# Macroprolactinemia in women with hyperprolactinemia: a 10-year follow-up

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*Submitted: 2012-10-12 Accepted: 2012-11-03 Published online: 2013-05-15*

*Key words:* **hyperprolactinemia; macroprolactin; amenorrhea; prolactinoma; polyethylene glycols**

Neuroendocrinol Lett 2013; **34**(3):207–211 **PMID:** 23685418 NEL340313A02 

## Abstract

**OBJECTIVES:** To determine the frequency of macroprolactinemia in a cohort of hyperprolactinemic women, describing 1) the association of macroprolactinemia with clinical variables and morphological changes in the pituitary gland and 2) clinical status and prolactin levels after 10 years of follow-up.

**DESIGN:** Blood samples were obtained from 32 patients for hormonal assessment. Treatment with cabergoline or bromocriptine was interrupted 3 months before the determination of serum prolactin and macroprolactin. Macroprolactin was measured using the polyethylene glycol (PEG) precipitation method. Computed tomography was performed in all patients.

**RESULTS:** The frequency of macroprolactinemia was 28.1%. In 19 patients prolactin remained elevated (persistent hyperprolactinemia). In 13, prolactin returned to normal (former hyperprolactinemia). Nine patients with PEG recovery between 40 and 50%, and the only two macroprolactinemic patients with previous hyperprolactinemia were excluded from the analysis of clinical outcomes. Only one of seven macroprolactinemic patients had an abnormal pituitary image (empty sella). None had galactorrhea. Main findings: Classic symptoms of hyperprolactinemia and abnormal imaging findings are not common in patients in whom macroprolactin is the predominant form of PRL.

**CONCLUSIONS:** Women with hyperprolactinemia, especially if asymptomatic, should be routinely screened for macroprolactinemia. Macroprolactinemia remains stable in the long term.

**Abbreviations:**

| | | | |
|---|---|---|---|
| BMI | Body mass index | LH | Luteinizing hormone |
| CT | Computed tomography | PEG | Polyethylene glycol |
| CV | Coefficient of variation | PRL | Prolactin |
| E2 | Estradiol | SD | Standard deviation |
| FSH | Follicle-stimulating hormone | SPSS | Statistical Package for the Social Sciences |
| Hprl | Hyperprolactinemia | T4 | Thyroxine |
| IRMA | Immunoradiometric assay | TSH | Thyroid-stimulating hormone |



ORIGINAL ARTICLE

## INTRODUCTION

Prolactin (PRL), a polypeptide hormone synthesized by lactotrophs in the anterior pituitary gland (Freeman *et al.* 2000), is best known for its role in inducing and maintaining lactation (Josimovich *et al.* 1987). Altered levels of circulating PRL are associated with menstrual irregularity, galactorrhea, and subfertility in women. Elevated serum prolactin in the absence of pregnancy, known as pathological hyperprolactinemia, is among the most common hypothalamo-pituitary disorders in women of reproductive age (Bronstein 2010).

Causes of hyperprolactinemia include pituitary adenomas (prolactinomas), use of drugs affecting the hypothalamic dopamine system and/or pituitary dopamine receptors, or the predominance of high molecular mass (>150 kDa) PRL isoforms over other circulating forms of PRL (Hauache *et al.* 2002). This latter condition, known as macroprolactinemia, is usually suspected if high PRL levels are found in the absence of classic hyperprolactinemia syndrome features (Gibney *et al.* 2005b).

Previous studies have shown that screening of macroprolactinemia (which accounts for an estimated 25% of biochemical hyperprolactinemia) may prevent patients from undergoing unnecessary imaging of the hypothalamus-pituitary region and also unnecessary treatment for hyperprolactinemia (Fahie-Wilson 2000; Leslie *et al.* 2001; Gibney *et al.* 2005a; Glezer & Bronstein 2012).

Therefore, the objectives of the present study were: 1) to determine the frequency of macroprolactinemia in a cohort of hyperprolactinemic women; 2) to describe the association of macroprolactinemia with clinical and hormonal variables and morphological changes in the anterior pituitary gland; and 3) to describe the clinical status and PRL levels of hyperprolactinemic patients after 10 years of follow-up.

