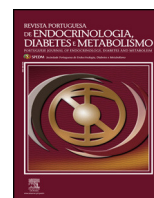




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Original article

Assessment of macroprolactinemia by polyethylene glycol precipitation method



Ana Maia Silva^{a,*}, Paula Martins da Costa^b, Ana Pacheco^b, José Carlos Oliveira^b, Cláudia Freitas^a

^a Department of Endocrinology, Diabetes and Metabolism, Hospital Santo António, Centro Hospitalar do Porto, Portugal

^b Department of Clinical Chemistry, Hospital de Santo António, Centro Hospitalar do Porto, Portugal

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ABSTRACT

Background: Macroprolactin is a molecule that results from association between monomeric prolactin (PRL) and immunoglobulin G (IgG). It has longer half-life and is considered biologically inactive, although it retains immunoreactivity, being detected in most available immunoassays.

Objective: To evaluate polyethylene glycol (PEG) precipitation method in routine detection of macroprolactin.

Methods: During 4 months a prospective study was performed in our hospital. Serum samples of PRL ≥ 30 ng/ml were collected and pre-treated with PEG. Initial PRL and post-PEG PRL (in the supernatant) were detected by electrochemiluminescence – Cobas e170®. Samples were classified as having a predominance of macroprolactin if recovery rate (RR) of PRL was $<40\%$ and an indeterminate result for the predominance of macroprolactin if RR was $40\text{--}65\%$.

Results: Ninety-six samples were enrolled, with median PRL 56.1 ng/ml (30.7–3667). PEG precipitation produced a decrease in PRL values in all of the cases (mean reduction of 22%). Two cases of macroprolactin predominance were detected (RR 4.9% and 16.1%) and 2 cases were indeterminate (RR 45.1% and 63.7%).

Discussion: PEG precipitation method is a simple and low-cost laboratory technique that can be routinely used in clinical practice. Macroprolactin accounting for hyperprolactinemia is a common cause of misdiagnosis. Screening for macroprolactin in hyperprolactinemic patients may avoid unnecessary investigation and inappropriate treatment.

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Avaliação de macroprolactina pelo método de precipitação com polietilenoglicol

RESUMO

Introdução: A macroprolactina é uma molécula que resulta da associação entre a prolactina (PRL) monomérica e a imunoglobulina G (IgG). Tem uma semi-vida superior e é considerada biologicamente inativa, apesar de ter imunorreatividade e ser detectada pela maior parte dos imunoensaios laboratoriais actualmente utilizados.

Objectivo: Avaliar o método de precipitação com polietilenoglicol (PEG) na avaliação laboratorial da macroprolactina.

Métodos: Foi elaborado no nosso hospital um estudo prospectivo com 4 meses de duração. Foram colhidas e pré-tratadas com PEG amostras de PRL sérica ≥ 30 ng/ml. Os valores de PRL inicial e de PRL pós-PEG (no sobrenadante) foram obtidos por electroquimioluminescência – Cobas e170®. As amostras foram classificadas como tendo predomínio de macroprolactina se a taxa de recuperação (RR) da PRL fosse $<40\%$ e como tendo um resultado indeterminado se a RR estivesse entre 40 e 65% .

Resultados: Noventa e seis amostras foram analisadas, com PRL mediana de 56.1 ng/ml (30.7–3667). A precipitação com PEG resultou numa diminuição no valor da PRL em todos os casos (redução média de 22%). Foram detectados 2 casos de predomínio de macroprolactina (RR 4.9% e 16.1%) e 2 casos com resultado indeterminado (RR 45.1% e 63.7%).

Palavras-chave:

Macroprolactina

Prolactina

Precipitação com Polietilenoglicol

* Corresponding author.

E-mail address: anamaiasilva@gmail.com (A.M. Silva).

Discussão: A precipitação com PEG é um método laboratorial simples e barato que pode ser utilizado por rotina na prática clínica. A macroprolactina é uma causa relativamente frequente de hiperprolactinemia que, quando detectada, pode permitir evitar custos adicionais com exames radiológicos ou tratamentos inapropriados.

