Research Article

Melahat Dirican*, Hacer Ebru Açıkgöz and Emre Sarandöl

Evaluation of percentage recovery together with modified reference range in hyperprolactinemia

Hiperprolaktinemide geri kazanım yüzdesi ve modifiye referans aralık değerlerinin birlikte kullanımı

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Abstract

Objective: Macroprolactinemia is an important cause of hyperprolactinemia. The aim of this study was to examine the added value of the consideration of modified reference range in determination of macroprolactinemia and true hyperprolactinemia.

Materials and methods: Three hundred and ninety patients with high and 131 with normal prolactin (PRL) levels were included in this study. PRL had been analyzed before and after polyethylene glycol precipitation (post-PEG PRL). Recovery percentage (R%) <40% and >60% had been reported as macroprolactinemia and true hyperprolactinemia, respectively. Post-PEG PRL levels were evaluated according to the modified reference range obtained from those of the normoprolactinemic subjects.

Results: According to the R% criterion; macroprolactinemia had been detected in 24.9% and true hyperprolactinemia in 67.4% of hyperprolactinemic patients. When the data were evaluated considering the post-PEG PRL levels according to the modified reference range obtained from those of the normoprolactinemic subjects; 13 (13.4%) of the 97 macroprolactinemia reports would be considered as true hyperprolactinemia and 6 (2.3%) of the 263 true hyperprolactinemia reports would be changed as macroprolactinemia.

Conclusion: Discrimination capacity of R% criterion for true hyperprolactinemia and macroprolactinemia is limited, and we suggest that, in accordance with R% criterion, laboratory reports should include the post-PEG PRL levels along with the modified reference range.

Keywords: Hyperprolactinemia; Macroprolactin; Prolactin; Monomeric prolactin; Polyethylene glycol.

Öz

Amaç: Makroprolaktininemi, hiperprolaktinineminin önemli bir nedenidir. Bu çalışmamızın amacı, makroprolaktininemi ve gerçek hiperprolaktinineminin belirlenmesinde modifiye referans aralığının katkısını incelenmesidir.

Gereç ve Yöntem: Retrospektif özellikteki bu çalışmaya prolaktin (PRL) düzeyi yüksek olan 390 ve normal olan 131 olgu dahil edildi. PRL düzeyi polietilen glikol presipitasyonundan önce ve hemen sonra (post-PEG PRL) ölçüldü. Geri kazanım yüzdesi (% R) <40% ve >60% olanlar sırasıyla makroprolaktininemi ve gerçek hiperprolaktininemi olarak rapor edildi. Post-PEG PRL düzeyi, normoprolaktininemik gruptan elde edilen modifiye referans aralığına göre değerlendirildi.

Bulgular: %R kriterine göre hiperprolaktininemik hastaların %24.9’unda makroprolaktininemi ve %67.4’ünde gerçek hiperprolaktininemi saptanmıştır. Veriler, post-PEG PRL seviyeleri dikkate alınarak modifiye referans aralığına göre yeniden değerlendirildiğinde; makroprolaktininemi olarak rapor edilen 97 olgudan 13‘ünün (%13.4) gerçek hiperprolaktininemi olarak ve gerçek hiperprolaktininemi
olan 263 olgu raporunun 6’sının (%2.3) makroprolaktinemi olarak değişmiş olduğu görüldü.

Sonuç: Gerçek hiperprolaktinemi ve makroprolaktineminin arımında %R kriteri kısıtlılığına sahiptir, bu nedenle laboratuvar raporlarının %R kriterinin yanı sıra, modifiye referans aralığı ile birlikte post-PEG PRL seviyelerini de içermesi gerekmektedir.

Anahtar Kelimeler: Hiperprolaktinemi; Makroprolaktin; Prolaktin; Monomerik prolaktin; Polietilen glikol.

Dedicated to: This work is dedicated to the memory of our colleague H. Ebru Acıkgöz.

