February 2018

Frequency of macroprolactin in hyperprolactinemia

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ORIGINAL ARTICLE

Frequency of Macroprolactin in Hyperprolactinemia

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ABSTRACT

Objective: To determine the frequency of Macroprolactin (MaPRL) in patients with increased total prolactin and its clinical and financial impact.

Study Design: Cross-sectional study.

Place and Duration of Study: Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital, Karachi, from March to May 2015.

Methodology: Patients with high total prolactin were screened by polyethylene glycol (PEG) precipitation for determination of MaPRL. Clinical history, imaging work-ups, and cost incurred in further investigations were collected by telephonic interview after verbal consent. Patients were stratified into true hyperprolactinemia and macroprolactinemia after PEG treatment, based on monomeric prolactin levels. Medical records of cases registered with AKUH were reviewed to confirm the diagnosis.

Results: Two hundred and thirty-nine patients were identified with high prolactin levels. Macroprolactinemia was identified in 145 (60.7%) and true hyperprolactinemia in 94 (39.3%) patients. Galactorrhea was significantly more in true hyperprolactinemic females (p=0.022), followed by visual disturbances (p=0.01) and headache (p=0.006). Moreover, as majority of population were females, the clinical features in the macroprolactinemia group as compared to true hyperprolactinemic group were mostly related to non-pituitary causes like drug intake [42.5% (54) vs. 37% (30)], heat intolerance due to thyroidal illness [41.7% (53) vs. 38.3% (31)] and surgery [26.8% (34) vs 22.2% (18)] in females. Further radiological workup (MRI, CT) were conducted in 35 (37.2%) patients with true hyperprolactinemia. Twenty-one (60%) of the patients were confirmed to have pituitary adenomas. In eight (5.5%) patients with MaPRL, only one had pituitary micro-adenoma on radiological workup. Total cost impact on the basis of investigations, was significantly higher in the group undergone imaging, despite 7 out of 8 individuals found to have normal imaging results. The median total cost in true hyperprolactinemic group undergone imaging was Rs. 4370 (IQR=2412.5, 22850) as compared to macroprolactinemic groups; Rs. 3,250 (IQR=2150, 4278). There was significant difference in the cost burden of both the groups (p <0.001).

Conclusion: High frequency of MaPRL was identified in patients with hyperprolactinemia. Screening with PEG precipitation in hyperprolactinemic sera is simple and cost-effective.

Key Words: *MaPRL. PEG precipitation. True hyperprolactinemia. Oligomenorrhea.*

INTRODUCTION

Prolactin (PRL) occurs in three isoforms, i.e. a monomeric PRL {MW~23 kDa}, a big PRL {MW~50 kDa} and a complex of monomeric prolactin and IgG known as macroprolactin (MaPRL) or as "big, big PRL" {MW <100 kDa}.[1] Circulating total prolactin hormone in normal and patients with increased prolactin levels mainly comprise of monomeric PRL (<85%) and MaPRL (less than 2%). MaPRL is biologically inert, as it is impermeable to the capillary blood barrier due to its large molecular size but is measured in the prolactin assay leading to falsely elevated prolactin levels.[2,3] However, in few cases of hyperprolactinemia; MaPRL becomes the dominant form, as it has been reported from 10% to 45% in hyperprolactinemic patients.[4-6]

The polythylene glycol (PEG) precipitation is used widely in clinical laboratories performing prolactin assay to screen for MaPRL.[2,3] PEG precipitation distinguishes patients with true hyperprolactinemia, which is due to increase of bioactive monomeric PRL, from those with MaPRL, in which monomeric prolactin is normal in concentrations. Inability of laboratories to perform PEG precipitation leads to overreporting of hyper-prolactinemia and consequent over investigation of the patient by treating physicians.[4,5]

PEG precipitation and analysis for MaPRL and monomeric prolactin of all samples with hyper-prolactinemia was started at the laboratory as a quality improvement initiative. This study was conducted to determine the frequency of MaPRL in patients with increased total prolactin and its impact on clinical and financial outcome.

