

Establishment of Reference Intervals of Monomeric Prolactin To Identify Macroprolactinemia in Chinese Patients with Increased Total Prolactin

Yao Hu

Huashan Hospital, Fudan University

Jiajin Ni

Huashan Hospital, Fudan University

Buyue Zhang

Huashan Hospital, Fudan University

Wei Cheng

Huashan Hospital, Fudan University

Huating Zhang

Huashan Hospital, Fudan University

Hongying Ye

Fudan University

Lijin Ji

Fudan University

Bin Lu

Fudan University

Ming Guan (✉ guanming88@yahoo.com)

Huashan Hospital, Fudan University

Research Article

Keywords: hyperprolactinemia, macroprolactinemia, reference interval

Posted Date: June 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-590536/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Macroprolactin is responsible for pseudohyperprolactinemia and is a common pitfall of the prolactin immunoassay. We aimed to determine the frequency of macroprolactinemia in Chinese hyperprolactinemic patients using monomeric prolactin discriminated by precipitation with polyethylene glycol (PEG).

Methods

Post-PEG monomeric prolactin gender-specific reference intervals were established for the Elecsys immunoassay method (Roche Diagnostics) using sera from healthy female (n = 120) and male (n = 120) donors. The reference intervals were validated using 20 macroprolactinemic (as assessed by gel filtration chromatography (GFC)) sera samples, and presence of monomeric prolactin was discriminated by GFC. Patients with high total prolactin were then screened by PEG precipitation to analyze macroprolactin. The demographic and biochemical details of patients with true hyperprolactinemia and macroprolactinemia were compared.

Results

Reference intervals for monomeric prolactin in females and males were 3.4–18.5 and 2.7–13.1 ng/mL, respectively. Among 1140 hyperprolactinemic patients, macroprolactinemia was identified in 261 (22.9%) patients while the other 879 (77.1%) patients were diagnosed with true hyperprolactinemia. Menstrual disturbances were the most common clinical feature in both groups. Galactorrhea, amenorrhea, and visual disturbances occurred more frequently in true hyperprolactinemic patients ($P < 0.05$).

Conclusions

The prevalence of macroprolactin in Chinese patients with hyperprolactinemia was described for the first time. Monomeric prolactin concentration, along with a reference interval screening with PEG precipitation, provides a diagnostic approach for hyperprolactinemia with improved accuracy.

Introduction

Heterogeneity has been described in the molecular size of prolactin in the majority of serum samples from healthy individuals and patients with hyperprolactinemia. Three major prolactin variants can be characterized, including monomeric, dimeric, and polymeric isoforms [1, 2]. Immunological and biological activity of prolactin in vivo may be almost exclusively attributed to the monomeric form [3]. Macroprolactin has a molecular weight > 100 kD and is a form of prolactin that is complexed with its

autoantibody [4]. Macroprolactinemia is mostly considered an extra-pituitary phenomenon. Patients with macroprolactinemia experience mild and nonspecific symptoms of hyperprolactinemia [3]. In these patients, monomeric prolactin concentrations are within reference values and most macroprolactin is confined to the vascular system, where it is biologically inactive [5]. Macroprolactin poses a major problem due to its interference with prolactin assays. This commonly results in misdiagnosis and mismanagement of patients, as well as wasted healthcare resources and unnecessary concern from both patients and clinicians [6]. High concentrations of macroprolactin appears to result from reduced clearance of antigen-antibody complexes of monomeric prolactin and immunoglobulin G. These complexes interfere with the interaction between prolactin and the capture and detection antibodies involved in the sandwich reaction of prolactin immunoassays [7, 8]. Consequently, it is essential for clinical laboratories to establish screening methods to detect macroprolactin and the monomeric prolactin component in all hyperprolactinemic serum samples [9].

Macroprolactinemia is primarily defined by a circulating prolactin level that is made up of greater than 60% macroprolactin [9]. The reference method for quantifying bioactive monomeric prolactin in sera is gel filtration chromatography (GFC). However, because this method is cumbersome and expensive, polyethylene glycol (PEG) serum precipitation is a simple, economical, and rapid method and has been widely used as a screening method to differentiate macroprolactinemia from true hyperprolactinemia [10–12]. Moreover, the laboratory priority should be to quantitatively determine the bioactive monomeric prolactin level, rather than simply to measure the percentage of macroprolactin present [13, 14].

