 to 4.68 µIU/mL, respectively. Serum prolactin was measured using immunometric immunoassay technique using VITROS Prolactin Reagent Pack and VITROS Prolactin Calibrators on the Vitros ECi immunodiagnostic system using Intellicheck technology. The minimal detectable concentration was 0.5 ng/mL. The assay range was 1.4 to 329 ng/mL. All samples where the results were > 329 ng/mL on initial testing were sequentially diluted and reassayed till we got the exact prolactin level. The immunometric immunoassay technique involves simultaneous reaction of prolactin present in the sample with a biotinylated sheep polyclonal antiprolactin antibody and a horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal antiprolactin). The antigen–antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing. The bound HRP conjugate is measured by a luminescent reaction. The light signals are read by the system. The amount of HRP conjugate bound is directly proportional to the concentration of the prolactin. The normal range of prolactin by this assay for males and females were 3.7 to 17.9 ng/mL and 3.0 to 18.0 ng/mL, respectively.

PEG Precipitation Test for Macroprolactin

Macroprolactin in the serum sample was detected using the PEG-6000 precipitation test. Precipitation with PEG was performed by adding 200 µL of serum to 200 µL of 250 g/L (25%) of PEG-6000 (Sigma Aldrich, United States). A 25% solution of PEG-6000 was prepared by dissolving 25 g of PEG-6000 crystals in 100 mL of phosphate buffered saline (PBS) pH 7.4. After thorough mixing and vortexing, the mixture was centrifuged at 1500 g for 30 minutes at 4°C in refrigerated centrifuge. The supernatant was removed for prolactin estimation. Recovery of prolactin after precipitation with PEG was determined by comparison with a dilution of 200 µL of serum in 200 µL of PBS (pH 7.4). Prolactin in the supernatant after precipitation of serum with PEG and in the diluted serum was measured as untreated serum using the manufacturer's guidelines. Ratio of the prolactin in supernatant to total prolactin in diluted serum was calculated and presented as percentage recovery of prolactin. The samples were defined to have macroprolactinemia when the post-PEG recovery was < 40%.[7,8]

Sample Size Calculation

A previous study from Chandigarh reported the prevalence of macroprolactinemia to be 15.7%.[9] Keeping a power of 80% and type-I error at 5%, it was calculated that we need to include at least 143 patients in our study ($n = z^2_{1-\alpha} * p * [1-p]/d^2$; where "$p$" stands for anticipated population proportion [15.7% or 0.157]; "d" stands for precision required on either side of proportion [5% or 0.05]; and "z" is a constant being 1.645 for one-sided test with power [1-β] of 80% and type-1 error of 5%).

Results

A total of 1,229 patients (male:female = 191:1038) having a mean age of 30.46 ± 10.14 years were detected to have serum prolactin > 18 ng/mL over a period of January 2019 to February 2020.

About 84.45% of patients with hyperprolactinemia were females. Further, 168 people (13.7%) were detected to have macroprolactinemia. Age quartiles-based analysis revealed no significant difference in the occurrence of macroprolactinemia across any of the age groups (quartile-1 [5–24 years], 34/325 [10.46%]; quartile-2 [24–28 years], 50/298 [16.77%]; quartile-3 [28–35 years], 48/318 [15.09%]; quartile-4 [35–80 years], 36/288 [12.5%]; $p = 0.104$).

A total of 134 out of 168 patients detected to have macroprolactinemia had serum prolactin in the normal range post-PEG precipitation. Hence, 34 patients (2.76%) continued to have raised serum prolactin levels, even after less than 40% recovery post-PEG precipitation. These patients primarily had underlying pituitary pathology (►**Table 1**). Further, 12 out of 191 males (6.28%) were detected to have macroprolactinemia, which was significantly lower as compared with females (156/1038, 15.03%; $p = 0.001$).

The occurrence of macroprolactinemia based on the underlying etiology or the reason for testing has been elaborated in ►**Table 1**. The four most common underlying etiologies for testing hyperprolactinemia were polycystic ovary syndrome ($n = 402$; 32.71%), pituitary adenomas ($n = 318$; 25.87%), drug-induced hyperprolactinemia ($n = 224$; 18.23%), and infertility ($n = 126$; 10.25%). The occurrence of macroprolactinemia in people with drug-induced hyperprolactinemia was significantly lower than all the other reasons for testing of hyperprolactinemia (►**Table 1**). It must be highlighted that all the 14 patients incidentally detected to have raised prolactin levels as a part of health package evaluation had raised prolactin levels due to macroprolactinemia. None of the patients with hyperprolactinemia secondary to uncontrolled primary hypothyroidism had macroprolactinemia. The median [25th–75th percentile] of prolactin before and after PEG precipitation test among people with different underlying etiology or reason for testing has been elaborated in ►**Table 2**.

