Research Article

Sema Ciftci Dogansen*, Gulsah Yenidunya Yalin and Sema Yarman

Assessment of macroprolactinemia in patients with prolactinoma
Prolaktinoma tanılı hastalarda makroprolaktineminin değerlendirilmesi

DOI 10.1515/tjb-2017-0062
Received October 19, 2016; accepted April 26, 2017; previously published online June 12, 2017

Abstract

Purpose: Macroprolactin, the high-molecular mass prolactin isoform, is considered to be an inactive product with extrapituitary origin. Although macroprolactinemia is considered a benign condition, there is evidence of overlapping clinical features among patients with hyperprolactinemia. Data on the prevalence of macroprolactinemia in prolactinomas is also quite limited. The aim of this study was to assess the prevalence of macroprolactinemia in our patients with prolactinoma.

Methods: The study included patients with macroprolactinoma (n = 50) and microprolactinoma (n = 16). Prolactin level was measured with an electrochemiluminescent immunoassay, and macroprolactinemia was defined as the percentage of prolactin recovery <40% after the polyethylene glycol precipitation.

Results: Macroprolactinemia was not detected in our patients with prolactinoma (the percentage of PRL recovery range; 55%–96%). The mean percentage of prolactin recovery was similar in patients with macroprolactinoma and microprolactinoma (67.7%±8.0% and 70%±9.4%, respectively, p = 0.96).

Conclusion: Macroprolactinemia is generally associated with negative findings on pituitary imaging. Although the monomeric prolactin is dominant, rarely macroprolactin may also be present in prolactinomas. We did not detect presence of macroprolactin in any of the patients and there was no statistically significant difference between micro- and macroprolactinomas in terms of prolactin recovery.

Keywords: Prolactinoma; Macroprolactinemia; Tumoral hyperprolactinemia; Big big prolactin; PEG precipitation method.

Özet

Amaç: Yüksek molekül ağırlıklı makroprolaktin, ekstrapitüiter orjinli inaktif prolaktin izoformudur. Makroprolaktinemi benign bir durum olarak bilinse de bazen hiperprolaktinemi klinik bulguları gözlenebilir. Ancak makroprolaktinemi ve prolaktinoma birlikteliği ile ilgili yayınlar oldukça sınırlıdır. Bu çalışmanın amacı; prolaktinomalarda makroprolaktinemi sıkılığı belirlemektir.

Yöntem: Bu çalışmaya 50 makroprolaktinoma ve 16 mikroprolaktinoma hastası dahil edilmiştir. Prolaktin ölçümü elektrokemilüminesans yöntemiyle yapılmıştır. Prolaktin ölçümü polietilenglikol (PEG) çöktürme yöntemi kullanılarak hesaplanan prolaktin recovery yüzde 40’ın altında olması makroprolaktinemi olarak tanımlanmıştır.

Sonuçlar: Hastalarımızın hiçbirinde makroprolaktinemi tespit edilmemiştir (hesaplanan prolaktin recovery aralığı %55–96%). Makroprolaktinomalardan ve mikroprolaktinomalardan ortalama prolaktin recovery yüzdesi sırasıyla %67.7±8.0 ve %70±9.4, p = 0.96)

Tartışma: Makroprolaktinemilerde genellikle hipofizde herhangi bir lezyon yoktur. Prolaktinomaldarda monomeric prolaktin dominant olmasına rağmen, nadiren makroprolaktin varlığı da olabilir. Biz prolaktinoma tanılı hastalarımızın hiçbirinde PEG çöktürme yöntemiyle makroprolaktinemiye rastlamadık, ayrıca makro- ve mikroprolaktinomalarda prolaktin recovery yüzdesi açısından fark bulunmamıştır (sirasıyla %67.7±8.0 ve %70±9.4, p = 0.96).

*Corresponding author: Sema Ciftci Dogansen, Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Capa, 34090 Istanbul, Turkey, Phone: +90 212 214 20 00/1215, Fax: +90 212 533 56 06, e-mail: sdogansen@gmail.com

Gulsah Yenidunya Yalin and Sema Yarman: Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul, Turkey.
Anahtar kelimeler: Prolaktinoma; Makroprolaktinemi; Tümöral hiperprolaktinemi; Big big prolaktin; PEG çök-türme yöntemi.

