ORIGINAL ARTICLE

Reporting of Hyperprolactinaemia Post-polyethylene Glycol (PEG) Precipitation at Hospital Tengku Ampuan Rahimah Klang, Malaysia

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ABSTRACT

Introduction: Macroprolactinaemia is defined as hyperprolactinaemia due to excess macroprolactin in the presence of normal monomeric prolactin. Failure to identify macroprolactinaemia may result in patients being subjected to unnecessary investigations and inappropriate treatment for hyperprolactinaemia. In our centre, screening for macroprolactinaemia is currently performed at the request of the treating physician. The study thus aimed to determine the frequency of macroprolactinaemia in samples with serum prolactin ≥700 mIU/L in Hospital Tengku Ampuan Rahimah (HTAR) and to determine the presence of true hyperprolactinaemia in these cases. **Methods:** A cross-sectional study among hyperprolactinaemic subjects in HTAR, using serum specimens received by the laboratory for measurement of prolactin between October 2018 and September 2019. Samples with prolactin ≥700 mIU/L were screened for macroprolactinaemia using the polyethylene glycol (PEG) precipitation technique. Macroprolactinaemia was present when the percentage recovery of prolactin post-PEG was <40%. Assay-specific post-PEG monomeric prolactin levels were also reported, with levels above the upper limit of reference intervals indicated the presence of true hyperprolactinaemia. **Results:** A total of 101 samples were subjected to PEG precipitation. Macroprolactinaemia was found in four (4%) samples, whilst eight (7.9%) were categorised as indeterminate (percentage recovery of 40-60%). The remaining 89 (88.1%) samples had a percentage recovery >60%, hence considered negative for macroprolactinaemia. All four samples with macroprolactinaemia also had raised monomeric prolactin levels indicating the co-existence of macroprolactinaemia in subjects with true hyperprolactinaemia. Similarly, in the indeterminate group, all eight had raised monomeric prolactin levels. **Conclusion:** In one-year period in HTAR, macroprolactinaemia was detected in 4% of subjects with prolactin ≥700 mIU/L, all of whom also had raised monomeric prolactin levels. The common occurrence of both macroprolactinaemia and true hyperprolactinaemia warrants the reporting of both percentage recovery and monomeric prolactin levels post-PEG.
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INTRODUCTION

Prolactin is an anterior pituitary hormone, best known for its function in lactogenesis. Accordingly, high serum prolactin concentrations occur as part of normal physiology during pregnancy and lactation. Elevated prolactin levels may also be a sign of underlying disease, one being prolactin-secreting pituitary adenoma. Other common causes of hyperprolactinaemia include overt primary hypothyroidism and the use of antipsychotics and antidepressants. Clinical features of true hyperprolactinaemia include galactorrhoea, menstrual irregularities and infertility in women, while galactorrhoea and impotence in men. Prolactin measurement is therefore imperative in the assessment of patients presenting with such symptoms.

Prolactin circulates in three major forms; monomeric prolactin (molecular mass 23 kDa), big prolactin (molecular mass 50–60 kDa) and macroprolactin (molecular mass 150–170 kDa) (1). The latter is mainly a complex of monomeric prolactin and immunoglobulin, typically IgG (2). In contrast to the biologically active monomeric isoform, macroprolactin is considered physiologically inactive and therefore, patients do not usually exhibit symptoms characteristic of hyperprolactinaemia (3). The current prolactin immunoassays are unable to discriminate macroprolactin from the active monomeric prolactin,

and hence a high macroprolactin level results in apparent hyperprolactinaemia (3). Macroprolactinaemia, defined as hyperprolactinaemia due to excess macroprolactin in the presence of normal monomeric prolactin may result in patients being misdiagnosed as true hyperprolactinaemia and subjected to unnecessary investigations and inappropriate treatment (4).

Macroprolactinaemia has been reported to be present in 3.7% in the general population and increases to 4-40% in cases of hyperprolactinaemia (2,3,5). A recent systematic review and meta-analysis study reported a frequency of 18.9% among hyperprolactinaemia cases (6). Heterogenous indications of screening for macroprolactinaemia partly contributes to the vast difference in the detection (2,5,7). In some centres, the strategy is to screen all hyperprolactinaemia samples for macroprolactinaemia, while in others, screening is only performed if the prolactin level is above a specific cut-off set by the laboratory (2,7). Another strategy is a clinician-guided approach, whereby screening for macroprolactinaemia is performed only for asymptomatic patients with hyperprolactinaemia (2,7).

The gold-standard method for detecting macroprolactin is gel filtration chromatography (GFC), which allows for the quantification of all three variants of prolactin. Unfortunately, this method is labour-intensive and not suitable for routine use. In contrast, the PEG precipitation technique is relatively simple and has widely been used for the screening of macroprolactin in clinical laboratories (8,9). PEG precipitates immunoglobulin-bound prolactin, leaving the bioactive monomeric molecules in the supernatant, which can subsequently be measured. The post-PEG precipitation result is conventionally reported as the percentage of total prolactin recovered after PEG treatment, with <40% typically indicating the presence of macroprolactinaemia whilst a value >60% rules it out (10,11). Although commonly used, the existence of an intermediate or grey zone (percentage recovery between 40-60%), creates difficulties in its interpretation (10). Misinterpretation of the results can also occur when there are excessive amounts of both macroprolactin and monomeric prolactin. Thus, it has recently been suggested that the concentration of monomeric prolactin post-PEG and the assay-specific reference interval be reported together with the percentage recovery to avoid misinterpretation of the results (2,7).