Introduction

Human serum prolactin (PRL) is known to exist in three major molecular variants, which are monomeric PRL (little or free PRL, 23 kDa), dimeric PRL (big PRL, 50–60 kDa) and big-big PRL (macroprolactin, 150–170 kDa). Monomeric PRL is the main circulating form of PRL (approximately 80–95% of total PRL) and accounts for the majority of PRL bioactivity and immunoreactivity in the serum of most patients with hyperprolactinemia as well as healthy individuals. In normal sera, dimeric PRL and macroprolactin account for 10–15% and <1% of total prolactin, respectively [1]. Macroprolactin is considered to have minimal bioactivity, however, it retains immunoreactivity with varying degrees depending on the specificity of the assay used to measure PRL levels [2, 3].

Macroprolactinemia, increased levels of macroprolactin, is one of the causes of hyperprolactinemia which usually requires no treatment. However, there are no reliable clinical features or laboratory tools to differentiate patients with macroprolactinemia from patients with elevated levels of biological active monomeric PRL (true hyperprolactinemia) [4]. This may lead to concerns for both the patient and clinician, unnecessary drug treatments and interventions [5]. Therefore, when serum prolactin is high, it is recommended that laboratories screen for macroprolactinemia to discriminate between those patients with true hyperprolactinemia and those with macroprolactinemia [6].

The reference method for the measurement of high molecular mass forms of prolactin is gel filtration chromatography (GFC). But this method is impractical for routine performance in clinical laboratories because it is labor-intensive, time-consuming and expensive [7]. In recent years, several laboratories have been performing polyethylene glycol (PEG) precipitation method to determine the ratio of post-PEG PRL (PRL concentration after PEG treatment) to total PRL levels which is both cheap and easy to perform in clinical laboratories. The PEG precipitation technique is based on the removal of the macromolecular form of PRL by precipitation with PEG [7–9]. In this technique, PRL levels are measured both in the serum (total PRL) and in the supernatant, which is obtained after precipitation with PEG (post-PEG PRL). The ratio of post-PEG PRL levels to serum total PRL is identified as the percentage of recovery (R%). There are several studies, comparing the results of PEG precipitation with GFC [9, 10], that yielded promising results and R% <40–50% has been suggested as the threshold for the determination of macroprolactinemia [5, 11–13]. However, increased monomeric PRL levels may be present accompanied by macroprolactinemia. It is therefore critical not only to detect the presence of macroprolactinemia by using R% value but also to measure the amount of the monomeric PRL, as well. For this purpose, it is suggested to evaluate if the post-PEG PRL level fall in the range obtained from the PEG-treated supernatant of healthy individuals (modified reference range) to confirm the absence of true hyperprolactinemia accompanying with macroprolactinemia [5, 14–16].

This retrospective study aimed to examine the added value of the consideration of post-PEG PRL levels in the determination of macroprolactinemia and true hyperprolactinemia. For this purpose, we examined the macroprolactin reports of our laboratory in the last 35 months and created a modified reference range for the post-PEG PRL obtained from the data of healthy controls. We reevaluated data of patients with hyperprolactinemia considering this modified reference range.

Materials and methods

Study design

In this retrospective study, the laboratory reports of the patients, between February 2014 and December 2016 were reviewed and screened for macroprolactinemia on the database of the laboratory information system of the Clinical Biochemistry Laboratory of the Uludag University Medical Faculty. Over a 35 months period, among the serum samples submitted to the laboratory for measurement of serum PRL concentrations, 390 patients (82 males and 308 females) with high levels of PRL were received as the patient group. Those patients were non-pregnant
adults with normal liver and renal function. The sources of the requests were as follows: endocrinology (68%), neurosurgery (20%), internal medicine (5%), psychiatry (3%), gynecology (2%) and others (2%). Sixty-two percent of the patient group is composed of patients with a pituitary tumor (macroprolactinoma, microprolactinoma, and nonfunctioning pituitary adenoma) and 38% of patients with functional hyperprolactinemia (hypothyroidism, polycystic ovary syndrome, drugs-induced-hyperprolactinemia and idiopathic origin). The control group (normoprolactinemic group) consisted of 131 (46 males and 85 females) apparently healthy subjects without known metabolic or endocrine disorders and having no medical treatment. Healthy subjects were recruited by local announcements in the hospital and the university. Laboratory investigation of the control group was performed in the serum obtained after 8–10 h fasting state and analyzed in the fresh samples. The modified reference range was determined using the post-PEG PRL levels of the control group.