Introduction

Human prolactin (PRL) consists of three major circulating forms, termed as monomeric PRL (mPRL), big PRL (bPRL), and “big big” PRL (bbPRL) or macroprolactin (MPRL) [1]. The monomeric form has a molecular weight of 23 kDa and accounts for most of the total PRL immunoreactivity in the serum of both normal subjects and patients with hyperprolactinemia. The bPRL has a molecular weight of 48–56 kDa and accounts for 10%–15% of PRL immunoreactivity. MPRL is defined as PRL with a molecular mass of ≥150 kDa which is a large antigen–antibody complex consisting of monomeric PRL and anti-PRL autoantibodies [1, 2]. MPRL molecules may also occur in different sizes and compositions. These include aggregates of PRL monomers with varying degrees of glycosylation and covalent or noncovalent bonding along with additional isoforms [3]. Normally, MPRL represents a negligibly small percentage of the total PRL amount but MPRL may be predominant in some serum specimens [4, 5]. This phenomenon is called macroprolactinaemia [6]. Current commercial PRL immunoassays may exhibit variable degrees of immunoreactivity with MPRL [7]. The prevalence of macroprolactinemia in general population was reported as 4% while the incidence of macroprolactinaemia in patients with hyperprolactinemia ranges from 10% to 40% when samples from different reference laboratories are assayed [7–11].

Both Current Endocrine Society and Pituitary Society Guidelines recommend MPRL measurement only in patients with asymptomatic hyperprolactinemia [12, 13]. Due to low biological activity and slower clearance rate of MPRL, symptoms related with hyperprolactinemia are not present in patients with macroprolactinemia and most authors do not recommend treatment or long-term follow up. However, recently several studies showed the co-occurrence of macroprolactinemia after systematic screening for macroprolactinemia in patients with prolactinoma [14–18]. Therefore, we aimed to assess the prevalence and clinical significance of macroprolactinemia in our patients with prolactinoma by using the polyethylene glycol (PEG) precipitation method.

Materials and methods

This is a retrospective study. The patients who were being followed up with diagnosis of prolactinoma (n = 66) in the pituitary out-patient clinic of the Istanbul Medical Faculty and who were investigated for presence of macroprolactinemia with MPRL measurements were enrolled in the study. The diagnosis of prolactinoma was confirmed according to the typical clinical signs and symptoms and radiographic signs (pituitary adenoma confirmed by magnetic resonance imaging) and laboratory tests (high PRL levels in at least two different blood samples) [12]. Serum PRL levels were measured with an electrochemiluminescent immunoassay (ECLIA; Elecsys, Modular Analytics, Roche Diagnostics) with reference ranges of 4.04–15.2 ng/mL and 4.7–23.3 ng/mL for adult males and females, respectively. Presence of MPRL was assessed with PEG precipitation of the serum samples according to the procedure which has been previously described by Hattori et al. [19] and extensively validated by Olukago and Kane [20]. The procedure was as follows: 200 μL of serum was added to 200 μL of 25 g/dL PEG 6000 solution, after thorough mixing and centrifugation at 3000 rpm for 30 min, the supernatant was removed for analysis, and PRL assay was performed immediately. PEG precipitates PRL molecules with a molecular weight more than 100 kDa, therefore PRL in the supernatant is considered to be free of MPRL. The results of the precipitation test was compared with those obtained from the unprecipitated serum samples. Percentage of MPRL was calculated using the following formula: MPRL% = (PRL serum – PRL supernatant) × 100/PRL serum. Results of the PEG precipitation test were presented as the percentage of PRL recovery (free PRL) = 100% – MPRL%. Macroprolactinemia was considered present when recovery % was <40% [16, 20–22].

Statistical analyses were performed using SPSS version 21.0. Categorical variables were defined by frequency and percentage rate, and numeric variables with mean ± standard deviation (SD). In dual independent group comparisons, Student’s t-test was used for normally distributed numeric variables and the Mann-Whitney U test was used for non-normally distributed data. Categorical variables were compared using the χ² test. Statistically significant results were defined with presence of p value <0.05.

Results

The mean follow up time of the patients with prolactinoma (43 female/23 male) was 77 ± 61 months (range 6–257 months). The baseline PRL levels ranged between 101 and 10,385 ng/mL. The mean age of diagnosis was 33 ± 1.2 years (range 17–71 years). Primary amenorrhea
(5%), secondary amenorrhea (90%), galactorrhea (83%), headache (35%) and infertility (11%) were symptoms and signs at presentation in female patients. Impotence and decreased libido (95%), infertility (9%) and headache (83%) were symptoms and signs at presentation in male patients. Visual field defects were detected in 15% of all patients. Sixteen patients (24%) had microprolactinoma and 50 patients (76%) had macroprolactinoma. The mean maximum tumor diameter of microprolactinoma and macroprolactinoma were $6.4 \pm 1.1$ mm (range 5–8 mm), and $16.2 \pm 10.7$ mm (range 10–52 mm), respectively. Comparison of PRL levels and the percentage of PRL recovery in micro- and macroprolactinomas are summarized in Table 1. Microprolactinomas were detected more commonly in female patients whereas macroprolactinomas were more common in male patients ($p = 0.03$). The mean calculated percentage of PRL recovery was similar in patients with macroprolactinoma and microprolactinoma ($67.7 \pm 8.0\%$ and $70\% \pm 9.4\%$, respectively, $p = 0.96$). Macroprolactinemia was not detected in any of these patients.

