0021-972X/01/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 86, No. 6
Printed in U.S.A.

Laboratory and Clinical Experience in 55 Patients with Macroprolactinemia Identified by a Simple Polyethylene Glycol Precipitation Method

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ABSTRACT

PRL exists in different forms in human serum. The predominant form is little PRL (molecular mass 23 kDa) with smaller amounts of big PRL (molecular mass 50–60 kDa) and at times big big or macroprolactin (molecular mass 150–170 kDa). The frequency and clinical consequences of macroprolactinemia have not been clearly established, mainly because of difficulty in identifying these patients biochemically. This previously required the use of gel filtration chromatography, which could not be used routinely. Recently, a screening test using polyethylene glycol (PEG) has been used to identify macroprolactin in serum. Consequently, this study was designed to examine the use of PEG precipitation in the identification of patients with a predominance of macroprolactin and to establish the clinical characteristics of such a cohort.

Over 12 months, 18,258 requests for serum PRL were received and of these 1225 patients had a serum PRL more than 700 mU/L. A total of 322 of these patients (26%) had a percentage recovery after PEG precipitation of less than 40%, thus indicating the presence of a predominance of macroprolactin.

Fifty-five of these patients were referred for detailed clinical assessment. Symptoms typical of hyperprolactinemia were not common in this cohort. None had sustained amenorrhea and eight have had oligomenorrhea at age less than 40 yr. One had galactorrhea. All had pituitary imaging, and four had a microadenoma with none having a macroadenoma.

PEG precipitation allows easy identification of macroprolactin in routine clinical practice. As the clinical consequences of this entity at this stage seem relatively benign, referral and intensive investigation of these patients may not be necessary. However, follow-up of a large cohort is required to ensure that the long-term outlook is likewise benign. This would have important implications for both patients and healthcare systems. (*J Clin Endocrinol Metab* **86:** 2743–2746, 2001)

SYNDROMES ASSOCIATED WITH hyperprolactinemia have been identified over the last 20–30 yr. Hyperprolactinemia occurs mainly because of pituitary or hypothalamic disease but can also occur secondary to hypothyroidism and drug medication. In human serum, three main species of PRL have been identified by gel filtration chromatography. These are monomeric PRL (molecular mass 23 kDa), big PRL (molecular mass 50–60 kDa), and big big PRL or macroprolactin (molecular mass 150–170 kDa) (1). In most normal individuals and in the majority of patients with hyperprolactinemia, monomeric PRL (molecular mass 23 kDa) is the major circulating form. However, it has been known for many years that in some patients with hyperprolactinemia, macroprolactin or big big PRL predominates.

The structure of macroprolactin has yet to be fully defined with various molecular forms having been described. Earlier studies reported an oligomeric form of little PRL whereas more recent work described macroprolactin as a complex of monomeric PRL with an IgG antibody (antiprolactin autoantibody) (2–5). Hattori (6) in 1996 identified a pregnant woman whose macroprolactin was a heterogeneous complex of covalently and noncovalently bound PRL with increased glycosylation. These findings suggest that differing etiologies may be involved in the formation of macroprolactin.

The frequency and clinical consequences of macroprolactinemia have not been clearly established, mainly because of difficulty in identifying these patients biochemically. This has required the use of gel filtration chromatography, which is a labor-intensive, expensive technique. Consequently, laboratories have not differentiated routinely between the different forms of PRL. This has meant that published experience of this condition consists of case reports and of small groups of patients (up to n = 17) (7–13).

Recently, a screening test using polyethylene glycol (PEG), a chemical used for many years in RIA to precipitate large molecular mass proteins, has been used to identify the presence of macroprolactin in serum. Macroprolactin, if present in serum, is precipitated by PEG leaving reduced levels in the supernatant (7, 13, 14). This is a simple inexpensive test that can easily be integrated into laboratory practice. We have used this methodology routinely and have been able to identify a large series of patients with this condition. In this report we identify the clinical presentations and radiological findings in a cohort of 55 patients.

