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Macroprolactin: an overlooked reason of hyperprolactinemia

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Abstract

Background: Immunoassays show variability in the detection of macroprolactin. The aim of this study was to detect the frequency of macroprolactinemia in hyperprolactinemic patients and the problems encountered in routine clinical practice.
Methods: The screening of macroprolactinemia was performed by precipitation with polyethylene glycol (PEG) in 900 patient samples with hyperprolactinemia over a period of approximately 6 months. Recovery values of less than 40% and greater than 60% were considered as macroprolactinemia and predominantly monomeric prolactin (PRL), respectively.
Results: A total of 900 (17.9%) of the 5007 PRL results were out of reference range. Thirty-one (3.4%) of the patients had less than 40% recovery after screening of all patients with hyperprolactinemia. However, the macroprolactin test was requested by clinics from only 171 patients and seven of these patients had less than 40% recovery. We also detected predominantly macroprolactin in 24 samples, overlooked in routine practice. The patients with PRL above 100 ng/mL had no macroprolactinemia.
Conclusions: The screening for macroprolactinemia of hyperprolactinemic patients who have <100 ng/mL and also with unexplained hyperprolactinemia should be the first approach before any further research or treatment is initiated. Thus, unnecessary test repetition, investigation and inappropriate treatment can be avoided. Each laboratory should inform clinicians about the frequency of macroprolactinemia.

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Keywords: hyperprolactinemia; macroprolactin; PEG precipitation.

A brief summary: Immunoassays show variability in the detection of macroprolactin. Overlooked macroprolactinemia may give rise to the misdiagnosis and mistreatment of patients with hyperprolactinemia. Every laboratory should investigate the frequency of macroprolactinemia and define a procedure that can be followed to eliminate macroprolactin problem in routine laboratory.

Introduction

In circulation, three forms of prolactin (PRL) have been identified by gel filtration chromatography (GFC). These forms are monomeric PRL (molecular weight [MW] 23 kDa), big PRL (MW 50–60 kDa) and big-big PRL or macroprolactin (macro-PRL, MW >150 kDa). Monomeric PRL constitutes the main form in most subjects with hyperprolactinemia. However, macro-PRL may be responsible for a significant portion of total PRL [1].

Macro-PRL is commonly defined as a complex constituted by monomeric PRL and immunoglobulin G (IgG) [2]. Hyperprolactinemia seen in subjects with macroprolactinemia is generally owing to the delayed clearance as a conclusion of the elevated MW or the deficiency of influential binding of macro-PRL to PRL receptors, removing the hypothalamic negative feed-back mechanism [3].

The variable detectability of macro-PRL among immunoassay systems may be associated with the type of monomeric PRL-immunoglobulin complex (IgG or non-IgG type) and different degrees of masking by anti-PRL autoantibody of epitopes [4].

The gold standard method for measuring monomeric PRL is GFC, but it is costly and not suitable for routine use. Therefore, the most frequently utilized procedure is precipitation with polyethylene glycol (PEG). This technique is easy, rapid and cheap, and has shown a good concordant with GFC [5–7].

Macroprolactinemic subjects usually lack the usual symptoms of hyperprolactinemia, because monomeric PRL-immunoglobulin complex cannot cross the endothelium and not reach to the PRL receptors [3, 8]. However, the presence of hyperprolactinemia-related symptoms in some patients with macro-PRL predominance may be clarified by intermittent decomposition of the macro-PRL complex or the synchronous presence of increased monomeric PRL and macro-PRL. In this condition, it is hard to discriminate macroprolactinemia from true hyperprolactinemia [1, 8, 9]. Overlooked macroprolactinemia may give rise to misdiagnosis and improper treatment of patients with hyperprolactinemia [4].

The purpose of this study was to examine the frequency of macro-PRL in patients with hyperprolactinemia and the impact of screening of all hyperprolactinemic patients for macro-PRL on clinical practice.