## Discussion

Macroprolactinemia is generally associated with negative findings on pituitary imaging and clinical presentation or bioactivity of MPRL are still debated [23]. Some studies have determined that serum samples containing high molecular mass PRL may exhibit lower, higher or similar biological activity compared with serum samples containing mPRL and may sometimes be associated with signs and symptoms of hyperprolactinemia [3, 24]. In the literature, a small proportion of patients with macroprolactinemia have symptoms of galactorrhea (20%) or oligo/amenorrhea (45%), and MPRL is associated with pituitary adenomas only in 20%–27% [23, 25–27]. Furthermore, although the mPRL is dominant, MPRL may also be present in prolactinomas [14–16, 28]. In the current literature dominance of MPRL was firstly described by Rogol and Rosen [28] in their study with prolactinoma patients. Then, Ohnami et al. [14] showed presence of tumor originated bbPRL in patients with PRL-secreting pituitary adenomas. In subsequent studies on this subject, generally the presence of prolactinoma have been evaluated in patients with macroprolactinoma. In a series of 106 patients with macroprolactinemia studied by Vallette-Kasic et al. [9], prolactinoma has been identified in only four patients and it was proven immunohistochemically.

There are several methods for detection of MPRL such as gel filtration chromatography (GFC), PEG precipitation method, ultrafiltration method, immunoadsorption with protein G-sepharose, protein A-sepharose or anti-hIgG-agarose [11, 29, 30]. The three forms of PRL are identified by GFC of serum or extracts of normal pituitary gland and pituitary tumors. MPRL identification by GFC is gold standart method but it is difficult, time-consuming, expensive, and not currently used in practice [8, 31]. Whereas, PEG-precipitation test is a simple and inexpensive method that can easily be integrated into laboratory practice and this test is proposed by Current Endocrine Society Guideline [12]. Furthermore one current study has reported high sensitivity (up to 100%), but suboptimal specificity levels with this method [31]. PEG precipitation method is also known as the method revealing the most consistent results with GFC measurements [11, 29, 30]. However, anti-PRL antibody should be present in circulation in order to use this method. Mounier et al. [15] showed that none of the patients with prolactinoma had autoantibodies by the PEG precipitation method, including patients who had predominantly bbPRL in serum analysis performed with the GFC method. Despite the fact that they have demonstrated the existence of bbPRL in tumor extracts by the GFC method. Similarly, we did not detect macroprolactinemia in any of our patients with the

### Table 1: The comparison of macro- and microprolactinomas.

|                          | Macroprolactinoma ($n=50$) | Microprolactinoma ($n=16$) | p-Value |
|--------------------------|----------------------------|----------------------------|---------|
| Age at diagnosis (years) | $32 \pm 6$                 | $33 \pm 12$                | 0.67    |
| Mean± SD (range)                                   | (22–44)                       | (15–71)                    |         |
| Sex (Female/Male)                              | 29/21                          | 14/2                        | 0.03    |
| Baseline PRL levels (ng/mL)                     | $1335 \pm 2120$              | $159 \pm 54$              | 0.002   |
| Mean± SD (range)                                      | (196–10,385)                          | (101–255)                     |         |
| The percentage of PRL recovery after PEG          | $67.7 \pm 8$                  | $70 \pm 9.4$               | 0.96    |
| Mean± SD (range)                                     | (58–91)                          | (55–96)                        |         |

*p < 0.05 statistically significant. Significant p-values are shown in bold.*
PRL, prolactin; PEG, polyethyleneglycol.
PEG precipitation method. However, recently, using this method, the frequency of macroprolactinemia in prolactinoma patients was reported to be 7.6% [16].