There have been no previous reports pertaining to the reference interval of monomeric prolactin based on the Chinese population. Therefore, we have undertaken this cross-sectional investigation to screen for macroprolactin with higher accuracy.

Materials And Methods

Reference intervals of monomeric prolactin establishment and validation

For the monomeric prolactin reference interval study, 240 blood samples (120 females, 120 males) from healthy donors between 18 and 60 years old were collected into serum gel tubes (Vacutainer 5.0 mL; BD). Females who were in hormone treatment for menopause or using estrogen-containing contraceptives were excluded. Samples were left to coagulate at room temperature for at least 30 min and were then centrifuged at $3000 \times g$ for 10 minutes within 2 hours. The serum was then divided into aliquots and stored at $-30\text{ }^{\circ}\text{C}$ until analysis.

Prevalence of macroprolactin in hyperprolactinemia

Serum samples with total prolactin levels above normal range were obtained from routine analysis, and presence of monomeric prolactin was identified by gel filtration chromatography. All samples were stored

frozen at -30 °C until assay-specific prolactin measurements were carried out.

PEG-precipitation

PEG-precipitation was performed by adding 200 μ L of serum to an equal volume of 25% (w/v) PEG 6000 dissolved in 0.9% normal saline. After vortexing for 10 min at room temperature, specimens were centrifuged at 3000 \times g for 10 minutes. The post-PEG monomeric prolactin level was determined by multiplying the prolactin concentration in the supernatant by two to correct for the dilution with PEG. Prolactin recovery was calculated by dividing the post-PEG monomeric prolactin concentration by the total prolactin result. If the post-PEG monomeric prolactin was 60% or less of total prolactin, the specimen was considered negative for macroprolactin. To validate the established post-PEG reference intervals, we compared the classifications of macroprolactinemia obtained using our reference interval to the those obtained using GFC.

Prolactin assay

Total and post-PEG serum prolactin concentrations were measured using the Elecsys® Prolactin II assay from Roche Diagnostics (Cobas 8000; Mannheim, Germany). Prolactin immunoassays were calibrated against the World Health Organization (WHO) third international standard for prolactin (IS 84/500). The assay has an intra-assay imprecision (coefficient of variation; CV) of 5.8% and 2.9% at prolactin concentrations of 6.2 and 34.3 ng/mL, respectively. The inter-assay CVs in our experiments were 3.0% and 6.4%. All analyses were conducted using standardized laboratory procedures.

Demographic and biochemical details collection

All patient charts were reviewed to record clinical signs and symptoms associated with hyperprolactinemia, including galactorrhea, menstrual disturbances, visual disturbances and the evidence of pituitary adenoma by imaging studies.

Statistical analysis

Means and standard deviations (SD) were calculated for continuous variables. Serum prolactin concentrations were non-normally distributed and therefore were presented as medians (M) and quartiles (QR). Serum monomeric prolactin reference intervals were defined as 95% confidence limits. Patients were stratified under macroprolactinemia groups and true hyperprolactinemia groups, according to monomeric prolactin reference range after PEG treatment. The Chi-square test was used for categorical variables. Differences were considered statistically significant at a P value of less than 0.05. Data were analyzed using the SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Reference intervals of post-PEG serum monomeric prolactin

Data were collected from eligible participants, including 120 females (age 18–55 years, median 31 years) and 120 males (age 18–60 years, median 34 years). The demographic characteristics, total prolactin, and post-PEG monomeric prolactin at baseline are presented in Table 1. Female and male total and monomeric prolactin data were non-normally distributed according to histograms. The 95% reference intervals for absolute levels of monomeric prolactin in females and males, respectively, ranged from 3.5 to 18.4 ng/mL and from 2.5 to 13.8 ng/mL. The reference values of total and post-PEG monomeric prolactin in male and female sera are illustrated in Table 2, along with manufacturer-provided reference intervals. There were no significant differences between the established total prolactin reference intervals and manufacturer range in either gender. In terms of post-PEG monomeric prolactin, we observed significant differences between females and males ($P < 0.05$).