Materials and Methods

Subjects and methods

Our laboratory receives requests for serum PRL from hospital clinics and general practitioners from all areas of Northern Ireland (population

Received March 2, 2000. Revision received August 24, 2000. Rerevision received January 23, 2001. Accepted January 30, 2001.

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1.5 million). During a 1-yr period all samples with a PRL more than 700 mU/L were submitted to the PEG screening test.

PRL assay

PRL was determined by an immunofluorimetric assay (AutoDELFIA PRL; Wallac, Inc. Oy, Turku, Finland). The normal reference range for unstressed males and females was less than 340 mU/L. The intraassay coefficient of variation (CV) and interassay CV were less than 5% (range, 40–9000 mU/L).

PEG precipitation test

Equal volumes (200 μL) of a 25% solution of PEG (molecular mass 6000 kDa) and patients' sera were mixed and centrifuged at 1500 $\times$ *g* for 30 min. Immunoreactive PRL was measured in the supernatant, and the results after correction for dilution compared with those obtained from unprecipitated serum. The results were expressed as the percent of PRL recovery. Recovery less than or equal to 40% was taken as evidence that a significant level of macroprolactin was present in the serum. Two patient samples, with macroprolactin present, were each submitted to 10 PEG precipitation tests. The within-assay CV for PRL recoveries were 3.7% and 2.7%, respectively.

Clinical assessment

Fifty-five patients identified as having macroprolactinemia were referred to our unit for endocrine assessment. Each had a full clinical assessment that included drug history, menstrual and fertility history, and assessment of free T_4 and TSH levels and visual acuity by Snellen testing. Visual fields were assessed by confrontation. Imaging of the pituitary fossa was performed by computed tomography (CT) scan in the majority and magnetic resonance imaging (MRI) in the remainder. CT was performed by a Somaton Plus-S scanner (Siemens, Erlangen, Germany) with 2-mm incremental slices in the coronal plane following iv contrast. MRI was performed by a GE 1.5 Tesla scanner (GE Medical Systems, Milwaukee, WI), visualizing the pituitary in both sagittal and coronal planes following administration of iv gadolinium.

Results

There were 18,258 requests for serum PRL over a 12-month period (January 1998 to December 1998). Of these, 1225 had serum PRL more than 700 mU/L. Figure 1 shows the percentage of recovery after PEG precipitation. In the 1225 patients the recovery was less than 40% in 322 patients (26%). Figure 2 shows the serum PRL levels in these 322 patients identified as having macroprolactinemia. Serum PRL levels ranged from 700–21,000 mU/L. Fifty-five of these patients were referred to our unit for detailed endocrine assessment. All were females with median age 41 yr (range, 18–55 yr) and had levels of PRL similar to that of the whole group with macroprolactinemia.

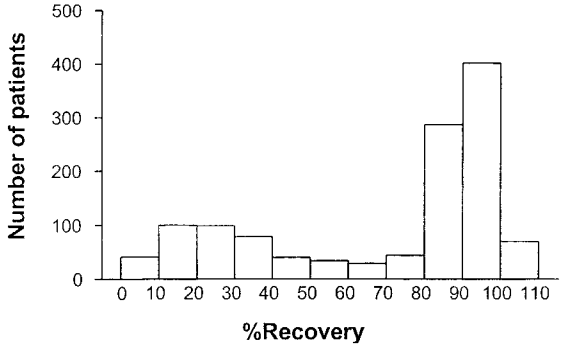

FIG. 1. The percentage of recovery after PEG precipitation in 1225 patients with PRL more than 700 mU/L.