The frequency of macroprolactinemia is higher in patients with microadenoma, but association of macroprolactinemia with macroadenoma is a much rarer situation [9, 18, 26, 27]. We did not detect MPRL in both of our patients with micro- and macroprolactinoma and there was no statistically significant difference in terms of PRL recovery. It is also unclear whether pituitary lesions are microprolactinomas or nonfunctional microadenomas with macroprolactinemia. Two recent studies revealed the 10 year follow up data of patients with macroprolactinoma. Abnormal imaging findings were reported to be rare and progression was not detected in the presence of microadenoma, and regression of microadenoma was reported even in the absence of treatment. These results support presence of incidental nonfunctional microadenomas with macroprolactinemia rather than presence of microprolactinoma with macroprolactinemia [32, 33]. In contrast to these, Elenkova et al. [16] evaluated 10 patients who had both prolactinoma and macroprolactinemia, four of these patients had macroprolactinoma and one of them had an invasive macroprolactinoma [17]. However, the presence of MPRL could not be clearly defined whether from tumor or peripheral origin, due to the fact that PEG precipitation method was used only on the serum samples and not on the tumor extracts [16, 17].

Consequently, our results suggest that prolactinoma may not be associated with macroprolactinemia. On the contrary to the recent publications implicating coexistence of prolactinoma and macroprolactinemia, we agree with the guidelines that suggest the assessment of MPRL levels merely in the evaluation of asymptomatic hyperprolactinemia. However, the limitations of our study are that the study is under power and further studies with larger patient series is needed in order to make stronger suggestion and also we used the PEG precipitation method in the investigation of macroprolactinemia, whereas the most qualified method in the assessment of macroprolactinemia is defined as the GFC method. Furthermore, especially as it has also been proven to be present immunohistochemically in the tumor extracts, presence of macroprolactinemia should also be kept in mind in the evaluation of prolactinomas.

Acknowledgement: The authors are grateful to Associate Professor Aysegul Terli from Istanbul University, Istanbul Medical Faculty, Department of Clinical Biochemistry for her consultation on technical issues of this article.

Conflict of interest statement: No conflict of interest was declared by the authors.

Financial disclosure: The authors declared that this study has received no financial support.