Materials and methods

Patients

All patients who were admitted to Erciyes University Hospital and diagnosed as having hyperprolactinemia in a period of 6 months were included in this study. However, we excluded patients who were pregnant. Demographic and clinical information were obtained by examining of patient records. Serum samples of patients with hyperprolactinemia were separated and then stored at –20 °C until they were analyzed for macro-PRL.

Polyethylene glycol precipitation method

The samples with hyperprolactinemia were screened by utilizing the PEG method [5]. Serum samples mixed with equal volume of 25% PEG (PEG 6000, Sigma, St. Louis, MO, USA) were incubated for 10 min at room temperature and then centrifuged at 14,000 *g* for 10 min. The supernatants including non-precipitated PRL were used for analysis. Pre-PEG and post-PEG PRL levels were detected with 2nd-generation assay kit by the electrochemiluminescence (ECLIA) method on Cobas 8000 e-602 module (Roche Diagnostics, GmbH Sandhoferstrasse 116 D-68305 Mannheim, Germany). Recovery of PRL was determined by the following formula: (post-PEG-PRL × 2/pre-PEG-PRL) × 100.

Samples were classified as macroprolactinemia when recovery of PRL was <40% and as true or monomeric PRL predominance when recovery was >60%. Patients with recovery between 40 and 60% (borderline values) were accepted in the gray zone, requiring GFC to approve the presence of macro-PRL. The classification based on recovery values was rearranged by considering reference intervals for monomeric PRL values obtained after PEG precipitation by Beltran et al. [10]. Thus, the subjects in the gray zone were reclassified as patients with and without high monomeric PRL.

We used pools of serum with PRL levels within and above the reference range to determine the repeatability of the PEG precipitation method. The within-run coefficients of variation (CV) for 16.2 ng/mL and 57.8 ng/mL PRL were 4.3% and 5.3%, respectively.

We measured total PRL concentrations with a CV of 1.7% at 28 ng/mL. The total PRL reference ranges are 4.79–23.3 ng/mL for women and 4.04–15.2 ng/mL for men [11].

Approval for this study was obtained from the Research Ethics Committee, Medical Faculty of the University of Erciyes (2017/431). We have complied with the World Medical Association Declaration of Helsinki regarding the ethical conduct of research involving human subjects.

Statistical analysis

Histogram, Q-Q plot and the Shapiro-Wilk test were used to determine whether data distribution was normal. Numerical variables with and without normal distribution were presented as mean ± standard deviation and median (25^{th}–75^{th} percentile) or (min–max), respectively. The independent T-test or the Mann-Whitney U-test was used to compare the two groups. Categorical variables were presented as frequency and percentage (%), and the chi-square (χ^2) exact method was utilized to compare these variables.

Results

PRL levels were analyzed in 5007 consecutive samples and 900 (17.9%) of them exhibited hyperprolactinemia during the 6-month follow-up period. Macroprolactinemia was detected in 31 (3.4%) of the 900 patients with hyperprolactinemia (Figure 1).

The macro-PRL test request was performed by clinics in 171 (19%) of the 900 patients with hyperprolactinemia over a period of 6 months and seven of the 171 patients had less than 40% recovery. After screening of all patients with hyperprolactinemia, we additionally detected macro-PRL in 24 (77%) patient samples, overlooked in routine practice (Figure 1).

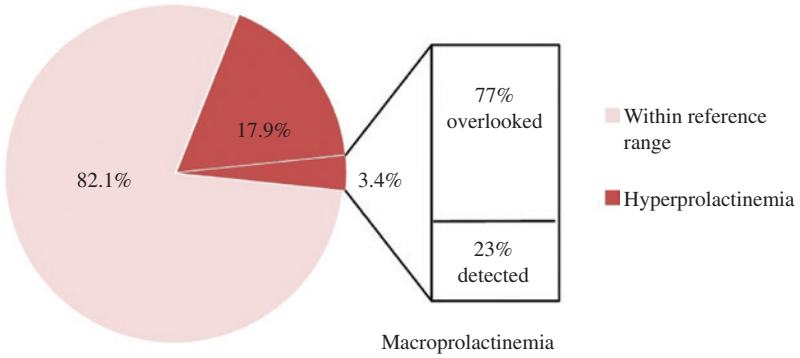

Figure 1: The percent of macroprolactinemia identified by clinics and detected after screening all patients with hyperprolactinemia (n = 900). Total number of patients: 5007.