## MATERIALS AND METHODS

### *Patients*

The population consisted of 32 women who were followed-up at the Gynecological Endocrinology Unit at the Hospital de Clínicas de Porto Alegre. All the patients had a diagnosis of hyperprolactinemia (>26ng/mL on at least two occasions). Exclusion criteria were hypothyroidism, hyperprolactinemia secondary to drugs, and pregnancy.

The study protocol was approved by the local Ethics Committee of Hospital de Clinicas de Porto Alegre (University Hospital) and informed consent was obtained from all subjects.

### *Study protocol*

The protocol included investigation of age, menstrual cycle, fertility, pregnancies, headache and galactorrhea. Anthropometric measurements included body weight and height and body mass index (BMI) (current weight in kg divided by height in $m^2$).

Blood samples were drawn at the end of follow-up for determination of 17β estradiol (E2), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroxine (T4) and PRL. Treatment with dopamine agonists cabergoline or bromocriptine was interrupted for 3 months (washout period) for determination of serum prolactin and macroprolactin levels. All the blood samples were obtained after the 3-month washout period at the beginning of the follicular phase (between the first and tenth days of the menstrual cycle) in normally cycling women, or on any day in amenorrheic patients.

### *Assays*

PRL levels were measured using an immunoradiometric assay (IRMA) (Adaltis Italia SpA, Italy) with sensitivity of 0.3 ng/mL and inter- and intra-assay coefficient of variation (CV) of 6% and 3.2%, respectively. For precipitation of macroprolactin, samples were treated with a 12.5% polyethylene glycol (PEG) /saline solution (250 µL of serum were mixed with 250 g/L PEG 6000 solution in 0.9% NaCL, kept at 4 °C), mixed for 1 min with a vortex mixer, and centrifuged for 5 min at 8,000 rpm. Monomeric PRL was determined in the supernatant, using IRMA.

Macroprolactin was determined by comparing the amount of total PRL obtained by IRMA with the amount of monomeric PRL recovered after PEG precipitation. Recovery of monomeric prolactin >50% (Olukoga & Kane 1999; Hauache *et al.* 2002) indicated predominance of the monomer isoform, whereas a recovery rate ≤40% (Vieira *et al.* 1998; Olukoga & Kane 1999) indicated predominance of big-big prolactin (macroprolactinemia). A recovery rate between 40 and 50% was considered as indeterminate (Vieira *et al.* 1998).

### *Computed tomography*

Computed tomography (CT) was performed in all patients at the beginning and at the end of the follow-up period.

### *Statistical analysis*

The results were expressed as median and interquartile range for variables with a non-Gaussian distribution, or mean and standard deviation (SD) for Gaussian variables. Clinical features were analyzed using the chi-square test for categorical variables and the Kruskal-Wallis H test for continuous variables. A $p \leq 0.05$ was considered to be significant. All the analyses were performed with the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL).

## RESULTS

Macroprolactinemia was detected in 9 of our 32 patients (frequency: 28.1%) using the PEG method. Mean age at baseline was 31.91±6.91 years (40.38±8.63 years at the end of the study), and mean BMI was 26.1±4.45.