METHODOLOGY

A retrospective cross-sectional study was carried out at Section of Clinical Chemistry, Department of Pathology

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Received: March 25, 2017; Accepted: December 11, 2017.

and Laboratory Medicine, The Aga Khan University, Karachi. The study was approved by the Ethical Review Committee of The Aga Khan University Hospital. Patients' sera having high total prolactin levels (>25-200 ng/ml in females and >15-200 ng/ml in males) were screened by PEG precipitation for MaPRL determination. Patients were contacted by telephone and those who gave verbal consent, were interviewed about clinical history, imaging workups and cost incurred in further investigations. Medical records of cases registered at AKUH were reviewed to confirm the diagnosis.

Serum samples with increase prolactin concentration were mixed with an equal volume of 25% PEG in saline, and incubated for 10 mins at room temperature. The monomeric PRL level in the supernatant was quantified by enzyme-amplified chemiluminescent immunoassay (ECLIA) on Immulite 2000, from Siemens, Germany.

Analytical sensitivity of the assay was 0.5 ng/mL. An Intra-assay coefficient of variation (CV) at the PRL concentration of 11.9 ng/mL was 4.8% and inter-assay CV at the concentration of 22.3 ng/mL was 4.0%. Reference ranges used in the laboratory were 1.9-25.0 ng/mL for women and 2.5-17.0 ng/mL for men.

The reproducibility of the PEG precipitation procedure was monitored by inclusion of control sera in each assay. Absolute levels of monomeric prolactin in sera after PEG precipitation were used for reference range i.e. 3.6-12.4 ng/ml in males and 4-18.5 ng/ml in females.[7]

The data was analyzed on SPSS (version 21.0). Macroprolactinemia accounts for up to 15% to 30% in frequency so for sample size calculation, taking 95% confidence interval with 5% type 1 error, 196 number of patients were required to achieve the target population. Two hundred and thirty-nine patients were recruited for better spread of data. Frequencies and percentages for categorical variable, mean and standard deviation (SD) for discrete or continuous variables and for non-parametric data, median with interquartile range were calculated. Patients were stratified under macro-prolactinemia group and true hyperprolactinemia group, according to monomeric reference range after PEG treatment. Cost comparison between both true hyperprolactinemia and those with MaPRL were performed by mean expenditure in performing imaging studies. The Chi-square test used for categorical variables; in case sample size or frequency found less than <5 then Fishers' exact test was employed for continuous variables. Additionally, for non-parametric data, Mann-Whitney and Kolmogorov-Smirnov tests were used to check normality of data. P-value <0.05 was taken as level of significance in analysis.