For serum total PRL, we use reference ranges obtained from local population which is almost in agreement with the reference ranges provided by the manufacturer (3.46–19.4 μg/L for male, 5.18–26.53 μg/L for female). In our laboratory, the upper limit of normoprolactinemia was 29 μg/L for female and 19 μg/L for male, and in our daily practice, we screen for macroprolactinemia in samples that have higher than above-mentioned levels for each gender. Furthermore, patients with PRL levels above 250 μg/L were also excluded since those patients were recommended to be investigated for prolactinoma [17]. The study was approved by the Ethics Committee of Uludag University Medical Faculty.

### Methods

Serum PRL levels were measured by Architect i2000 immunoanalyzer using the Abbott Architect 7K76 PRL chemiluminescent microparticle immunoassay (Abbott Lab., Chicago, IL, USA). The Architect PRL assay was a two-step chemiluminescent immunoassay and standardized to the WHO 3rd International Standard IS 84/500. The sensitivity of the assay was 0.6 μg/L, the intra-assay coefficient of variation (CV) ranged between 2.1 and 3.0%, and inter-assay CV ranged between 3.9 and 4.7% at concentrations of 11.1 and 39.6 μg/L, respectively.

Prolactin was analyzed before (serum total PRL) and immediately after PEG precipitation (in the supernatant). In the PEG-precipitation test, 300 μL of serum mixed with an equal volume of 25% (weight/volume) PEG (Polyethylene Glycol 6000, Merck) solution was incubated for 10 min at room temperature and was centrifuged (after thorough vortex mixing) at 1800 × g for 30 min at 20°C. The supernatant was analyzed for PRL (post-PEG PRL). After correcting for dilution, the results were expressed as percentage recovery (R%) by dividing the PRL concentration in the PEG supernatant by that of untreated serum. An R% value <40% indicated the presence of a substantial amounts of macroprolactin in serum (macroprolactinemic subjects); whereas, an R% value >60% was considered as indicative of substantial absence of macrolactin (true hyperprolactinemic subjects). R% values in the range 40–60% (a gray area of PEG test) were considered as suspicious results [8].

We reevaluated the data of the hyperprolactinemic patients with the alternative recovery criterion 50% which identifies patients R% <50% as macroprolactinemia and ≥50% as true hyperprolactinemia to examine its consistency with the modified reference range [12].

Reproducibility of the PEG precipitation test was investigated in four serum samples. The intra-assay CV for R% ranged between 1.1 and 1.3% (n = 10) and inter-assay CV was <3.5% (n = 5).

Post-PEG PRL (accepted as the active monomeric PRL) is the PRL concentration in the supernatant after PEG treatment. Post-PEG PRL levels of the patient group were evaluated according to the modified reference range obtained from those of the healthy control group for each sex.

### Statistical analysis

SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Comparison of proportions was performed using the Pearson chi-squared test. Conformity of variables with normal distribution was tested with the Kolmogorov–Smirnov test. Results were expressed as mean ± standard deviation or median (interquartile range), depending on data distribution. Results were considered statistically significant at p < 0.05.

### Results

The results obtained in hyperprolactinemic and normoprolactinemic subjects are summarized in Table 1. PRL recovery (R%) for the 390 patients with hyperprolactinemia assayed after PEG-precipitation ranged from 2 to 106%. The 95% reference ranges (2.5th–97.5th percentiles)
for PRL in healthy subjects after PEG treatment ranged from 2.5–20.5 μg/L in females and 1.9–14.5 μg/L in males (Table 2).

Using the cut-off <40% for recovery, the prevalence of macroprolactinemia in hyperprolactinemic patients were 24.9%; whereas 67.4% of patients showed a true hyperprolactinemia (R > 60%) and 77% were evaluated as suspicious (40% < R < 60%). Figure 1 shows the recovery % frequency distribution in hyperprolactinemic subjects. When we evaluated our data according to gender, macroprolactinemia was detected in 76 patients (24.7%) and true hyperprolactinemia was detected in 210 patients (68.2%) within 308 hyperprolactinemic female patients. 22 of these patients (7.1%) were evaluated as suspicious. However, in 82 male patients with hyperprolactinemia, 21 patients (25.6%) were identified as macroprolactinemia, 53 patients (64.6%) as true hyperprolactinemia and 8 patients (9.8%) were considered as suspicious. Accordingly, there was no statistically significant difference between genders in terms of macroprolactinemia prevalence (p = 0.137).