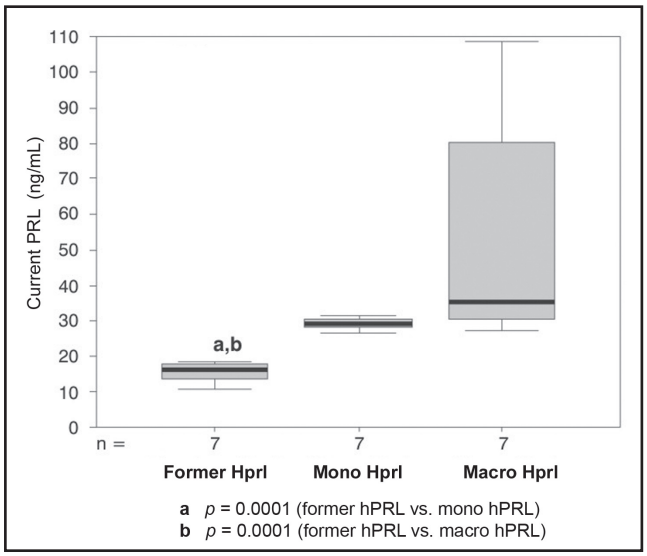


**Fig. 1.** Serum PRL concentration (ng/mL) in hyperprolactinemic women.

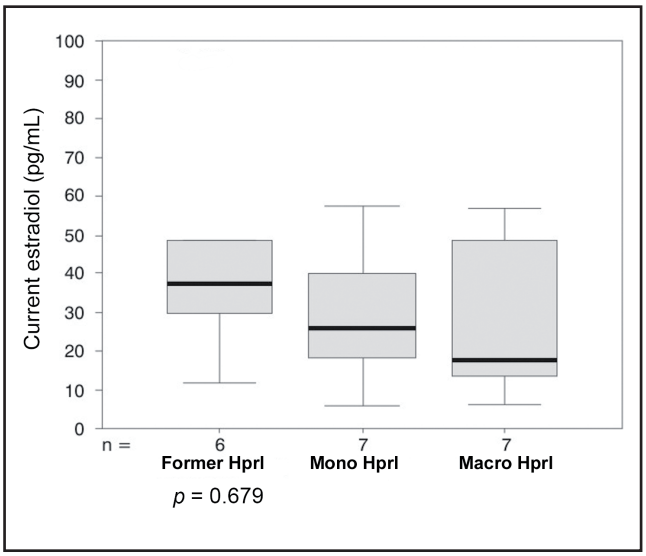


**Fig. 2.** Serum estradiol concentration (pg/mL) in hyperprolactinemic women.

**Tab. 1.** Clinical, hormonal and pituitary CT results in patients with former hyperprolactinemia.

| Variable | Patient no. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5** | 6 | 7 |
| Age | 41 | 45 | 22 | 44 | 52 | 50 | 40 |
| Cycle | Regular | Regular | Regular | Regular | Amenorrhea | Regular | Regular |
| Pregnancies/Parity | 2/2 | 0 | 0 | 4/4 | 0 | 2/2 | 1/0 |
| Headache | No | No | Yes | No | Yes | No | No |
| Galactorrhea | No | No | No | No | No | No | No |
| Pituitary CT | 5 mm§ | Normal | 8 mm§ | Rathke's cyst | Normal | Normal | Normal |
| PRL (ng/mL) | 16.44 | 15.84 | 24.42 | 18.52 | 16.06 | 11.05 | 10.85 |
| E2 (pg/mL) | 11.93 | 48.44 | 33.59 | 29.7 | – | 132 | 40.84 |
| TSH (μUI/mL) | – | 3.14 | 1.54 | 0.97 | 2.08 | 5.23 | 2.99 |
| T4 (μg/dL) | – | 11 | 6.5 | 4.45 | 6.87 | 9.26 | 6.20 |
| FSH (μUI/mL) | 9.10 | 6 | 3.3 | 17.2 | – | 3.8 | 9.47 |
| LH (μUI/mL) | 3.10 | 1.3 | 8.29 | 0.70 | – | 1.4 | 5.35 |

**Menopausal patient; § Microprolactinoma.

For analysis of clinical outcomes, patients were initially divided into two groups according to PRL level: 19 patients with consistently elevated serum PRL levels were included in the persistent hyperprolactinemia (Hprl) group, and 13 patients whose PRL levels had returned to normal at the end of the study were included in the former Hprl group. Nine patients with PRL recovery between 40 and 50% (5 in the persistent Hprl group and 4 in the former Hprl group) were excluded from this analysis, as well as the only two macroprolactinemic patients in the former Hprl group.