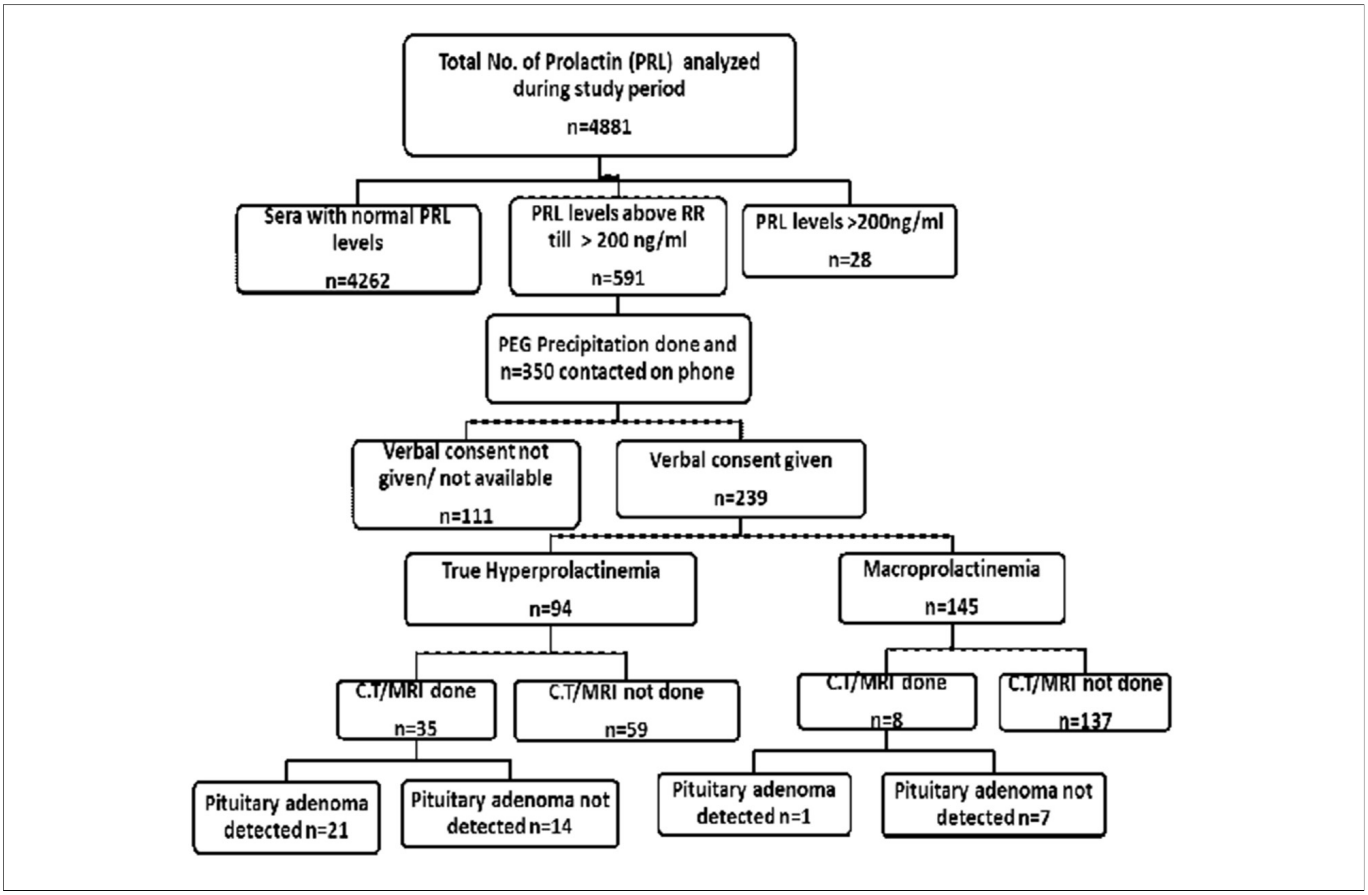

Figure 1: Consort diagram of study participants. The flow of patients enrolled, screened and imaged is shown in accordance to the CONSORT statement.

RESULTS

Figure 1 shows the total number of patients tested for serum prolactin at the Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine during the study period, with breakup of those screened and enrolled in the study.

Three hundred and fifty patients, out of 591 with PRL levels between normal range and till 200 ng/ml, were screened for MaPRL by PEG precipitation. Out of these, 239 gave informed consent and provided the clinical details. Median age of the patients was 28 years, (IQR=24, 35) and male/female ratio was 31/208, with female preponderance. MaPRL was present in 60.7% (n=145) of hyperprolactinemic patients. The monomeric PRL levels are significantly different (p <0.001) from 26.7 ng/ml (IQR=21.2, 44.9) to 12.9 ng/ml (IQR=9.8, 15.1) in true hyperprolactinemia and macroprolactin after PEG precipitation.

Table I compares the demographic and biochemical details of patients with true hyperprolactinemia and macroprolactinemia. Total prolactin was significantly higher (p <0.001) in patients with true hyperprolactinemia as compared to patients with MaPRL and this difference was maintained after treatment with PEG. There was also significant difference in the cost burden of both the groups (p <0.001). The median total cost in true hyperprolactinemic group undergone imaging was Rs. 4,370 (IQR=2412.5, 22850) as compared to macroprolactinemic group was Rs. 3,250 (IQR=2150, 4278).