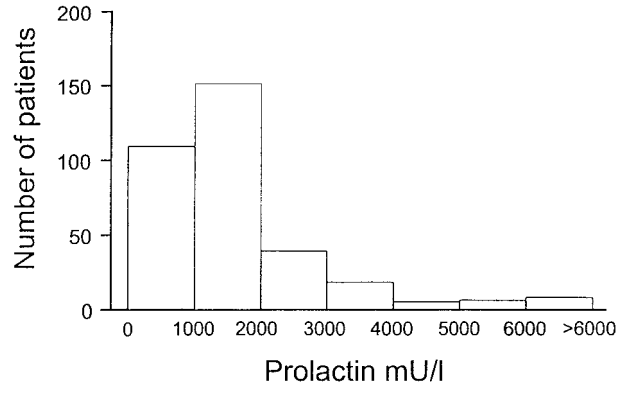

FIG. 2. Serum PRL in 322 patients with a predominance of macroprolactin identified by PEG precipitation.

TABLE 1. The reason stated for the initial request for PRL in 55 patients with macroprolactinemia

Reason test performed	No.
Menopausal symptoms	16
Oligomenorrhea	10
Fatigue	7
Menorrhagia	7
Subfertility	1
Galactorrhea	1
Headaches	1
Others	12

Clinical assessment

The reason for the initial test request is shown in Table 1.

Drug history

Of the 55 patients, 6 were currently or had recently been on a combined oestrogen progestogen contraceptive pill, 3 were on hormone replacement therapy, and 4 were on antidepressants (1 dothiepin, 1 nefazodone, and 2 sertraline).

Bromocriptine- and cabergoline-treated patients

Eleven patients had taken bromocriptine at some stage, whereas one had taken cabergoline. Of these, three patients had taken bromocriptine for headaches, with two reporting improvement. Another of the 11 patients had amenorrhea and had return of periods after 5 months of treatment; she subsequently became pregnant, discontinued her bromocriptine, and has had normal periods in the 3 yr since.

Five patients were prescribed bromocriptine because of oligomenorrhea (defined for this study as a menstrual cycle of between 6 weeks and 6 months); three patients had improvement in period regularity, one having taken bromocriptine for a period of 4 months with periods returning to an irregular cycle (periods lasting 4–5 days every 56–63 days) on stopping bromocriptine, another for a period of 2 yr with periods also returning to an irregular cycle (period 4–5 days every 30–90 days) on stopping therapy, and the other having taken bromocriptine for the past 16 months. One patient with oligomenorrhea has been treated with bromocriptine for 13 yr with no effect on period regularity (remains 4 days every 60 days). The remaining patient with oligomenorrhea was treated with bromocriptine for 6 weeks

and became pregnant and has subsequently had a second pregnancy while not on bromocriptine.

One patient has begun taking bromocriptine for subfertility. Her periods have always been regular, and, despite 10 months of bromocriptine and normalization of her PRL (1160 decreased to 188 mU/L), she has not achieved pregnancy.

One patient was asymptomatic and had been prescribed bromocriptine on the finding of an elevated PRL alone. This has subsequently been discontinued.

One patient had been taking cabergoline for oligomenorrhea and, despite 9 months treatment, had not noted improvement in menstrual cycle.

Menstrual history

Menstrual pattern was regular in 33 patients. Of the remainder, 13 were oligomenorrheic. Five of these had been oligomenorrheic since menarche, with two having evidence of polycystic ovarian syndrome (hirsutism or acne and elevated LH to FSH ratio); there was no other apparent reason for oligomenorrhea in the other three patients.

Two patients developed irregular periods following childbirth. One of these had six periods per year since childbirth 13 yr previously and had continued reduced period frequency despite bromocriptine during this time; the other patient had oligomenorrhea (lasting 7 days every 42 days) for 1 yr after childbirth in 1996, thereafter reverting spontaneously to normal frequency.

Of the six remaining patients who had developed oligomenorrhea, one had proven polycystic ovarian syndrome and one had dysfunctional uterine bleeding. The other four patients had no other apparent cause for their oligomenorrhea.