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

Diagnosis	Macroprolactinemia absolute number (percentage)
Prolactinoma ($n = 192$)	18 (9.37%)
Nonprolactinoma pituitary adenoma ($n = 126$)	18 (14.28%)
PCOS ($n = 402$)	66 (16.41%)
Infertility ($n = 126$)	28 (22.22%)
Drug-induced hyperprolactinemia ($n = 224$)	2 (0.89%)
On psychiatry medications ($n = 32$)	4 (12.5%)
Gynecomastia ($n = 21$)	4 (19.1%)
Erectile dysfunction ($n = 16$)	4 (25%)
Primary hypothyroidism ($n = 26$)	–
Type 2 diabetes ($n = 26$)	8 (30.76%)
Prolactin tested as part of health package ($n = 14$)	14 (100%)
Others ($n = 24$)	2 (8.33%)

Abbreviation: PCOS, polycystic ovary syndrome.

Discussion

Less than 40% recovery after PEG precipitation of prolactin has been universally accepted to be the definition of occurrence of macroprolactinemia.[10] This is because this cutoff believed to have a 100% sensitivity in picking up macroprolactin.[7,10] Gel filtration chromatography, which is considered to be the gold standard test to pick up macroprolactin, but is not easily available, technically difficult, time consuming, and is much costlier, showed that only 6% of all blood samples having 40 to 60% of monomeric prolactin contain significant amount of macroprolactin.[7,9] Our study is the largest ever from India on the prevalence of macroprolactinemia in patients being evaluated and detected to have hyperprolactinemia. We documented a 13.7% prevalence of macroprolactinemia in such patients. In a previous study from New Delhi, the prevalence of macroprolactinemia was found to be 21.57% in a study of 102 patients with serum prolactin > 100 ng/mL.[11] In a study involving 1,163 women undergoing evaluation for infertility at Chandigarh, 15.7% ($n = 183$) women were detected to have hyperprolactinemia. About 11.5% of these 183 women with hyperprolactinemia were detected to have macroprolactinemia.[12]

Previous studies from other parts of the globe have reported the prevalence of macroprolactinemia to be 4 to 46% depending on the assay and the ethnicity, in people being evaluated for hyperprolactinemia.[7,10,13] Screening of general blood samples, and not samples that are specifically looking for hyperprolactinemia, has reported a lower occurrence of macroprolactinemia. In a large study evaluating 10,737 consecutive samples, Bjøro et al reported a 1.5% prevalence of macroprolactinemia.[14] In a study of 1,330 apparently healthy individuals with hepatitis-B positivity, a 3.6% prevalence of macroprolactinemia was documented.[15]

Macroprolactins are bioinactive and presence of macroprolactinemia leads to decreased bioavailability of bioactive monomeric prolactin.[10] Antiprolactin antibodies are primarily responsible for the formation of macroprolactins.[10] How and why these antiprolactin antibodies form is not well understood. Macroprolactinemia has not been associated with any of the autoimmune disorders like thyroid autoimmunity, rheumatoid arthritis, and lupus.[16-18] Age was not a factor for the occurrence of macroprolactinemia in our study. However, few studies have suggested increased occurrence of macroprolactinemia with age.[19] Also underlying etiology or the reason for testing for hyperprolactinemia was not a factor on the occurrence of macroprolactinemia.