For the 21 patients remaining in the study, mean age at baseline was 33.7±6.7 years, and mean BMI was 25.9±4.9 kg/m². Median follow-up was 10.7 (3.4–14.2) years. During the study period, four women entered menopause.

The final group of 21 women was further divided into three groups of 7 patients each: former Hprl, mono Hprl (monomeric PRL recovery >50%), and macro Hrpl (macroprolactinemia). Figure 1 shows PRL levels in the three groups. As expected, the former Hprl group had significantly lower PRL levels than the two other groups ($p$=0.0001). Figure 2 shows that the groups had similar estradiol levels ($p$=0.679). TSH, FSH and LH were also similar in all the groups. The four postmenopausal patients were not included in this analysis.

Table 1 shows the results for women in the former Hprl group. In this group, four patients became preg-

nant and one had a spontaneous abortion. Of seven patients, six had regular cycles. Patient no. 5 was considered to be menopausal based on age (52 years) and amenorrhea for over 1 year (E2, FSH and LH measurements were not available). None of the women in this group had galactorrhea. CT revealed Rahtke's cyst in one woman and microadenomas in two.

Of the seven patients included in the mono Hprl group (Table 2), four were amenorrheic (three menopausal) and one had irregular menses. Of the menopausal patients, two (no. 10 and 13) had elevated serum levels of FSH and LH. FSH and LH measurements were not available for patient no. 9. This 57-year old, amenorrheic patient was classified as menopausal. Two women did not have children. Patients no. 11 and 13 had one and four abortions, respectively. Two patients had galactorrhea. CT revealed a microadenoma in one patient.

Table 3 shows the results concerning the seven patients in the macro Hprl group. Two women were amenorrheic, one of which (patient 17) presenting postoperative panhypopituitarism. Patient 19 is currently undergoing investigation of amenorrhea, since she has proven fertility and a normal CT. In one patient with irregular cycles (no. 21) and idiopathic hirsutism, the dosage of spironolactone (not related to hyperprolactinemia) is currently being adjusted. Five patients did not have children. None had galactorrhea. CT revealed empty sella in one patient.

**Tab. 2.** Clinical, hormonal and pituitary CT results in patients with mono hyperprolactinemia.

| Variable | Patient no. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 8 | 9** | 10** | 11 | 12 | 13** | 14 |
| Age | 52 | 57 | 48 | 45 | 41 | 51 | 44 |
| Cycle | Amenorrhea | Amenorrhea | Amenorrhea | Regular | Regular | Amenorrhea | Irregular |
| Pregnancies/Parity | 0 | 2/3 | 0 | 4/3 | 2/2 | 7/3 | 1/1 |
| Headache | Yes | No | Yes | Yes | Yes | Yes | Yes |
| Galactorrhea | No | No | No | Yes | Yes | No | No |
| Pituitary CT | Normal | Normal | 5 mm§ | Normal | Normal | Normal | Normal |
| PRL (ng/mL) | 28.52 | 26.46 | 30.29 | 31.42 | 30.04 | 27.66 | 29.2 |
| E2 (pg/mL) | 57.64 | 11.93 | 60.7 | 46.5 | 33.85 | 25.95 | 24.56 |
| TSH (µUI/mL) | 0.56 | 1.62 | 1.49 | 3.89 | 0.75 | 2.72 | 1.42 |
| T4 (µg/dL) | 6.64 | – | 5.58 | 6.51 | 10 | 8.28 | 8.20 |
| FSH (µUI/mL) | 17.1 | – | 97.9 | 4.28 | 6.89 | 42.7 | 13.5 |
| LH (µUI/mL) | 6.01 | – | 19.9 | 2.63 | 0.70 | 11.4 | 5.82 |