Table 1

Descriptive statistics for total prolactin and post-PEG monomeric prolactin (ng/mL) in serum samples from healthy females (n = 120) and males (n = 120)

Characteristics	Minimum	Maximum	Mean	Median	SD	2.5th percentile	97.5th percentile
Total prolactin, ng/mL							
Females	4.0	29.1	12.2	10.4	5.5	4.5	25.6
Males	3.1	17.7	10.8	11.7	3.6	4.0	16.5
Post-PEG monomeric prolactin, ng/mL							
Females	3.0	20.2	9.4	8.0	4.2	3.5	18.4
Males	2.2	15.0	8.7	9.4	3.0	2.5	13.8

Table 2

Reference intervals (95%) for total prolactin and post-PEG monomeric prolactin (ng/mL) in serum samples from females and males

	Established parametric range		Manufacturer's range	
	Female	Male	Female	Male
Total prolactin, ng/mL	4.5–25.6	4.0-16.5	4.8–23.3	4.0-15.2
Post-PEG monomeric prolactin, ng/mL	3.5–18.4	2.5–13.8	N/A	N/A
N/A, not available				

Validation of the established monomeric prolactin reference intervals

Discriminated by GFC, 20 macroprolactinemia samples (15 female, five male) were analyzed and categorized by the monomeric prolactin reference intervals along with recovery cutoff method. We found that all macroprolactinemia sera (20/20) classified by the post-PEG monomeric prolactin reference intervals (Table 3) had initially elevated prolactin levels due to macroforms. Moreover, the monomeric reference interval method showed elevated discordant classification (Table 3).

Table 3

Discordant classification of macroprolactinemia as determined using the post-PEG reference interval and recovery methods.

	Macroprolactinemia samples, N	Macroprolactinemia classification, N (%)
Reference interval method	20	20 (100)
Recovery method cutoff, %		
40	20	16 (80)
60	20	14 (70)

Frequency of macroprolactin in hyperprolactinemia

Using PEG precipitation, 1140 patients with total prolactin levels above normal range were screened for macroprolactinemia. The median age of the patients was 33 years (interquartile range (IQR) = 27, 42) and the male/female ratio was 267/873 with female preponderance. The median total prolactin (ng/mL) was 29.1 (IQR = 21.3, 48.4) in males compared to 50.3 (IQR = 34.3, 88.7) in females. Macroprolactinemia was present in 22.9% (n = 261) of hyperprolactinemic patients with normal monomeric prolactin concentrations. True hyperprolactinemia was identified in 77.1% (n = 879) of patients. Based on serum total prolactin, all patients were divided into three groups. The prevalence of macroprolactinemia was 34.4%, 19.8%, and 6.4% in groups with total prolactin below 50.0, 50.0-100.0, and above 100.0 ng/mL, respectively. The frequency of macroprolactinemia was the highest in patients with total prolactin less than 50.0 ng/mL. A significantly higher proportion of macroprolactinemia patients had total prolactin below 100 ng/mL ($P < 0.001$). A summary of the prevalence is presented in Fig. 1.

Comparison of the demographic and biochemical characteristics of patients with true hyperprolactinemia and macroprolactinemia

The demographic and biochemical details of patients with true hyperprolactinemia and macroprolactinemia were compared (Table 4). Total prolactin was significantly higher ($p < 0.001$) in patients with true hyperprolactinemia compared to patients with macroprolactinemia, and this difference was maintained after treatment of serum with PEG.