Table 1: Characteristics and PRL levels of patients with macroprolactinemia (recovery <40%) and with monomeric PRL predominance (recovery >60%).

	Recovery >60, % n = 825	Recovery <40, % n = 31	p-Value
Age	33.5 ± 14.2	31.2 ± 10.6	NS
Sex			
Female	615 (75.5)	20 (65.5)	NS
Male	210 (25.5)	11 (35.5)	NS
PRL, ng/mL	30.65 (24.95–48.23)	32.60 (23.96–43.94)	NS
	30.65 (8–2278)[a]	32.60 (16.49–81.83)[a]	
PRL <30, ng/mL	401(48.6%)	15 (48.4%)	NS
PRL 31–50, ng/mL	236 (28.6%)	9 (29%)	NS
PRL 51–100, ng/mL	106 (12.9%)	7 (22.6%)	NS
PRL 101–150, ng/mL	38 (4.6%)	–	
PRL >150, ng/mL	44 (5.3%)	–	
Post-PEG (monomeric) PRL, ng/mL	24.4 (19.76–38.48)	10.44 (7.7–13.98)	<0.001
	24.4 (6.32–2026) [a]	10.44 (3.30–19.28)[a]	

Data were shown as [a]median (min-max), median (25%–75%), mean ± standard deviation n (%). NS, not significant; PEG, polyethylene glycol; PRL, prolactin.

After PEG precipitation, PRL levels decreased from 32.6 ng/mL (23.96–43.94) to 10.44 ng/mL (7.7–13.98) in patients with macroprolactinemia and from 30.65 ng/mL (24.95–48.23) to 24.4 ng/mL (19.76–38.48) in patients with monomeric-PRL predominance (Table 1).

No significant difference was found for age, sex or PRL values between samples with recovery >60% and <40% ($p > 0.05$). About 10% of the patients with monomeric PRL predominance had PRL values above 100 ng/mL. None of the hyperprolactinemic patients with PRL levels above 100 ng/mL had macroprolactinemia (Table 1).

The median recovery values for macro-PRL and monomeric PRL predominance were found to be 32% and 79.6%, respectively. Forty-four cases were in the gray zone, with a median recovery of 50.8%.

According to the reference range for post-PEG PRL (monomeric PRL male 2.96–11.51 ng/mL, female 3.52–17.9 ng/mL for Roche) reported by Beltran et al. [10], two patients' samples with recovery <40% (one had microadenoma) and 15 of the 44 patients with recovery 40–60% (gray zone) had high monomeric PRL values (Table 2).

When the patients in the gray zone were compared in terms of clinical information, there was no significant difference. Pituitary magnetic resonance (MR) imaging was applied to 11 (25%) of the 44 patients in the gray zone. Five of the 11 patients were evaluated as normal in MR imaging. Although four patients detected with microadenoma (n = 3) and macroadenoma (n = 1) had high monomeric PRL, two patients detected with microadenoma did not have high monomeric PRL after PEG precipitation.

Pituitary MR imaging was performed in nine (29%) of the macroprolactinemic patients. Seven of these patients were not tested for macro-PRL by clinics, but MR imaging was reported as normal. MR imaging findings revealed

Table 2: Comparison of clinical information of patients with high and low monomeric PRL after PEG precipitation.