**Menopausal patient; § Microprolactinoma.

**Tab. 3.** Clinical, hormonal and pituitary CT results in patients with macroprolactinemia.

| Variable | Patient no. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| Age | 39 | 48 | 39 | 34 | 30 | 30 | 45 |
| Cycle | Regular | Regular | Amenorrhea | Regular | Amenorrhea | Regular | Irregular |
| Pregnancies/Parity | 0 | 0 | 0 | 0 | 1/1 | 0 | 2/2 |
| Headache | No | Yes | No | No | Yes | Yes | No |
| Galactorrhea | No | No | No | No | No | No | No |
| Pituitary CT | Normal | Normal | Normal | Normal | Normal | Normal | Empty sella |
| PRL (ng/mL) | 30.95 | 160.71 | 27.05 | 52.11 | 108.95 | 29.54 | 35.35 |
| E2 (pg/mL) | 40 | 14.11 | 6.39 | 155 | 17.63 | 12.93 | 56.63 |
| TSH (µUI/mL) | 2.2 | 1.13 | 1.22 | 7.16 | 2.38 | 1.3 | 1.12 |
| T4 (µg/dL) | 9.4 | 9.4 | 8.47 | – | – | 13.28 | 8.94 |
| FSH (µUI/mL) | 10.8 | 6.99 | 0.44 | 6.76 | 4.5 | 11.6 | 1.77 |
| LH (µUI/mL) | 4 | 1.01 | 0.14 | 1 | 0.72 | 5.6 | 1.24 |

## DISCUSSION

The present report describes the long-term follow up of 32 hyperprolactinemic women. Since macroprolactinemia affects mostly women (Vallette-Kasic *et al.* 2002), we expected that the study of a strictly female cohort would reveal important information concerning this disorder.

As described in the literature (Vallette-Kasic *et al.* 2002; Gibney *et al.* 2005b; Hattori *et al.* 2010), patients in the previous Hprl group had significantly lower PRL levels than those observed in the other two groups. Also, a clear improvement in the regularity of menstrual cycles was recorded along the follow-up period in this group, a finding that is consistent with the normalization of PRL levels that occurred in the same period. Concerning the mono Hprl group, most continued to present irregular cycles or amenorrhea, even at the end of 10 years, confirming previous observations (Suliman *et al.* 2003; Hattori *et al.* 2010).

The frequency of macroprolactinemia encountered by us (28.1%) falls within the 15–46% range reported in the literature (Fahie-Wilson & Soule 1997; Olukoga & Kane 1999; Leslie *et al.* 2001; Vallette-Kasic *et al.* 2002; Glezer & Bronstein 2012). Our macroprolactinemic patients were also similar to other populations in terms of clinical heterogeneity (presence of symptoms that could be associated with menstrual disturbances not related to hyperprolactinemia) (Vallette-Kasic *et al.* 2002) and frequency of pituitary changes on CT (14,2% vs. 7 to 22% in the literature) (Olukoga & Kane 1999; Vallette-Kasic *et al.* 2002; Suliman *et al.* 2003). These findings support the notion that patients with hyperprolactinemia due to high levels of macroprolactin do not require specific treatment for this condition.

Screening of hyperprolactinemia is indicated in the presence of altered menses, amenorrhea, infertility and galactorrhea in women. In addition, determining PRL levels is also essential in the presence of hypothalamic-pituitary tumors (Gibney *et al.* 2005b). Previous studies, as well as the present results, indicate that macroprolactinemia screening would be useful to ensure that patients receive the most adequate treatment, especially taking into consideration that the most employed screening method, PEG precipitation, has proven simplicity, good reproducibility and correlation with the gold standard gel chromatography (Vieira *et al.* 1998). In the present study, PEG precipitation revealed the predominant form of circulating PRL in 71.8% of the cases (n=32).

In conclusion, this long term follow-up of hyperprolactinemic women showed that the classic symptoms of hyperprolactinemia are not common in patients in whom macroprolactin is the predominant form of PRL, and that macroprolactinemia remains stable in the long term. This suggests that women with hyperprolactinemia, especially if asymptomatic, should be screened for macroprolactinemia.

## ACKNOWLEDGMENTS

This work was partially supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq INCT 573747/2008-3). We thank Raphaella Migliavacca, Igor G Benedetto, and Fabiane Tiskievicz for their support with data collection.

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