Table 4
Comparison of demographic and biochemical characteristics of the patients

Characteristics	True hyperprolactinemia (n = 879)	Macroprolactinemia (n = 261)	P value
Age, years (IQR)	33(27, 43)	34(27, 42)	0.897
Gender, N (%)			
Male	210(23.9%)	38(21.8%)	0.712
Female	669(76.1%)	204(78.2%)	0.625
Total prolactin, ng/mL (IQR)	50.7(33.1, 97.3)	32.9(26.3, 45.0)	< 0.001
Monomeric prolactin, ng/mL (IQR)	37.2(24.5, 69.0)	12.0(8.1, 13.8)	< 0.001
Imaging studies (MRI, CT) *	201	30	
Adenoma detected, N (%)	120(59.7)	7(23.3)	< 0.001
Microadenoma	54	5	
*MRI, magnetic resonance imaging; CT, computed tomography			

The clinical presentations were diverse and varied between macroprolactinemic and true hyperprolactinemic patients (Fig. 2). Menstrual disturbances were the main clinical feature in macroprolactinemic and true hyperprolactinemic female patients (38.1% vs. 48.9%). Galactorrhea was significantly more common in true hyperprolactinemic patients ($P= 0.02$), as well as amenorrhea ($P= 0.01$) and visual disturbances ($P= 0.01$). There was no significant difference in the incidence of headache between the true hyperprolactinemic and macroprolactinemic patients. However, in the macroprolactinemic group, the most common clinical symptoms were heat intolerance due to thyroidal illness, infertility, and obesity, in addition to less common symptoms of headache and galactorrhea.

Imaging studies [magnetic resonance imaging (MRI), computed tomography (CT)] were conducted in 201 true hyperprolactinemic patients. However, only 30 patients with macroprolactinemia were directed to further radiological examination. Medical data showed that 120 (59.7%) of the 201 true hyperprolactinemic patients were confirmed to have pituitary adenomas. In contrast, in the group of macroprolactinemia cases, only five patients (17%) were identified with pituitary microadenoma, as shown in Table 4.

Discussion

In individuals with macroprolactinemia and normal levels of monomeric prolactin, nonspecific symptomatic progression was found during a 10-year clinical follow-up study. Therefore, macroprolactinemia can be considered a benign variant of hyperprolactinemia [15]. Failure to identify

these individuals prior to or during laboratory prolactin assays leads to misdiagnosis, unnecessary investigation, inappropriate treatment, and ultimately an increased cost of management. Consequently, routine diagnostic macroprolactin screening in all hyperprolactinemic patients has been authoritatively recommended and financially justified due to reduced use of both imaging and dopamine agonist prescription in these patients [16].

It is difficult to differentiate between true hyperprolactinemia and macroprolactinemia based on clinical symptoms alone, as most of the patients with macroprolactinemia are also symptomatic. Without the use of PEG pretreatment, no commercial prolactin kits have been developed to accurately classify macroprolactin from true hyperprolactinemia. Only reporting with the recovery after PEG treatment lacks specificity, and may be inappropriate and misinterpreted in cases in which excess macroprolactin occurs simultaneously with supraphysiological concentrations of monomeric prolactin [17]. Screening for macroprolactinemia by reporting a monomeric prolactin concentration along with a PEG-specific monomeric reference interval is a recommended good practice for laboratories because the presence of excess monomeric prolactin is of clinical concern. This approach would enable more rigorous definition of macroprolactinemia than recovery cutoff method, and would prevent confusion when excess biologically active prolactin is present along with macroprolactin. Furthermore, this may be more valuable and easier to interpret than the reporting of macroprolactin along with a recovered prolactin concentration [17, 18].

In the present study, gender-specific monomeric prolactin reference intervals were established to avoid any confusion as to whether biologically active prolactin is also increased when excess macroprolactin is present. The application of a monomeric prolactin reference interval for the PEG precipitation procedure allowed for the detection of two cohorts of patients: one with true hyperprolactinemia and the other in whom hyperprolactinemia could be accounted for entirely by macroprolactin. True hyperprolactinemia is defined by the presence of excess monomeric prolactin in serum. In our study, macroprolactinemia was characterized by the presence of excess serum macroprolactin together with monomeric prolactin concentration in the normal reference interval. To our knowledge, this is the first study to compare unselected macroprolactinemic patients with true hyperprolactinemic patients among the Chinese population. A high incidence of macroprolactinemia in this study was identified to be 22.9%. Other studies have reported very different macroprolactinemia incidences ranging from 10–45% [19–22].

