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UNCORRECTED PROOF

The frequency of macroprolactinemia among patients with hyperprolactinemia in a central laboratory of a training and research hospital

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ABSTRACT

OBJECTIVE: Macroprolactinemia is a well-described endocrine disorder, with its results leading to unnecessary tests and overtreatment. However, routine macroprolactin screening is not performed in many laboratories. Routinely used prolactin assays can result in false diagnosis of hyperprolactinemia in patients with no signs and symptoms related to hyperprolactinemia and clinicians should be aware of macroprolactinemia frequency encountered with the method in use. In this study, it was aimed to examine the frequency of macroprolactinemia among patients with hyperprolactinemia.

METHODS: Prolactin analyses were performed on Roche Cobas® e801 immunoanalyzer using the Elecsys Prolactin II electrochemiluminesence immunoassay (Roche Diagnostics, Mannheim, Germany). Samples were provided from 14 different hospitals in total and evaluated with the same method in a single central laboratory. In order to precipitate the samples for macroprolactin analysis, polyethylene glycol (PEG) 6000 was used.

RESULTS: In this study, we evaluated 1100 patients with hyperprolactinemia and determined the frequency of macroprolactinemia to be 9.6% (recovery cut-off value <40%), while 8.5% of the patients were in the gray zone (recovery cut-off value 40% to <60%).

CONCLUSION: Laboratories should consider regularly screening for macroprolactinemia in all hyperprolactinemic samples and collaborate with clinicians to raise awareness about the prevalence of this condition.

Keywords: Immunoassay; macroprolactin; polyethylene glycol.

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Prolactin (PRL), comprised of 199 amino acids, is a polypeptide hormone secreted by lactotroph cells in the anterior pituitary gland. Its primary function is to regulate lactation and breast development during pregnancy, but it also serves various other crucial biological roles such as osmoregulation and immunoregulation [1].

Hyperprolactinemia, a common endocrine disorder, is more common in women and its cause may be physiological, pathological, or pharmacological [2]. While causes such as pregnancy, breastfeeding, and stress stand out among physiological elevations, pituitary tumors, hypothyroidism, using medication (such as antipsychotic drugs) and macroprolactinemia stand out as pathological elevations.

PRL has three forms in circulation: monomeric prolactin, dimeric prolactin, and macroprolactin. Monomeric PRL, which makes up 85% of the total immunoreactive PRL, has a molecular weight of 23 kDa, while dimeric PRL has a molecular weight ranging from 48 to 56 kDa and comprises only 5% to 10% of circulating PRL [3]. Monomeric PRL, the dominant form in cir-

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culation, is responsible for biological and immunological activity and is also responsible for hyperprolactinemia findings such as irregular menstrual cycles, galactorrhea, low libido, and infertility.

Macroprolactin (macro PRL) has a molecular weight of approximately 150 kDa and consists of an antigen-antibody complex, immunoglobulin (most commonly IgG), and monomeric prolactin. However, the reason for the formation of prolactin autoantibodies in people with macroprolactinemia has not been fully determined. Factors that trigger the formation of autoantibodies are believed to be a genetic predisposition, as well as post-translational modifications in the PRL molecule [4]. In macroprolactinemia, the predominant form of PRL in serum is macro PRL instead of monomeric PRL. Macro PRL is thought to have no biological activity due to the difficulty of binding to biological receptors and passing through capillary walls. Due to their large molecular weight, macro PRL molecules cannot cross the blood-brain barrier and do not result in downregulation of monomeric PRL release; therefore, monomeric PRL levels generally remain within normal ranges. For all these reasons, the classic symptoms and signs of true hyperprolactinemia are not observed in hyperprolactinemia due to macro PRL. An additional effect of the increased molecular weight of macro PRL is a decrease in glomerular filtration and subsequent reduction in renal clearance compared to monomeric PRL, leading to remain in circulation for prolonged periods and higher serum macro PRL levels and thus hyperprolactinemia [3, 5, 6]. Routinely used prolactin assays cannot distinguish monomeric PRL form from macro PRL form, resulting in false diagnosis of hyperprolactinemia in patients with no signs and symptoms related to hyperprolactinemia. Therefore, macroprolactinemia is a well-described endocrine disorder that can result in excessive and unnecessary medical intervention, leading to potential iatrogenic harm.