One of the 55 patients had amenorrhea with return of periods after 5 months of bromocriptine treatment; she subsequently became pregnant and has had normal periods in the 3 yr since.

Of the remaining eight patients who no longer had a regular menstrual cycle, four had normal periods before hysterectomy and four had normal periods until menopause at normal age.

Fertility history

Thirty-nine of the 55 patients had attempted fertility and, of these, 34 had conceived without difficulty, whereas 5 patients had subfertility. Two of these five patients had regular periods at the time of assessment, and of the remaining three, one was postmenopausal and had had regular periods before this, a second had recently developed heavy, irregular bleeding and was found to have dysfunctional uterine bleeding (she also had a stillbirth 10 yr previously but was otherwise infertile), and the third has had irregular periods for 20 yr and despite bromocriptine normalizing period frequency for 2 yr remains infertile. Of the patients with regular periods, one has been taking bromocriptine for 10 months without yet achieving pregnancy despite achieving a normal PRL.

Galactorrhea

One patient had a period of self-limiting galactorrhea 4 yr before assessment. She had irregular periods (lasting 4–5 days every 30–90 days) for over 20 yr and complained of intermittent headaches.

Headache

Six patients complained of headache. Five of these were otherwise asymptomatic; the remaining patient had irregular periods and had had self-limiting galactorrhea. Another of the six patients had a microadenoma on CT scan; she was treated with bromocriptine for 10 yr (1988–1998). Her headaches settled and have not recurred on stopping bromocriptine.

Visual acuity and visual fields

Fifty of the 55 patients had normal visual acuity, and the remaining 5 had unrelated amblyopia of one eye. Visual fields were normal to confrontation in all 55 patients.

Thyroid function

Free T_4 and thyroid-stimulating hormone were normal in 52 patients, 2 had adequately treated primary hypothyroidism, and 1 patient had untreated subclinical hypothyroidism at presentation with a TSH of 28 mU/L and has recently been started on T_4 therapy.

Imaging of the pituitary fossa

All 55 patients had pituitary imaging (52 CT scanning and 3 MRI scanning). These were normal in 51 patients. In four patients, a microadenoma was found (three on CT scan and one on MRI). Of these, one was asymptomatic and one had been amenorrheic for 1 yr with periods restarting after 5 months of bromocriptine treatment. This patient subsequently became pregnant, and her periods remained regular on no treatment for 3 yr. One has had oligomenorrhea for 18 months with periods remaining irregular despite recent introduction of cabergoline and 1 has had less frequent periods (6 per year) since childbirth 13 yr ago with no change of pattern on bromocriptine.

Discussion

The finding of macroprolactin in 26% of 1225 of our patients with PRL more than 700 mU/L confirms the fact that macroprolactinemia is not at all unusual. Bjoro *et al.* (15) reported a similar prevalence of macroprolactin in a series of 605 patients with total PRL more than 1000 mU/L, whereas Fahie-Wilson and Soule (7) demonstrated a similar incidence in a much smaller series of 69 samples with a PRL more than 700 mU/L. Vieira *et al.* (16) found macroprolactin to be prevalent in 36% of 1279 samples with PRL more than 540 mU/L (15), whereas Olukoga and Kane (13) reported an incidence of 15% in 188 patients with serum PRL more than 430 mU/L.

In our study, the presence of macroprolactin was not confirmed by gel filtration chromatography. Most previous studies have demonstrated that recoveries less than 40% reliably identify the presence of substantial amounts of macroprolactin and recoveries more than 50% of the absence of macroprolactin (7, 8, 13). Wider cut-off limits have been suggested with Vieira *et al.* (16) classifying values between 30 and 65% as indeterminate, although these values have since been challenged (17). In our study only a small number of

patients belonged to this category, with 8 of the 55 patients in the cohort having recoveries between 30 and 40%. Most studies identify a gray area with recovery between 40 and 50% necessitating gel filtration chromatography to confirm or refute the presence of macroprolactin. Therefore, in our study, a cut-off recovery of less than 40% should mean that it is unlikely that any patients are misclassified as having macroprolactinemia. This is of obvious importance clinically, and it is also our policy to recommend repeat samples annually on all patients with macroprolactinemia; this is part of an on-going study.