References

1. Smith CR, Norman MR. Prolactin and growth hormone: molecular heterogeneity and measurement in serum. Ann Clin Biochem 1990;27:542–50.
2. Hattori N, Ishihara T, Ikekubo K, Moridera K, Hino M, Kurahachih. Autoantibody to human prolactin in patients with idiopathic hyperprolactinemia. J Clin Endocrinol Metab 1992;75:1226–9.
3. Sinha YN. Structural variants of prolactin: occurrence and physiological significance. Endocr Rev 1995;16:354–69.
4. Hattori N, Inagaki C. Anti-prolactin (PRL) autoantibodies cause asymptomatic hyperprolactinemia: bioassay and clearance studies of PRL-immunoglobulin G complex. J Clin Endocrinol Metab 1997;82:3107–10.
5. Bjoro T, Morkrid L, Wergeland R, Turter A, Kvistborg A, Sand T, et al. Frequency of hyperprolactinemia due to large molecular weight prolactin. Scand J Clin Lab Invest 1995;55:139–47.
6. Jackson RD, Wortsman J, Malarkey WB. Macroprolactinemia presenting like a pituitary tumor. Am J Med 1985;78:346–50.
7. Samson SL, Hamrahian AH, Ezzat S. American Association of Clinical Endocrinologists, American College of Endocrinology Disease State clinical review: clinical relevance of macroprolactin in the absence or presence of true hyperprolactinemia. Endocr Pract 2015;21:1427–35.
8. Fahie-Wilson MN, Soule SG. Macroprolactinemia: contribution to hyperprolactinaemia in a district general hospital and evaluation of a screening test based on precipitation with polyethylene glycol. Ann Clin Biochem 1997;34:252–8.
9. Vallette-Kasic S, Morange-Ramos I, Selim A, Gunz G, Morange S, Enjalbert A, et al. Macroprolactinemia revisited: a study on 106 patients. J Clin Endocrinol Metab 2002;87:581–8.
10. Shimatsu A, Hattori N. Macroprolactinemia: diagnostic, clinical, and pathogenic significance. Clin Dev Immunol 2012;167:1212.
11. Hattori N, Aisaka K, Shimatsu A. A possible cause of the variable detectability of macroprolactin by different immunoassay systems. Clin Chem Lab Med 2016;54:603–8.
12. Melmed S, Casanueva FF, Hoffman AR, Kleinberg DL, Montori VM, Schlechte JA, et al. Endocrine society. Diagnosis and treatment of hyperprolactinemia: an Endocrine society clinical practice guideline. J Clin Endocrinol Metab 2011;96:273–88.
13. Casanueva FF, Molitch ME, Schlechte JA, Abs R, Bonert V, Bronstein MD, et al. Guidelines of the pituitary society for the diagnosis and management of prolactinomas. Clin Endocrinol 2006;65:265–73.
14. Ohnami S, Eto S, Ohnami S, Soejima T, Nakata H. Characterization of “big prolactin” in serum and tumor extract in patients with PRL-secreting tumor. Endocrinol Jpn 1987;34:325–34.
15. Mounier C, Trouillas J, Claustrat B, Duthel R, Estour B. Macroprolactinemia associated with prolactin adenoma. Hum Reprod 2003;18:853–7.
16. Elenkova A, Genov N, Abadzhieva Z, Kirilov G, Vasilev V, Kalinov K, et al. Macroprolactinemia in patients with prolactinomas:
prevalence and clinical significance. Exp Clin Endocrinol Diabetes 2013;121:201–5.
17. Elenkova A, Abadzhieva Z, Genov N, Vasilev V, Kirilov G, Zacharieva S. Macroprolactinemia in a patient with invasive macroprolactinoma: a case report and minireview. Case Rep Endocrinol 2013;634349.
18. Lakatos G, Szűcs N, Kender Z, Czirják S, Rácz K. Macroprolactinemia associated with pituitary macroadenoma: treatment with quinagolide. Orv Hetil 2010;151:1072–5.
19. Hattori N, Ikekubo K, Ishihara T, Moridera K, Hino M, Kurahachi H. Effects of antiprolactin autoantibodies on serum prolactin measurements. Eur J Endocrinol 1994;130:434–7.
20. Olukoga AO, Kane J. Macroprolactinaemia: validation and application of the polyethylene glycol precipitation test and clinical characterization of the condition. Clin Endocrinol 1999;51:119–26.
21. Gibney J, Smith TP, McKenna TJ. The impact on clinical practice of routine screening for macroprolactin. J Clin Endocrinol Metab 2005;90:3927–32.
22. McCudden CR, Sharpless JL, Grenache DG. Comparison of multiple methods for identification of hyperprolactinemia in the presence of macroprolactin. Clin Chim Acta 2010;411:155–60.
23. Hattori N, Ishihara T, Saiki Y. Macroprolactinaemia: prevalence and aetiologies in a large group of hospital workers. Clin Endocrinol 2009;71:702–8.
24. Leite V, Cosby H, Sobrinho LG, Fresnoza MA, Santos MA, Friesen HG. Characterization of big prolactin in patient with hyperprolactinemia. Clin Endocrinol 1992;37:365–72.
25. Donadio F, Barbieri A, Angioni R, Mantovani G, Beck-Pecco P, Spada A, et al. Patients with macroprolactinaemia: clinical and radiological features. Eur J Clin Invest 2007;37:552–7.
26. Hauache OM, Rocha AJ, Maia AC Jr, Maciel RM, Vieira JG. Screening for macroprolactinaemia and pituitary imaging studies. Clin Endocrinol 2002;57:327–31.
27. Tamer G, Telci A, Mert M, Uzum AK, Aral F, Tanakol R, et al. Prevalence of pituitary adenomas in macroprolactinemic patients may be higher than it is presumed. Endocrine 2012;41:138–43.
28. Rogol AD, Rosen SW. Prolactin of apparent large molecular size: the major immunoreactive prolactin component in plasma of a patient with pituitary tumor. J Clin Endocrinol Metab 1974;38:714–7.
29. Kavanagh L, McKenna TJ, Fahie-Wilson MN, Gibney J, Smith TP. Specificity and clinical utility of methods for the detection of macroprolactin. Clin Chem 2006;52:1366–72.
30. Beda-Maluga K, Pisarek H, Romanowska I, Komorowski J, Świętosławski J, Winczyk K. Ultrafiltration – an alternative method to polyethylene glycol precipitation for macroprolactin detection. Arch Med Sci 2015;11:1001–7.
31. Lippi G, Plebani M. Macroprolactin: searching for a needle in a haystack? Clin Chem Lab Med 2016;54:519–22.
32. Radavelli-Bagatini S, Lhullier FL, Mallmann ES, Spritzer PM. Macroprolactinemia in women with hyperprolactinemia: a 10-year follow-up. Neuro Endocrinol Lett 2013;34:207–11.
33. Wallace IR, Satti N, Courtney CH, Leslie H, Bell PM, Hunter SJ, et al. Ten-year clinical follow-up of a cohort of 51 patients with macroprolactinemia establishes it as a benign variant. J Clin Endocrinol Metab 2010;95:3268–71.