We also evaluated our reports according to the alternative cut-off (50%) for R% and we observed 116 patients (29.7%) were in the macroprolactinemia range. According to the modified reference range for post-PEG PRL, 105 of the 390 hyperprolactinemic cases (26.9%) were identified as macroprolactinemia, i.e. the post-PEG PRL levels fell to less than 20.5 μg/L in sera from women and to less than 14.5 μg/L in samples from men after treatment with PEG (Table 3).

On the other hand, the prevalence of macroprolactinemia in normoprolactinemic subjects was 2.3% (n = 3), and 6.1% of participants (n = 8) showed an R% value in the 40–60% range.

Comparisons were also made between two interpretive approaches (recovery criteria and modified reference range) for categorizing subjects as having macroprolactinemia or true hyperprolactinemia. Of the 390 cases with hyperprolactinemia, 84 cases were identified as having macroprolactinemia, and 257 cases were identified as having true hyperprolactinemia using either the modified reference range or percentage recovery approach (Figure 2). Thirteen of the 97 macroprolactinemic subjects (13.4%) according to the 40% criterion were considered to be hyperprolactinemic using the modified reference range. Total PRL levels of these subjects were >70 μg/L. Six of the 263 hyperprolactinemic patients (2.3%) who were reported as true hyperprolactinemia according to the 40% criterion were considered to be macroprolactinemic by using the modified reference range. Serum total PRL levels in these six patients were <30 μg/L. On the other hand, we found that 15 suspicious results (50%) ended with a normal post-PEG PRL concentration after PEG precipitation (macroprolactinemic subjects), whereas in the remaining 15 cases the values remained elevated (true hyperprolactinemic subjects). We investigated the

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**Table 1:** Prolactin concentrations of normoprolactinemic and hyperprolactinemic subjects before (total) and after polyethylene glycol precipitation (post-PEG) and recovery values.

<table>
<thead>
<tr>
<th></th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoprolactinemia (n)</td>
<td>85</td>
<td>46</td>
</tr>
<tr>
<td>Total PRL (μg/L)</td>
<td>13.5 ± 6.0</td>
<td>10.7 ± 4.3</td>
</tr>
<tr>
<td>Post-PEG PRL (μg/L)</td>
<td>9.6 ± 4.5</td>
<td>8.1 ± 3.5</td>
</tr>
<tr>
<td>R%</td>
<td>71.9 ± 13.3</td>
<td>75.3 ± 10.9</td>
</tr>
<tr>
<td>Hyperprolactinemia (n)</td>
<td>308</td>
<td>82</td>
</tr>
<tr>
<td>Total PRL (μg/L)</td>
<td>57.9 (40.4–94.5)</td>
<td>36.4 (27.3–63.4)</td>
</tr>
<tr>
<td>Post-PEG PRL (μg/L)</td>
<td>33.7 (19.4–59.6)</td>
<td>19.8 (13.1–36.9)</td>
</tr>
<tr>
<td>R%</td>
<td>72.1 (39.6–80.2)</td>
<td>72.8 (39.7–82.8)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation or as median (interquartile ranges 25–75%). PRL, Prolactin; R%, percentage of recovery.

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**Table 2:** Gender-specific modified reference ranges for post-PEG prolactin in the present and other studies.

<table>
<thead>
<tr>
<th>Modified reference ranges for post-PEG PRL (μg/L)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>1.9–14.5 (n = 46)</td>
<td>2.5–20.5 (n = 85)</td>
</tr>
<tr>
<td>Beltran et al. [15]</td>
<td>3.4–10.9 (n = 53)</td>
<td>3.8–16.5 (n = 93)</td>
</tr>
<tr>
<td>Overgaard and Pedersen [16]</td>
<td>2.4–9.9 (n = 128)</td>
<td>2.8–10.7 (n = 96)</td>
</tr>
<tr>
<td>Whitehead et al. [18]</td>
<td>1.5–14.7 (n = 49)</td>
<td>1.9–20.1 (n = 52)</td>
</tr>
</tbody>
</table>

Post-PEG PRL, Prolactin concentration after polyethylene glycol treatment.
consistency of 50% recovery criterion with the modified reference range. We observed that 21 patients (18.1%) identified as macroprolactinemia with the 50% criterion were in the range of true hyperprolactinemia and 11 true hyperprolactinemia cases (4%) with the 50% criterion were in the range of macroprolactinemia according to the modified reference range (Figure 3).