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Introduction

Prolactin (PRL) is a hormone produced by lactotroph cells in anterior pituitary gland and its action is essential for human species survival. The main functions of PRL are exerted during pregnancy, enabling breast development, milk production and lactation. It has also reproductive and metabolic effects, like stimulation of immune response. PRL receptors are, therefore, expressed in several tissues, like breast, pituitary, adrenal cortex, prostate, gonads, liver and other essential ones.¹

The PRL molecule presents great heterogeneity with respect to molecular mass. Most human circulating PRL (about 85–95%) exist in a monomeric form of 23 kDa but high molecular mass isoforms including big-PRL, a dimer of 50 kDa and big-big-PRL or macroprolactin, a variant of 150–170 kDa, may also be present. These high-molecular weight forms have been described either in healthy subjects or in hyperprolactinemic patients.

Hyperprolactinemia refers to a state of elevated serum levels of PRL and may be caused, among others, by a prolactinoma, an autonomous PRL secreting pituitary adenoma, or by a loss of inhibitory effect of dopamine by other kinds of pituitary or sellar tumours. In some situations, however, no cause for elevated levels of PRL can be identified and patients are assumed to have idiopathic hyperprolactinemia.¹

Macroprolactinemia is another source of hyperprolactinemia. It accounts for a variable amount of all reported cases of hyperprolactinemia (10–46%), depending on the immunoassay used for laboratory determination.^{2–5}

Macroprolactin is most frequently a complex formed by a monomeric PRL and an immunoglobulin G molecule but post-translational modification of pituitary PRL with varying glycosylation and phosphorylation degrees have also been described.^{6,7}

This tertiary structure modification of PRL, responsible for retention in vascular tree and reduced tissue availability, contributes to its clinical inactivity. However, as the PRL part of PRL–IgG complex still preserves immunoreactivity in most available immunoassays used nowadays and as it has lower clearance rate and augmented half-life, it may cause high PRL levels in routine tests.

Sometimes, most of the circulating PRL may be in the macroprolactin form, leading, in those circumstances, hyperprolactinemia to be called macroprolactinemia.

The gold standard test to determine the presence of macroprolactinemia is gel filtration chromatography, but more available and less expensive alternatives have been described, like precipitation with polyethylene glycol (PEG).⁸

The aim of this study was to evaluate PEG precipitation method in routine detection of macroprolactin in a central hospital.

Materials and methods

Starting in August 2012, a 4-month prospective study was performed in our hospital, which consisted on collecting serum samples with high PRL levels (PRL $\geq$ 30 ng/ml; reference range: ♀ 4.79–23.3 ng/ml and ♂ 4.04–15.2 ng/ml) from individuals independently of their sex, diagnosis or treatment till then. Twenty-five

grams of PEG 6000 (Merck® ref. 807491) was dissolved in 60 ml of distilled water at room temperature (18–25 °C) and mixed at vortex, with volume fulfilled till 100 ml of solution. Two hundred and fifty microliters of that 25% PEG solution were added at room temperature (20–25 °C) to equal volume of patients' sera. After thorough vortex mixing and 30 min stabilization, the solution was centrifuged at 9500 $\times$ g for 10 min. Initial-PRL (pre-PEG) and supernatant-PRL (post-PEG) were detected by electrochemiluminescence – Cobas e170®. PRL recovery rate (RR) was determined by the ratio: supernatant-PRL/initial-PRL $\times$ 100, after correction of post-PEG PRL result for PEG dilution factor of 2. Samples were classified as having a predominant macroprolactin form if RR of PRL was <40%. RR > 65% indicated monomeric PRL predominance and RR 40–65% was classified as indeterminate. Positive control for macroprolactin (RR 37%) was obtained by UK NEQAS® – the United Kingdom National External Quality Assurance Scheme, sample G970.

Results

Among a total of 678 samples of PRL analyzed in our laboratory over 4 months of study, 96 consecutive samples of PRL $\geq$ 30 ng/ml were identified (14.2%), with median initial-PRL 56.1 ng/ml (30.7–3667) and median post-PEG PRL 43.1 ng/ml (2–2966).