Our study demonstrated that, while menstrual disturbances occurred more frequently in patients with true hyperprolactinemia, they also occurred in 38.1% of macroprolactinemic patients. Galactorrhea occurred less frequently in patients with macroprolactinemia compared to those with true hyperprolactinemia. Although this difference was statistically significant, it is clearly not sufficient to distinguish between the two groups. Out of a total of 30 macroprolactinemic patients who underwent MRI or CT scan, adenoma was detected in seven patients (23.3%) and microadenoma was detected in five patients (17%). Consistent with our observation, Hauche et al. [23] observed that abnormal pituitary CT scans occurred in 21% of macroprolactinemic patients. These results indicate that patients with macroprolactinemia can have microadenomas, and this should be evaluated with detailed clinical context and careful follow-up.

Our study has certain advantages compared to previous studies. First, a large cohort of big data was used in the present study. Secondly, the reference interval of monomer prolactin based on Chinese population was established for the first time.

Conclusions

It is important for clinical laboratories to perform routine screening for macroprolactinemia in all hyperprolactinemic sera. The identification of macroprolactinemia by monomeric prolactin specific reference intervals based on pretreatment with PEG precipitation is an improved methodology with several advantages over the current standard methodology of GFC.

Abbreviations

PEG

polyethylene glycol; GFC:gel filtration chromatography.

Declarations

Acknowledgements

We would like to thank all the nursing staff at the Huashan Hospital who helped recruit patients into the study and for collecting samples throughout.

Authors' contributions

YH carried out the design of the study and drafted the manuscript. BZ and WC performed the immunoassays. JN performed statistical analysis. LJ, BL and HZ collected clinical history and imaging workups. MG and HY conceived the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final version of the manuscript.

Funding

This study was supported by the Innovation Group Project of Shanghai Municipal Health Commission (2019CXJQ03); Program of Shanghai Academic/ Technology Research Leader (18XD1400900).

Availability of data and materials

All data generated or analyzed during this study are included in this published article and information about experimental sessions and results are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Our study was approved by the Huashan Hospital Foundation Ethical Committee (reference number KY2019-395). Written informed consent was obtained from all participants. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Consent for publication

Patient consent for research use of data was obtained for each case presented.

Competing interests

The authors declare that they have no competing interests

Author details

¹Department of Laboratory Medicine, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, 200040, China. ²Department of Nursing, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, 200040, China. ³Department of Endocrinology, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, 200040, China.

References

1. Kavanagh, L; McKenna, TJ; Fahie-Wilson, MN; et al. Specificity and clinical utility of methods for the detection of macroprolactin. [J]. *Clin Chem*.2006,52(7):1366–72
2. McCudden, CR; Sharpless, JL; Grenache, DG; Comparison of multiple methods for identification of hyperprolactinemia in the presence of macroprolactin. [J]. *Clin Chim Acta*.2010,411(3–4):155–60
3. Chen, YJ; Song, GZ; Wang, ZN; A new criteria for screening macroprolactinemia using polyethylene glycol treatment combined with different assays for prolactin. [J]. *Eur Rev Med Pharmacol Sci*.2016,20(9):1788–94
4. Sherazi, NA; Baig, MZ; Khan, AH; Frequency of Macroprolactin in Hyperprolactinemia. [J]. *J Coll Physicians Surg Pak*.2018,28(2):93–97
5. Kasum, M; Oreskovic, S; Cehic, E; et al. Laboratory and clinical significance of macroprolactinemia in women with hyperprolactinemia. [J]. *Taiwan J Obstet Gynecol*.2017,56(6):719–724
6. Beshyah, SA; Sherif, IH; Chentli, F; et al. Management of prolactinomas: a survey of physicians from the Middle East and North Africa. [J]. *Pituitary*.2017,20(2):231–240
7. Barth, JH; Lippiatt, CM; Gibbons, SG; et al. Observational studies on macroprolactin in a routine clinical laboratory. [J]. *Clin Chem Lab Med*.2018,56(8):1259–1262
8. Sostaric, M; Bokulic, A; Marijancevic, D; et al. Optimizing laboratory defined macroprolactin algorithm. [J]. *Biochem Med (Zagreb)*.2019,29(2):020706
9. Vilar, L; Vilar, CF; Lyra, R; et al. Pitfalls in the Diagnostic Evaluation of Hyperprolactinemia. [J]. *Neuroendocrinology*.2019,109(1):7–19