Clinical information	Recovery 40–60, % Gray zone (n = 44)		p-Value
	High monomeric PRL after PEG precipitation (n = 15)	Low monomeric PRL after PEG precipitation (n = 29)	
Headache	3 (20)	2 (6.9)	NS
Infertility	5 (33.3)	8 (27.6)	NS
Menstrual disturbance	4 (26.7)	7 (24.1)	NS
PCOS	2 (13.3)	2 (6.9)	NS
Hirsutism	1 (6.7)	1 (3.5)	NS
Obesity	–	3 (10.3)	NS
Clinically unexplained PRL rise	–	6 (20.7)	NS

NS, not significant; PCOS, polycystic ovary syndrome, n (%); PEG, polyethylene glycol; PRL, prolactin. According to the reference range for post-PEG PRL (monomeric PRL male 2.96–11.51 ng/mL; female 3.52–17.9 ng/mL for Roche) reported by Beltran et al. [10].

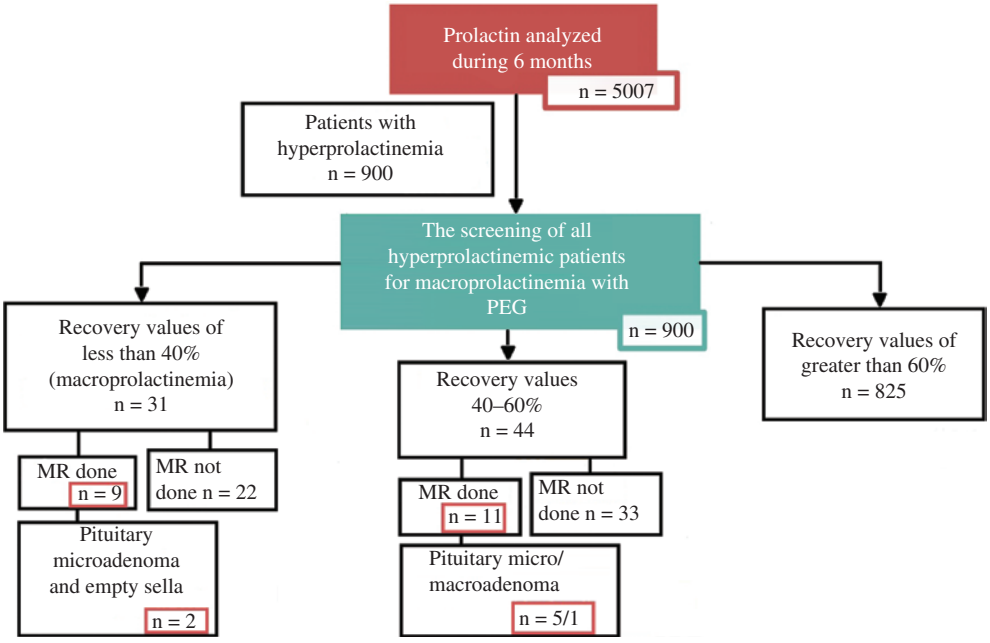

Figure 2: Diagram of patients screened and imaged.

that two patients (6.4%) among 31 hyperprolactinemic patients with predominant macro-PRL had pituitary lesions (one had a microadenoma and other had empty sella lesions) (Figure 2).

Discussion

Predictions of the prevalence of macroprolactinemia in clinical practice vary widely. Macroprolactinemia is present in approximately 4% of the general population [12]. However, the frequency of macroprolactinemia is detected in 4–46% of patients with hyperprolactinemia, relating to the method applied and the type of population investigated [13]. Hattori et al. [4] determined that macro-PRL was predominant in 4.02% of patients who visited an obstetric and gynecological hospital. In the present study, we screened all patients with hyperprolactinemia and detected macroprolactinemia in 31 (3.4%) of the 900 hyperprolactinemic patients. This ratio was low, when compared to the literature [13].

Although some immunoassay systems are more specific than others for monomeric prolactin, a specific

immunoassay system for monomeric PRL is not currently available.

Screening of all hyperprolactinemic samples for macro-PRL has been recommended by numerous groups [13–17]. However, some studies suggested that patients who had PRL concentrations below the threshold of 85 ng/mL [18] or between 25 and 150 ng/mL and every asymptomatic patient with hyperprolactinemia should be tested for macro-PRL [13].