Discussion

This retrospective study demonstrated that, 24.9% of hyperprolactinemic samples reported by our laboratory in a 35 months period were identified as macroprolactinemia according to the 40% criterion. The ratio of macroprolactinemia in hyperprolactinemic subjects which is believed to be assay dependent, has been reported between 10 and 46% in several studies [4, 11, 12, 19–21]. All these data, including ours, indicate that macroprolactinemia is a common cause of hyperprolactinemia. In addition, we observed macroprolactinemia in 3 (2.3%) of the normoprolactinemic subjects which is comparable with some other reports [22, 23]. Hattori et al. [22], reported that the prevalence of macroprolactinemia in healthy population was 3.86% in women and 3.13% in men; those authors also reported that there were no differences between males and females. However, in our study, all of the three participants of normoprolactinemic subjects with macroprolactinemia were female but the number of samples is low to make any significant comments. We would like to point out that the significance of macroprolactinemia in normoprolactinemic subjects remains unknown.

Reference ranges for monomeric PRL levels in the post-PEG supernatant (post-PEG PRL) have been reported for widely used automated immunoassays [15, 16, 18]. We compared our modified reference ranges with the ones that were obtained by the Abbott Architect assay that we had been using (Table 2). Gender-specific reference ranges, particularly upper limits, were similar with those found by Whitehead et al. [18]. However, the upper limits of reference ranges were considerably higher than those established by Beltran et al. [15] and Overgaard and Pedersen [16].

The findings of this study revealed that the number of samples identified as macroprolactinemia would not significantly change ($p = 0.126$), if the patients had been reported related to the modified reference range derived from normal subjects instead of 40% criterion (26.9% and 24.9%, respectively) as we had already been doing. However, it was observed that 13 subjects that had been identified as macroprolactinemia using 40% criterion had higher post-PEG PRL levels than those of the healthy controls. Since those patients should have been reported as true hyperprolactinemia accompanying with macroprolactinemia, there might be a latency in their treatment process. These subjects account for the 13.4% of macroprolactinemic patients reported according to the 40% criterion and had total PRL levels >70 μg/L. Consistent with this observation, McCudden et al. [24] suggested that when the PRL concentration was >85 μg/L clinicians...
should not be concerned with macroprolactinemia, but focus on routine evaluation of hyperprolactinemia. The coexistence of true hyperprolactinemia and macroprolactinemia emphasizes the importance of modified reference range for the post-PEG PRL [25]. On the other hand, six subjects (2.3%) who were reported as having true hyperprolactinemia using 40% criterion were macroprolactinemic when evaluated with the modified reference range. Although R% classification of hyperprolactinemia has advantages and benefits in daily laboratory practice, it seems like it is unsatisfactory to distinguish macroprolactinemia and true hyperprolactinemia when reports solely include R% criterion. Furthermore, there is a grey zone for R% (40–60%) in which the method has almost no efficiency and it is stated that those samples should be subjected to GFC for a definitive diagnosis [8]. In order to cope with this problem, some authors also suggest 50% cut-off for R% [26]. When we evaluated our reports according to the cut-off 50%, we observed 116 patients were in the macroprolactinemia range however, according to the modified reference range 21 of these patients (18.1%) had true hyperprolactinemia. So, the ratio increased from 13.4 to 18.1% for the misdiagnosis of coexistence of true hyperprolactinemia and macroprolactinemia when we used 50% instead of 40% cut-off for recovery.

In conclusion, these data show that macroprolactinemia is a common cause of hyperprolactinemia, however discrimination capacity of R% criterion for true hyperprolactinemia and macroprolactinemia is limited, particularly when macroprolactinemia coexist with true hyperprolactinemia. We suggest that, in accordance with R% criterion, laboratory reports should include the post-PEG PRL levels along with the modified reference range of this component derived from healthy individuals. It is important that adding this data to their reports will not cause any expense and will not bring any extra workload since these values have already been obtained if the laboratory reports the R% criterion of macroprolactinemia.

**Declarations of interest:** The authors declare no conflict of interest.

**References**