Two cases of macroprolactin predominance (2.1%) were detected in this study, with RR of 4.9% (pre-PEG/post-PEG prolactin 40.8/2.0 ng/ml, respectively) and RR of 16.1% (pre-PEG/post-PEG prolactin 57.4/9.26 ng/ml, respectively). Two cases were indeterminate, with RR of 45.1% (pre-PEG/post-PEG prolactin 37.96/17.12 ng/ml, respectively) and RR of 63.7% (pre-PEG/post-PEG prolactin 33.2/21.16 ng/ml, respectively). The remaining 92 cases showed RR between 69.1% and 93.5%.

After initial results, a second analysis was performed in two patients who were able to cooperate (one case of positive result and one case indeterminate). Second/first RR were similar: 15.6/16.1% and 41.6/45.1%, respectively).

Treatment with PEG produced a decrease in PRL values in all sera, with mean reduction of 22% (or 20% considering only the 92 cases of monomeric PRL predominance).

Macroprolactin positive cases are now being reviewed by their endocrinologists and a brief description is made as follows:

Case 1

Female, aged 40 years, sent to the endocrinology department because of mild galactorrhea and persistent hyperprolactinemia (PRL 142 ng/ml, reference range 4.79–23.3), but with regular menses and no infertility background. Sellar magnetic resonance imaging (MRI) showed an irregular area of 4 mm, with contrast enhancement, probably a pituitary microadenoma, according to previous clinical information of hyperprolactinemia. She started on dopamine agonist (DA) treatment with prolactinemia reduction but it never normalized (PRL 40.6 ng/ml under 5 mg of bromocriptine). After PEG precipitation, final PRL was 2 ng/ml (RR 4.9%).

Case 2

Female, aged 38 years, being followed in the endocrinology department for the last 18 years because of obesity (BMI 30.3 kg/m²) and irregular menses. She had no galactorrhea. PRL was 155.2 ng/ml (reference range 4.79–23.3) but no abnormal lesion was seen in the pituitary imaging study. She started on bromocriptine with PRL normalization but oligomenorrhea persisted and hyperprolactinemia recurred after DA withdrawal (PRL 57.4 ng/ml). No significant weight reduction was seen during follow-up time. After PEG precipitation, final PRL was 9.3 ng/ml (RR 16.1%).

The case of indeterminate result that was confirmed as inconclusive in this study referred to a 32-year-old female patient observed in the endocrinology department because of mild hyperprolactinemia, confirmed in subsequent analysis (PRL 37.93 ng/ml). She had oligomenorrhea but no galactorrhea and no abnormal images in cerebral computerized tomography (CT). She was diagnosed with autoimmune thyroiditis with subclinical hypothyroidism and is now medicated with levothyroxine 75 µg/day but in the last year she had some irregular menses, PRL 37.98 ng/ml and borderline TSH results (TSH 3.9–6.2 µUI/ml, reference range 0.27–4.2 µUI/ml).

Discussion and conclusions

Macroprolactin is a high-molecular weight molecule that was first described in 1981 by Wittaker et al.⁹ It is a PRL-IgG immunocomplex in which endogenous IgG molecule is directed against epitopes on N- and C-terminal residues of monomeric PRL.¹⁰

The likelihood that current immunoassays will detect macroprolactin depends on the previous binding of PRL to endogenous immunoglobulin and the availability of PRL epitopes to bind the assays' antibody. Whenever the assay's antibody binds an available epitope in PRL molecule, it is accounted as monomeric PRL and contributes to total PRL levels. That is what determines the immunoassay's sensitivity to differentiate monomeric PRL from macroprolactin.