10. Saleem, M; Martin, H; Coates, P; Prolactin Biology and Laboratory Measurement: An Update on Physiology and Current Analytical Issues. [J]. Clin Biochem Rev.2018,39(1):3–16
11. Overgaard, M; Pedersen, SM; Serum prolactin revisited: parametric reference intervals and cross platform evaluation of polyethylene glycol precipitation-based methods for discrimination between hyperprolactinemia and macroprolactinemia. [J]. Clin Chem Lab Med.2017,55(11):1744–1753
12. Beltran, L; Fahie-Wilson, MN; McKenna, TJ; et al. Serum total prolactin and monomeric prolactin reference intervals determined by precipitation with polyethylene glycol: evaluation and validation on common immunoassay platforms. [J]. Clin Chem.2008,54(10):1673–81
13. Chanson, P; Maiter, D; The epidemiology, diagnosis and treatment of Prolactinomas: The old and the new. [J]. Best Pract Res Clin Endocrinol Metab.2019,33(2):101290
14. Fahie-Wilson, M; In hyperprolactinemia, testing for macroprolactin is essential. [J]. Clin Chem.2003,49(9):1434–6
15. Wallace, IR; Satti, N; Courtney, CH; et al. Ten-year clinical follow-up of a cohort of 51 patients with macroprolactinemia establishes it as a benign variant. [J]. J Clin Endocrinol Metab.2010,95(7):3268–71
16. Gibney, J; Smith, TP; McKenna, TJ; The impact on clinical practice of routine screening for macroprolactin. [J]. J Clin Endocrinol Metab.2005,90(7):3927–32
17. Suliman, AM; Smith, TP; Gibney, J; et al. Frequent misdiagnosis and mismanagement of hyperprolactinemic patients before the introduction of macroprolactin screening: application of a new strict laboratory definition of macroprolactinemia. [J]. Clin Chem.2003,49(9):1504–9
18. Hauache, OM; Rocha, AJ; Maia, AC; et al. Screening for macroprolactinaemia and pituitary imaging studies. [J]. Clin Endocrinol (Oxf).2002,57(3):327–31
19. Fahie-Wilson, M; Smith, TP; Determination of prolactin: the macroprolactin problem. [J]. Best Pract Res Clin Endocrinol Metab.2013,27(5):725–42
20. Donadio, F; Barbieri, A; Angioni, R; et al. Patients with macroprolactinaemia: clinical and radiological features.[J].Eur J Clin Invest.2007,37(7):552–7
21. Sherazi, NA; Baig, MZ; Khan, AH; Frequency of Macroprolactin in Hyperprolactinemia. [J]. J Coll Physicians Surg Pak.2018,28(2):93–97
22. Aisaka, K; Tsuchiya, F; Sueta, M; et al. Impact of macroprolactin on galactorrhea and the rate of patients possibly affected by macroprolactin.[J].Endocr J.2018,65(2):203–211