Wallace et al. [19] followed 51 patients with macroprolactinemia for a 10-year period and they finalized that macroprolactinemia was a benign condition. However, macroprolactinemia causes confusion in diagnosis when it coexists with nonspecific symptoms of hyperprolactinemia [9, 17, 20].

Kavanagh et al. [21] compared the performance of some methods in the detection of macro-PRL and detected that PEG precipitation was superior and showed the best concordance with GFC.

PEG precipitation has been widely utilized to determine the interference caused by endogenous antibodies [22]. However, it is well documented that assay-specific reference ranges for post-PEG PRL must be used because approximately 20% of the monomeric PRL in serum is coprecipitated with IgG [14]. Therefore, Beltran et al. [10] determined monomeric PRL reference ranges in males and females for six different analyzers. They reported that the concordance between GFC and post-PEG reference intervals in patient classifications was 96%.

Recent studies indicated that there were no differences in terms of hyperprolactinemia-related symptoms between patients with macroprolactinemia and monomeric hyperprolactinemia [8].

In some conditions, it is not reliable to discriminate macroprolactinemia from true hyperprolactinemia based on clinical findings. The use of post-PEG reference intervals may be useful in the determination of patients who have both macroprolactinemia and monomeric hyperprolactinemia and in the evaluation of patients in the gray zone according to the recovery ratio.

Our findings also showed that clinical findings did not show significant difference between patients with high monomeric PRL and low monomeric PRL according to the post-PEG reference interval in the gray zone. In the present study, 15 patients with high monomeric PRL values needed further investigations.

The present study indicated that when macro-PRL screening was performed on request or by only clinical suspicion, 77% of patients with macroprolactinemia were overlooked. In other words, we additionally detected 24 patients with macroprolactinemia overlooked in routine clinical practice after screening all of the patients with hyperprolactinemia.

Pituitary imaging studies are frequently performed in patients with retrospectively detected macroprolactinemia. Pituitary microadenomas have been identified in this group but its true clinical relationship is uncertain, as pituitary microadenomas are also detected in about 10–20% of the general population at autopsy [9, 20, 23]. Suliman et al. [20] reported that 93% of identified macro-PRL underwent computed tomography (CT) or MR imaging to determine the reason of the hyperprolactinemia. This ratio was reported to be 88% by Olukoga and Kane [7].

The prevalence of abnormal MR imaging or CT scans was detected as 7–22% in patients with macroprolactinemia in cohort studies [9]. The prevalence of macroprolactinemia did not differ between newly diagnosed patients with prolactinoma and healthy controls. The coexistence of a pituitary adenoma and macroprolactinemia or macro-PRL production by the adenoma itself has been proposed as a comment in these uncommon cases [24].

MR imaging findings revealed that 26.7% of patients with macroprolactinemia had pituitary lesions. One of these patients had empty sella lesions and others had a microadenoma [25].

Leslie et al. [23] reported that 7.3% of macroprolactinemic patients had microadenomas, but none had macroadenoma. In our study, pituitary imaging was applied to 29% of patients with macroprolactinemia, and 89% of these had normal MR imaging.

Our study had some limitations: (1) GFC is regarded as the gold standard and this reference assay could not be applied to patients detected in the gray zone with the PEG precipitation method. However, 29 patients according to the post-PEG reference interval had normal monomeric PRL values. (2) Recovery <40% was accepted as macroprolactinemia by the PEG precipitation method, but other endogen interferences such as heterophilic antibodies were also precipitated by PEG in the present study.

On the basis of the findings: (a) macroprolactinemia should be considered in the differential diagnosis of hyperprolactinemia to avoid unnecessary expensive examination; (b) the screening for macro-PRL should be the first approach especially in patients who have <100 ng/mL PRL concentrations and also with unexplained hyperprolactinemia before any further research or treatment is initiated; (c) the use of post-PEG reference intervals by clinical laboratories may be useful in some cases; (d) clinicians should be informed about the macro-PRL problem.

Finally, we propose that every laboratory should investigate the frequency of macroprolactinemia and

define a procedure that can be followed to eliminate the problem of macro-PRL in routine laboratory.

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