Treatment of sera with high concentrations of PEG may overcome this problem. PEG precipitates out high-molecular weight compounds including immunoglobulin. As it can precipitate macroprolactin, leaving reduced levels in the supernatant, it has been applied to assess cases of elevated PRL due to macroprolactin predominance. This method is cheap, reproducible and easily performed and it seems the one that most tightly correlates with concentrations measured by gel filtration chromatography (GFC), the gold standard for macroprolactin assessment.^{4,11} GFC is not feasible to use in routine laboratory practice as it is too expensive and technically demanding.⁴

Monomeric PRL may also precipitate during technical processing with PEG, reducing post-PEG PRL values in all serum samples.⁴ This lack of specificity, responsible for 5–44% reduction in final PRL even in normoprolactinemic sera,¹² was also demonstrated in the present study, with mean 20% reduction in all 92 cases of monomeric PRL predominance. To minimize this gap, new reference intervals for PRL could be obtained after PEG precipitation of PRL in normoprolactinemic individuals' sera¹² or new calibration curves could be obtained after treating assay calibrators by the same PEG procedure.¹³

In the present study, we used the classic approach of macroprolactin assessment by PEG precipitation, establishing a particular cut-off for RR of PRL.¹⁴ Some authors have taken into account the macroprolactin-specific RR that was achieved after PEG precipitation of positive macroprolactin samples in GFC.^{15,16} Others use RR previously validated, like in the present study. Most frequently, results of RR <30%¹⁴ or RR <40%^{5,17,18} have been published. In this

study, RR <40% was considered positive for macroprolactinemia, as it seemed the more reliable and agreed criterion. More variety has been described in literature in what concerns to the interval for indeterminate presence of macroprolactin, although the upper limit of that interval seems frequently an arbitrary option. Consensually, indeterminate results would imply GFC confirmation.

Some situations of hyperprolactinemia can represent simultaneous macroprolactinemia and elevated pituitary PRL secretion (because final PRL result, even if macroprolactinemia is present, may still be above the upper limit of normal).¹⁹ These cases imply the same clinical approach than isolated true hyperprolactinemia, in order to diagnose organic lesion responsible for it.

The incidence of macroprolactinemia depends on the nature of the study centre, as some receive samples of elevated prolactinemia for macroprolactin confirmation. In the present study, macroprolactinemia was present in a lower rate than usually reported (2.1% vs 10–46%).^{5,11,18,20–22} The fact that all hyperprolactinemic samples were collected independently of DA treatment or the presence of pituitary macroadenomas may have contributed to it.

Bioactivity of macroprolactin molecule has been questioned but it is still a confusing area of discussion. Its high molecular mass confines it to the intravascular space probably limiting its bioavailability and biological activity but intermittent dissociation of macroprolactin from IgG molecule has been suggested.²³ Although macroprolactinemic patients may have symptoms similar to those with true hyperprolactinemia, as symptoms are themselves the motive for serum PRL evaluation, they are sometimes nonspecific and are also seen in general population. Galactorrhea, irregular menses, headache or infertility have been identified in macroprolactinemic and true hyperprolactinemic subjects but seems significantly less common in the former group.^{5,12,24} Clinical features alone may not reliably distinguish macroprolactinemic patients from patients with true hyperprolactinemia.

Sellar imaging studies are frequently performed in patients with retrospectively identified macroprolactinemia. Pituitary microadenomas have been described in this population but its true clinical relevance is unclear, as pituitary incidentalomas are also found in about 10–20% of the general population at autopsy.^{2,5,12}

The beneficial effect of DA in macroprolactinemic patients has been described and seems more evident for patients with associated galactorrhea^{12,25}; although spontaneous improvement of symptoms may also occur in those patients.²⁴

In conclusion, macroprolactinemia is a misdiagnosed cause of hyperprolactinemia. Its identification may lead clinicians to reconsider the likelihood of requesting sophisticated and expensive imaging studies, even in apparently symptomatic patients.

Applying PEG precipitation to hyperprolactinemic sera is an easy and reliable method of screening for macroprolactin that we validated in our institution.

In the present study, we validated this technique and found two cases of macroprolactinemia predominance, with RR of 4.9% and 16.1% and two cases of indeterminate results, needing GFC confirmation.

We understand that screening for macroprolactinemia may be very important in daily clinical practice, especially in clarifying persistent hyperprolactinemia in apparently healthy individuals. Being so, in our hospital we are now able to measure macroprolactin by PEG precipitation whenever appropriate, preferentially before any investigation or pharmacologic therapy is initiated.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work centre on the publication of patient data and that all the patients included in the study received sufficient information and gave their written informed consent to participate in the study.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors have no conflicts of interest to declare.

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