Figures

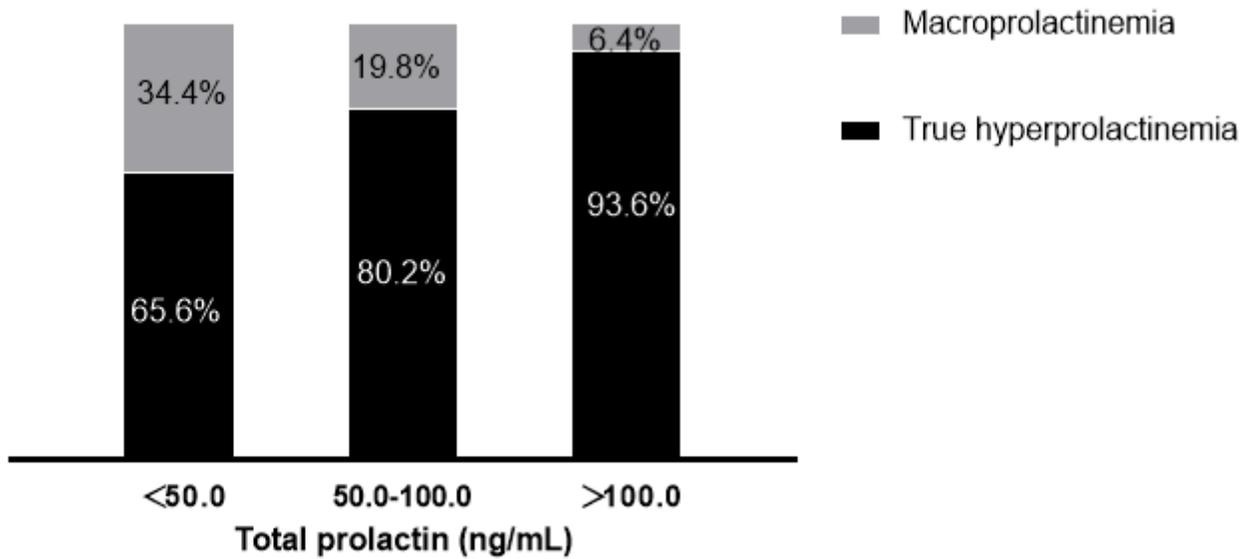


Figure 1

The prevalence of true hyperprolactinemia and macroprolactinemia among patients with increased total prolactin.

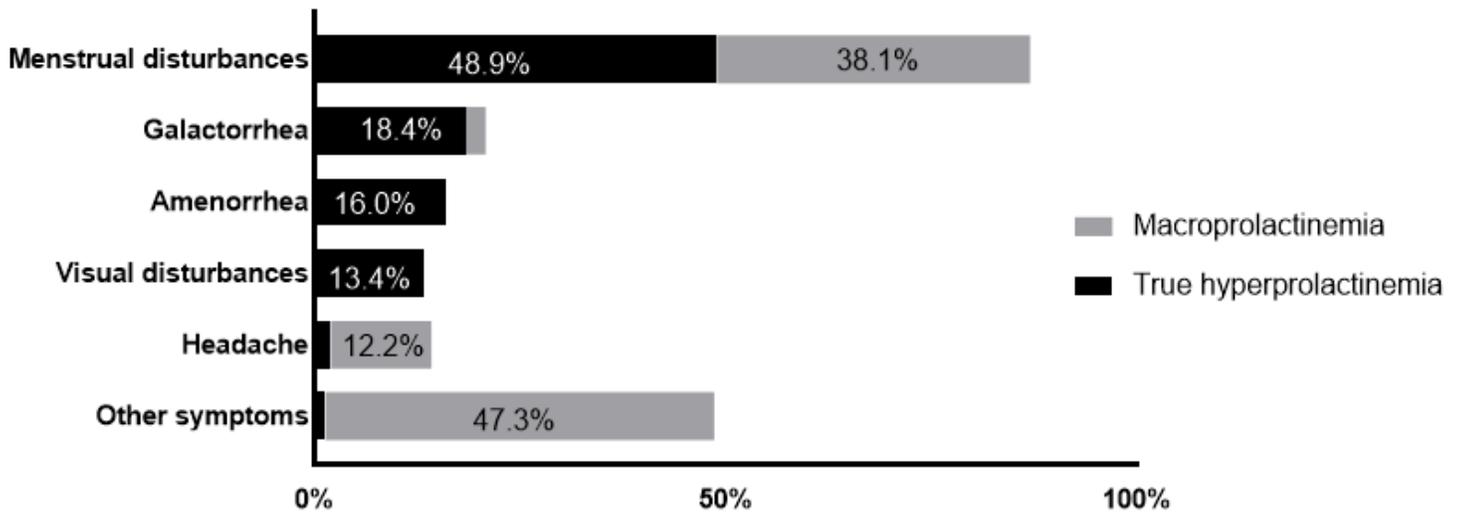


Figure 2

Clinical features of macroprolactinemic and true hyperprolactinemic patients.