Table I: Comparison of demographic, biochemical and expenses in patients with true hyperprolactinemia due to MaPRL screened at Aga Khan University Hospital Clinical Laboratories (n=239).

Variables	True Hyperprolactinemia (n=94)	MacroPRL (n=145)	p-value
Age (years)	28 (IQR=24, 35)	28 (IQR=24, 35)	0.846
Gender			
Male	13 (13.8%)	81 (86.2%)	0.750
Female	18 (12.4%)	127 (87.6%)	
Marital Status			
Married	68 (72.3%)	118 (81.4%)	0.101
Single	26 (27.7%)	27 (18.6%)	
Total Prolactin (ng/ml)	49.3 (IQR=35.4,81.9)	27 (IQR=24,32)	<0.001**
Monomeric Prolactin (ng/ml)	26.7 (IQR= 21.2,44.9)	12.9 (IQR= 9.8,15.1)	<0.001**
Radiological (MRI+CT)	35 (37.2%)	8 (5.5%)	
Adenoma detected	21	1	<0.001**
Adenoma not detected	14	7	
Total cost (PKR)	4370 (IQR=2412.5, 22850)	3250 (IQR=2150, 4278)	<0.001**

***Highly significant; *Significant.*

The indications for testing of prolactin were diverse and varied between males and females (Figures 2a and 2b). Overall in females, predominantly menstrual disturbances, infertility, oligomenorrhea were the main indications for testing followed by heat intolerance, obesity, headache, cold intolerance, visual disturbance and galactorrhea.

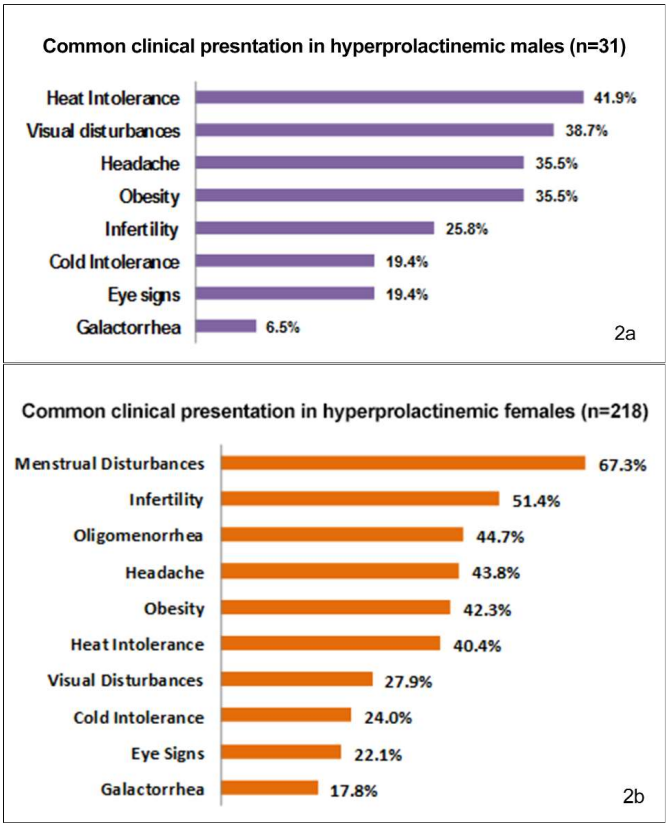

Figure 2: Indications for screening of patients for hyperprolactinemia and frequency comparison between true hyperprolactinemia and patients with MaPRL.