The clinical implication of macroprolactinemia has remained a confusing area for many years, with some reports documenting associated galactorrhea and menstrual disturbances, and others suggesting that patients remain asymptomatic despite marked macroprolactinemia. Previous series have all been small. Leite *et al.* (2) reported on 11 patients with a predominance of macroprolactin in whom 7 had galactorrhea, menstrual disturbance, or both. Olukoga and Kane (13) in a recent paper identified 17 patients with macroprolactinemia in whom a substantial number had symptoms predominantly affecting the menstrual cycle. However, at least 4 of these had an alternative explanation for their symptoms such as polycystic ovarian syndrome or discontinuation of the oral contraceptive pill, and it is not clear how many of the others had other reasons for their symptomatology (13). There are other reports of small numbers of patients who have been either asymptomatic or have demonstrated minimal symptoms (4, 5, 9–11). Lower biological activity of macroprolactin has been suggested as the reason for the lack of symptoms typical of hyperprolactinemia. This has been supported by *in vitro* studies comparing the bioactivity of little PRL with macroprolactin in the NB_2 lymphoma cell bioassay (2, 10). However, some centers have reported similar *in vitro* bioactivity of little and macroprolactin, and this may indicate biological heterogeneity inherent in macroprolactin (18, 19).

We have been able to assess in detail the clinical characteristics of 55 patients with macroprolactinemia, the largest series to date. Classical symptoms of hyperprolactinemia were uncommon. Oligomenorrhea was present in 13 (23.6%) patients, and in 4 of these another condition such as polycystic ovarian syndrome was the possible explanation. One patient had had a 1-yr period of amenorrhea. Six (10.9%) patients had new headache and only one (1.8%) had had galactorrhea. Macroprolactinemia was not associated with a pituitary macroadenoma in any of our cases. The finding of four small microadenomas may or may not be relevant as CT and MRI scanning series of normal individuals frequently demonstrate minor abnormalities (20).

We intend to follow this group on a long-term basis. It is difficult to know whether a trial of bromocriptine is indicated in the patients with oligomenorrhea. If it is given, it should be for a defined length of time and should regular menstruation not occur then it should be withdrawn. We feel that the finding of macroprolactinemia should be reported to gynecologists in such a way that they do not delay other subfertility investigations in patients identified as having this particular form of hyperprolactinemia.

In summary, the use of PEG precipitation allows detection of macroprolactin to be made easily and inexpensively, and we now use it routinely. Using this technique, 26% of patients with serum PRL more than 700 mU/L were shown to have a predominance of macroprolactin. Our initial series suggests that classical symptoms of hyperprolactinemia are uncommon and the outlook for these patients seems benign. If the longer follow-up of a large cohort continues to be without problems then endocrinology referral and intensive investigation may be unnecessary.