In view of above, in this retrospective study, we aimed to examine the frequency of macroprolactinemia and show the added value of the consideration of post-polyethylene glycol (post-PEG) PRL levels in the determination of macroprolactinemia.

Highlight key points

- Routinely used prolactin assays can result in false diagnosis of hyperprolactinemia.
- Macroprolactinemia is a well-described endocrine disorder that can result in excessive and unnecessary medical intervention, leading to potential iatrogenic harm.
- Clinicians should be aware of macroprolactinemia frequency encountered with the method in use.
- Laboratories should consider regularly screen for macroprolactinemia in all hyperprolactinemic samples and collaborate with clinicians to raise awareness about the prevalence of this condition.

MATERIALS AND METHODS

Study Design

Using the database of the laboratory information system of the Goztepe Prof. Dr. Suleyman Yalcin Training and Research Hospital, the laboratory test reports of the patients between September 2022 and September 2023 were examined and checked for macroprolactinemia in this retrospective study. Samples were provided from 14 different hospitals in total and evaluated with the same method in a single central laboratory. During this period, this study consisted of 1100 patients (170 men and 930 women) whose prolactin levels were above URLs and were evaluated for macroprolactinemia. The study was approved by the Goztepe Prof. Dr. Suleyman Yalcin Training and Research Hospital Clinical Research Ethics Committee (date: 25.10.2023, number: 2023/0721) and carried out in compliance with the Helsinki Declaration.

Laboratory Analyses

PRL analyses were performed on Roche Cobas® e801 immunoanalyzer using the Elecsys Prolactin II electrochemiluminesence immunaasay (Roche Diagnostics, Mannheim, Germany). Elecsys PRL assay on Roche Cobas® e801 was a sandwich principle immunoassay and standardized to the WHO 3rd International Standard IS 84/500. The sensitivity of the assay was 0.094 ng/mL, coefficient of variation (CV%) ranged between 2.0% and 2.0% for repeatability and 2.6% and 4.4% for intermediate precision at PRL concentrations of 11.9 ng/mL and 41.1 ng/mL, respectively. According to the manufacturer's package insert, the upper reference limits (URLs) for the Elecsys Prolactin II test on the Cobas® e801 Immunoassay System were respectively 15.2 ng/mL and 23.3 ng/mL for males and females.

PEG-Precipitation

PEG-precipitation was performed by adding 200 µL of serum to an equal volume of 25% (w/v) PEG6000 (Polyethylene Glycol 6000, Merck). The solution was centrifuged (after thorough vortex mixing) at 1500 × g for 30 min at 20°C. PRL analysis was performed in the supernatant

Table 1. Prolactin concentrations of subjects before and after polyethylene glycol (PEG) precipitation

	True hyperprolactinemia (n=900)	Gray zone (n=94)	Macroprolactinemia (n=106)	p
Age	32.1 / 13.2	33.7 / 10.8	32.9 / 11.4	0.532
Prolactin-1*	39.2 (23.6–63.1)	34 (28.5–48.7)	42.2 (33.5–57.3)	0.095
Polactin-2**	31 (22.3–50.1)	18.2 (14.3–23.8)	10.5 (7.3–15.1)	**<0.001**

*: Prolactin-1: Prolactine concentrations before PEG; **: Prolactine-2: Prolactine concentrations after PEG.

(post-PEG PRL). The value of PRL after the use of PEG was calculated by multiplying the initial PRL result by 2 to account for the dilution caused by PEG [7]. To calculate PRL recovery, the post-PEG PRL result is divided by the initial PRL result and multiplied by 100. The criterion for diagnosing macroprolactinemia was the utilization of a PEG-precipitation ratio exceeding 60% (recovery less than 40%). Following PEG precipitation procedure, post-PEG recovery <40% was accepted as positive for macro PRL, 40–60% as gray zone and >60% as negative [8].

Statistical Analysis

All statistical analyses were carried out using the R Statistical language (version 4.2.1; The R Foundation for Statistical Computing, Vienna, Austria). To assess the normality of the data, Shapiro-Wilk's test and Q-Q plots were performed. Results were expressed as n (%), mean±SD and median and interquartile ranges (IQR), depending on data distribution. A value of p less than 0.05 was considered statistically significant.