While in males, indications for testing were predominantly heat intolerance, visual disturbance, infertility and obesity followed by headache, cold intolerance and galactorrhea. Monomeric and MaPRL levels in patients with hyperprolactinemia did not differ when compared with clinical presentation in either males or females. Upon stratification, Galactorrhea was significantly more in true hyperprolactinemic females (p=0.022), followed by visual disturbances (p=0.01) and headache (p=0.006). Moreover, as majority of population were females, the clinical features in the macroprolactinemia group as compared to true hyperprolactinemic group were mostly related to non-pituitary causes like drug intake [42.5% (54) vs. 37% (30)], heat intolerance due to thyroidal illness [41.7% (53) vs. 38.3% (31)] and surgery [26.8% (34) vs. 22.2% (18)] in females.

Imaging studies (MRI, CT) were conducted in 35 (37.2%) patients with true hyperprolactinemia. However, only 8 (5.5%) patients with MaPRL were directed for further imaging. Statistically significant difference (p <0.001) in financial impact was seen between the two groups. Among the 35 patients, who underwent MRI/ CT scan, medical records showed that 21 (60%) of the patients were confirmed to have pituitary adenomas. Whereas, in the group of patients with hyperprolactinemia due to MaPRL, only one patient was identified with pituitary microadenoma as shown in Table I.

DISCUSSION

A high frequency of patients with hyperprolactinemia was identified due to MaPRL in our patient population. Out of the 145 patients diagnosed with MaPRL, 1 out of 8 patients who underwent MRI or CT scan were identified with microadenoma on CT scan. Previous reports also described minor CT or MRI scan abnormalities consistent with the presence of a microadenoma in macroprolactinemic patient. Consistent with this observation, abnormal pituitary CT scans 21% vs. 75% are reported in macroprolactinemic and true hyperprolactinemic patients, respectively. Such patients need follow-up scans and monitoring of pituitary microadenoma, as surgical intervention is needed in growing microadenomas/macroadenomas.[10-12]

MaPRL is the complex of monomeric prolactin attached to IgG, which results in increased size of prolactin molecule and hinders its renal clearance leading to increased levels of total PRL. As shown in this study, it is difficult to differentiate between true hyperprolactinemia and that due to MaPRL by clinical judgment alone; as most of the patients with MaPRL were also symptomatic. The absence of MaPRL screening by laboratories performing prolactin assay leads to over investigating patients with imaging studies and; hence, increase cost of management. High frequency of MaPRL is identified in this study and as reported by other studies also, menstrual disturbances, infertility and galactorrhea remains the most common clinical findings as mentioned in Table II.

Table II: Comparison of frequency of main clinical findings in women with Macroprolactinemia in our study and other major publications.

Symptoms	Vallette-Kasic et al.[7] (n=96)	Gibney et al.[19] (n=32)	Isik et al.[13] (n=84)	Present study (n=127)
Infertility	32%	22%	4.9%	54.3% (69)
Menstrual disturbances	39%	59%	38.9%	66.9% (85)
Galactorrhea	46%	22%	39.2%	13.4% (17)

Cost-effectiveness of macroprolactin screening is already established in literature and mean cost was higher in normal macroprolactin individuals' undergone imaging, which was cost burden due to unnecessary investigations.[13-15] As seen in our study, the mean expenditure was much less in MaPRL group, who did not need to go for imaging after PEG screening.

It is important that clinical laboratories performing prolactin testing should screen for MaPRL in all hyperprolactinemic sera. It is equally important that clinicians involved in managing these patients should be aware of this potential diagnostic pitfall and insist on macroprolactin screening. However, the results should be evaluated in detailed clinical context as few patients with MaPRL can have microadenomas, which are identified on MRI or CT scan and need careful monitoring and follow-up.

PEG precipitation is a simple, economical and a rapid method for the detection of MaPRL. Screening for MaPRL in all prolactin assays above reference range is a recommended good laboratory practice by most organizations. Method specific reference intervals are better than percent recovery method.[7,8,10]

CONCLUSION

High frequency of MaPRL was identified in patients with hyperprolactinemia. Screening with PEG precipitation in hyperprolactinemic sera is simple and cost-effective.

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