References

1. **Smith CR, Norman MR.** 1990 Prolactin and growth hormone: molecular heterogeneity and measurement in serum. Ann Clin Biochem. 27:542–550.
2. **Leite V, Cosby H, Sobrinho LG, Fresnoza MA, Santos MA, Friesen HG.** 1992 Characterisation of big big prolactin in patients with hyperprolactinaemia. Clin Endocrinol (Oxf). 37:365–372.
3. **Cavaco B, Leite V, Santos MA, Arranhado E, Sobrinho LG.** 1995 Some forms of big big prolactin behave as a complex of monomeric prolactin with an immunoglobulin G in patients with macroprolactinemia or prolactinoma. J Clin Endocrinol Metab. 80:2342–2346.
4. **Hattori N, Ishihara T, Ikekubo K, Moridera K, Hino M, Kurahachi H.** 1992 Autoantibody to human prolactin in patients with idiopathic hyperprolactinemia. J Clin Endocrinol Metab. 75:1226–1229.
5. **Bonhoff A, Vuille JC, Gomez F, Gellersen B.** 1995 Identification of macroprolactin in a patient with asymptomatic hyperprolactinemia as a stable PRL-IgG complex. Exp Clin Endocrinol Diabetes. 103:252–255.
6. **Hattori N.** 1996 The frequency of macroprolactinemia in pregnant women and the heterogeneity of its eitiologies. J Clin Endocrinol Metab. 81:586–590.
7. **Fahie-Wilson MN, Soule SG.** 1997 Macroprolactinaemia: contribution to hyperprolactinaemia in a district general hospital and evaluation of a screening test based on precipitation with polyethylene glycol. Ann Clin Biochem. 34:252–258.
8. **Olukoga AO, Kane J.** 1995 Antiprolactin autoantibodies and hyperprolactinaemia. Eur J Endocrinol. 133:463–464.
9. **Whittaker PG, Wilcox T, Lind T.** 1981 Maintained fertility in a patient with hyperprolactinemia due to big big prolactin. J Clin Endocrinol Metab. 53:863–866.
10. **Jackson RD, Wortsman J, Malarkey WB.** 1985 Characterization of a large molecular weight prolactin in women with idiopathic hyperprolactinemia and normal menses. J Clin Endocrinol Metab. 61:258–264.
11. **Fraser IS, Lun ZG, Zhou JP, et al.** 1989 Detailed assessment of big big prolactin in women with hyperprolactinemia and normal ovarian function. J Clin Endocrinol Metab. 69:585–592.
12. **Hattori N, Ikekubo K, Ishihara T, Moridera K, Hino M, Kurahachi H.** 1992 A normal ovulatory woman with hyperprolactinemia: presence of antiprolactin autoantibody and the regulation of prolactin secretion. Acta Endocrinol. 26:497–500.
13. **Olukoga AO, Kane JW.** 1999 Macroprolactinaemia: validation and application of the polyethylene glycol precipitation test and clinical characterization of the condition. Clin Endocrinol. 51:119–126.
14. **Hattori N, Ikekubo K, Ishihara T, Moridera K, Hino M, Kurahachi H.** 1994 Correlation of the antibody titers with serum prolactin levels and their clinical course in patients with anti-prolactin autoantibody. Eur J Endocrinol. 130:438–445.
15. **Bjoro T, Morkrid L, Wergeland R, et al.** 1995 Frequency of hyperprolactinaemia due to large molecular weight prolactin (150–170 kD PRL). Scand J Clin Lab Invest. 55:139–147.
16. **Vieira JGH, Tachibana TT, Obara LH, Maciel RMB.** 1998 Extensive experience and validation of polyethylene glycol precipation as a screening method for macroprolactinemia. Clin Chem. 44:1758–1759.
17. **Fahie-Wilson MN.** 1999 Polyethylene glycol precipitation as a screening method for macroprolactinemia. Clin Chem. 45:436–437.
18. **Hattori N, Inagaki C.** 1997 Anti-prolactin (PRL) autoantibodies cause asymptomatic hyperprolactinemia: bioassay and clearance studies of PRL-immunoglobulin G complex. J Clin Endocrinol Metab. 82:3107–3110.
19. **Whitaker MD, Klee GG, Kao PC, Randall RV, Heser DW.** 1984 Demonstration of biological activity of prolactin molecular weight variants in human sera. J Clin Endocrinol Metab. 58:826–830.
20. **Hall WA, Luciano MG, Doppman JL, Patronas NJ, Oldfield EH.** 1994 Pituitary magnetic resonance imaging in the normal volunteers: occult adenomas in the general population. Ann Intern Med. 120:817–820.