RESULTS

A total of 1100 patients with hyperprolactinemia were included in the study (Table 1). Among the individuals with hyperprolactinemia included in the study, 930 (84.5%) were female and 170 (15.5%) were male. The frequency of macroprolactinemia was 9.6% (n=106) while 8.5% was in the gray zone (n=94). As shown in Table 1, when hyperprolactinemia patients were divided into 3 groups: true hyperprolactinemia, gray zone and macroprolactinemia, no statistical difference was observed in prolactin concentrations among groups before the procedure with PEG (p=0.095). As expected, prolactin concentrations were significantly different between groups after PEG precipitation (p<0.001).

DISCUSSION

In routine laboratories, hyperprolactinemia is determined by immunoassay systems, but macroprolactinemia is one of the factors that cause falsely high prolactin measurements. Macro PRL is a significant factor of interference, which can result in incorrect diagnosis and improper treatment in patients with hyperprolactinemia. In this study, we evaluated 1100 patients with hyperprolactinemia and determined the frequency of macroprolactinemia to be 9.6% (recovery cut-off value <40%), while 8.5% of the patients were in the gray zone (recovery cut-off value ≥40% and <60%).

Gel filtration chromatography, an expensive and labor-intensive method, is the gold standard for determining macro PRL [9]. Another approach to detecting macroprolactinemia is through reanalysis after precipitating with PEG and estimating the recovery. This method is relatively more convenient and cost-effective, making it a suitable option for routine screening of macroprolactinemia [10]. Less than 40% recovery after PEG precipitation of prolactin, believed to have 100% sensitivity, has been accepted as the universal cut-off for macroprolactinemia [4, 11]. Individuals diagnosed with hyperprolactinemia and having a notable presence of macroprolactin do not necessitate pituitary imaging or prolonged administration of dopamine agonist therapy [12].

Previous research from various regions around the world have indicated that the prevalence of macroprolactinemia in individuals undergoing assessment for hyperprolactinemia ranges from 0% to 56% across different studies, varying based on the assay used in the analysis and population ethnicity [7, 13–16]. In the study conducted by Sharma et al. [4] in patients with hyperprolactinemia, the frequency of macroprolactinemia was found to be 13.7%. Jassam et al. [17] reported the rate of macroprolactinemia as 4% in 409 patients with hyperprolactinemia

using the Advia Centaur autoanalyzer in the UK. In a study conducted in South Africa, analyzed with the Advia Centaur autoanalyzer, the frequency of macroprolactinemia was stated to be 28% in hyperprolactinemia [18].

It has been stated that the frequency of hyperprolactinemia varies depending on the assay used in the analysis. Smith et al. [19] reported that Roche users reported the highest PRL levels, while the lowest PRL levels were reported by Access, Centaur and Bayer ACS:180 systems. Similarly, in a study conducted by Akbulut et al. [11], the frequency of prolactinemia was 13.9% in the Roche Cobas e601 autoanalyzer, while it was 8.1% in the Beckman Coulter UniCel® DxI800 autoanalyzer. In the same study, while the frequency of macroprolactinemia was 7.6% in the Roche Cobas system, this rate was 0.7% in the DxI800 autoanalyzer [11]. Several factors, including interference, variations in antigenic epitopes on reagent antibodies, and the degree of immunoreactivity between the reagent antibody and macro PRL, may contribute to variations in the measurement of prolactin concentrations among different immunoassay systems [19–21].

The limitations of our study are, firstly, that macro PRL was not evaluated in gel filtration chromatography, which is the reference method. Secondly, prolactin concentrations were not measured using a different assay other than Roche Cobas.

Conclusion

According to the results of current study, it can be clearly said that clinical laboratories should consider regularly screening for macroprolactinemia in all hyperprolactinemic samples and collaborate with clinicians to raise awareness about the prevalence of this condition, clinicians should be aware of the method used in the laboratory. It is crucial in the field of general medicine and primary care for physicians to possess an effective resource that enables them to gain a deeper comprehension of the laboratory tests employed for diagnosing frequently encountered endocrine disorders. This knowledge could greatly benefit patients by ensuring accurate diagnoses and subsequent treatments.

Ethics Committee Approval: The Goztepe Prof. Dr. Suleyman Yalcin Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 25.10.2023, number: 2023/0721).

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