Table 2 Prolactin levels before and after polyethylene glycol (PEG) precipitation test among people with different underlying etiology or reason for testing

Diagnosis	Prolactin (baseline) (ng/mL)	Prolactin (post PEG precipitation) (ng/mL)
Prolactinoma (*n* = 192)	177.05 (80.1–316.10)	112 (72.97–217.72)
Nonprolactinoma pituitary adenoma (*n* = 126)	42.5 (31.9–65)	25 (19.2–39.1)
PCOS (*n* = 402)	39.30 (30.00–56.22)	21.11 (16.72–29.65)
Infertility (*n* = 126)	34.7 (30.1–50.70)	20 (16.3–27.9)
Drug-induced hyperprolactinemia (*n* = 224)	45.85 (25.3–67.73)	29.60 (19.00–45.12)
On psychiatry medications (*n* = 32)	54.1 (31.4–97.42)	32.45 (20.8–56.47)
Gynecomastia (*n* = 21)	36.3 (30.05–42.4)	19.6 (16.9–25.7)
Erectile dysfunction (*n* = 16)	28.9 (27.37–69.27)	15.7 (9.8–30.01)
Primary hypothyroidism (*n* = 26)	39.2 (32.27–66.57)	30.00 (24.63–54.82)
Type 2 diabetes (*n* = 26)	55.5 (45.82–66.95)	24.20 (15.80–31.90)
Prolactin tested as part of health package (*n* = 14)	71.2 (58.5–84.90)	10.5 (8.1–12.1)
Others (*n* = 24)	35.2 (27.03–73.37)	19.9 (16.95–34.55)

Abbreviation: PCOS, polycystic ovary syndrome.
All values expressed as median (25th–75th percentile).

An important observation of this study is that none of the patients with hyperprolactinemia secondary to uncontrolled primary hypothyroidism had associated macroprolactinemia. It is well known that uncontrolled primary hypothyroidism is associated with hyperprolactinemia that needs no evaluation or treatment.[20] Restoration of euthyroidism with levothyroxine supplementation leads to spontaneous correction of this hyperprolactinemia. Our study also showed that the occurrence of macroprolactinemia was very low in people with drug-induced hyperprolactinemia. This study hence highlights the importance of good history taking in the setting of evaluation of hyperprolactinemia, as testing for macroprolactinemia is not warranted in easily reversible cause of secondary hyperprolactinemia like uncontrolled primary hypothyroidism or drug-induced hyperprolactinemia. A lack of a primary underlying alternation in prolactin metabolism in people with primary hypothyroidism and drug-induced hyperprolactinemia may explain the low occurrence of macroprolactinemia in them. The different pharmacologic agents cause reversible hyperprolactinemia by different mechanisms. They may be involved in increased transcription of prolactin gene, antagonism of dopamine receptor, dopamine depletion, or inhibition of dopamine reuptake. The hyperprolactinemia caused by drugs is of mild nature and is due to nonimmunologic mechanisms. The hyperprolactinemia caused by drugs is of reversible nature, that is, prolactin values return to normal as soon as the causative drug/s discontinued.[21] We must remember that both subclinical and overt primary hypothyroidism have been linked to benign reversible hyperprolactinemia secondary to trophic effect of thyrotropin releasing hormone-mediated prolactin release from lactotrophs in pituitary.[22,23] Strengths of this study are the large number of patients evaluated. Limitations being the testing for macroprolactinemia were limited to patients detected to have hyperprolactinemia and not the general population. Also, the clinical details and etiology details were collected from the patient records and not from the patients directly, which may lead to under reporting of events.

The small number of 34 patients (2.76%), who had macroprolactinemia with true hyperprolactinemia (raised serum prolactin even after PEG precipitation), had underlying primary pituitary pathology like pituitary adenoma. This is in accordance with a previous study where 36% of women with macroprolactinemia and true hyperprolactinemia had underlying pituitary abnormalities on magnetic resonance imaging brain, as compared with 4% in the group with macroprolactinemia but normal prolactin levels after PEG precipitation.[24] Evaluation for macroprolactinemia is not warranted in people with underlying pituitary pathology in routine clinical practice. Hence, less than 40% recovery of serum prolactin post-PEG-precipitation along with normalization of serum prolactin levels post-PEG precipitation is mandatory before we define the patient to have macroprolactinemia. Macroprolactinemia screening primarily has a role in a totally asymptomatic patient detected to have hyperprolactinemia.

Conclusion

To conclude, it may be said that macroprolactinemia is not uncommon in people being tested for hyperprolactinemia. Hence, we should not hesitate to screen for macroprolactinemia in patients who have been incidentally been detected to have hyperprolactinemia, but otherwise do not have clinical features suggestive of hyperprolactinemia.

Authors' Contribution

The study was planned by LKS and DD. The study protocol was designed by BK, DD, and LKS. Data collection was done by RS, LKS, SL, RS, and NS. Data analysis was done by DD, RS, and LKS. All authors contributed equally to the manuscript preparation.

Conflict of Interests

None.

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