In HTAR, serum prolactin is measured by a two-site sandwich immunoassay using direct chemiluminometric technology [Advia Centaur (Siemens Healthineers)] and screening for macroprolactinaemia is only performed following the request of the treating physician (clinician-guided approach). In such cases, the specimen will be outsourced to an external laboratory for PEG precipitation, which currently only reports the percentage recovery. The study's main aim was thus to determine the frequency of macroprolactinaemia in samples with serum prolactin ≥700 mIU/L and the presence of true hyperprolactinaemia in these cases by interpreting the post-PEG precipitation results by both percentage recovery and post-PEG serum monomeric prolactin concentration.

MATERIALS AND METHODS

Study design

This was a cross-sectional study among hyperprolactinaemic subjects in HTAR, using serum specimens received by the laboratory for measurement of prolactin concentration from October 2018 until September 2019.

Data collection

All serum specimens received for measurement of prolactin with concentrations of ≥700 mIU/L, from patients aged 18 years and above irrespective of the patient's gender, diagnosis, and treatment were included. The ≥700 mIU/L cut-off was based on the recommendations and common practices in other centres (12,13). These samples were stored at -20 degrees celsius until analysis for PEG precipitation. Exclusion criteria were insufficient sample and grossly haemolysed sample.

PEG and sample preparation

To prepare a 25% (weight/volume) PEG solution, 25g of PEG-6000 (Sigma ref. 81260) was dissolved in 60 ml distilled water at room temperature, and the volume was filled up to 100 ml after mixing. Frozen samples were allowed to thaw at room temperature, following which 250 µL was extracted and mixed with an equal volume of fresh 25% PEG solution. The solution was subsequently mixed using a vortex, centrifuged at 3000 rpm for 30 minutes, and the supernatant was measured for prolactin. Prolactin level for paired untreated serum was also measured in the same batch.

Prolactin assay

The Advia Centaur (Siemens Healthineers) prolactin assay was used for the measurement of serum prolactin before and after treatment with PEG. It is a two-site sandwich immunoassay using direct chemiluminometric technology and was calibrated against the World Health Organization 3rd International Standard 84/500. During the study period, the coefficients of variation (CV) for prolactin assay were between 1.5% and 2.2%.

Samples were classified as having macroprolactinaemia if the prolactin percentage recovery post-PEG precipitation was <40% and excluded if the result was >60%. It is reported as intermediate if the percentage recovery was between 40% and 60%. The calculation for percentage recovery was as follows:

$$\frac{\text{(Prolactin concentration post-PEG precipitation x 2)}}{\text{Prolactin concentration pre-PEG precipitation}} \times 100$$

Post-PEG prolactin concentration was adjusted by a factor of two to correct for the dilution step that took place during sample pretreatment. In addition, we also reported the monomeric prolactin level (prolactin level post-PEG precipitation) for each sample. An increase in the monomeric prolactin level i.e., a level above the reference interval for males (61-196 mIU/L) and females (66-278 mIU/L) indicates true hyperprolactinaemia (14).

Data analysis

Data were analysed using the standard statistical software package, IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. Exploratory data analysis was conducted to determine the distribution of numerical data and frequency of categorical data. Numerical data with normal distribution were presented as mean and standard deviation (SD), whereas for skewed data, they were presented as median and interquartile range (IQR).

Ethical Clearance

Ethical approval to conduct the study was obtained from the Malaysian Research Ethical Committee (MREC) Ministry of Health (KKM/NHISSEC/P19-456(6).

RESULTS

A total of 1631 samples were received for prolactin measurement during the study period. Among these, 148 (9.1%) samples had prolactin levels ≥700 mIU/L. Two samples belonged to subjects <18 years old, three had insufficient volume while 42 were repeat requests; therefore, in total, only 101 samples were subjected to PEG precipitation. The median age of the subjects was 35-year-old and ranged between 21 and 75 years old. The majority were females (n=97, 96%) and Malay (n=54, 54.5%). The most requests for prolactin measurements came from the Medical Outpatient Department (MOPD) (n=46, 45.5%). The demographic characteristics of the subjects are presented in Table I.

The median [interquartile range (IQR)] prolactin concentrations for pre-PEG and post-PEG precipitation were 1470.5 mIU/L (IQR=1265.2) and 1000.0 mIU/L (IQR=786.3), respectively. Figure 1 shows the distribution of serum prolactin concentrations before precipitation with PEG.

Frequency of macroprolactinaemia and true hyperprolactinaemia

Table II demonstrates the distribution of subjects based on the results of prolactin percentage recovery

Table I : Demographic characteristics of subjects according to gender, ethnicity and requesting department (N=101)

Characteristics	n	%
Gender		
- Male	4	4.0
- Female	97	96.0
Ethnicity		
- Malay	54	53.5
- Chinese	25	24.8
- Indian	19	18.8
- Others	3	3.0
Requesting Department		
- MOPD	46	45.5
- Obstetrics and Gynaecology	24	23.8
- Psychiatric	22	21.8
- Others	9	8.9

Table II : Frequency of macroprolactinaemia and true hyperprolactinaemia in HTAR (N=101)

	Recovery of prolactin post-PEG		
	<40%	40-60%	>60%
Total, n (%)	4 (4.0)	8 (7.9)	89 (88.1)
Post-PEG monomeric prolactin levels			
- Within reference interval	0 (0)	0 (0)	0 (0)
- Elevated (true hyperprolactinaemia)	4 (100)	8 (100)	89 (100)

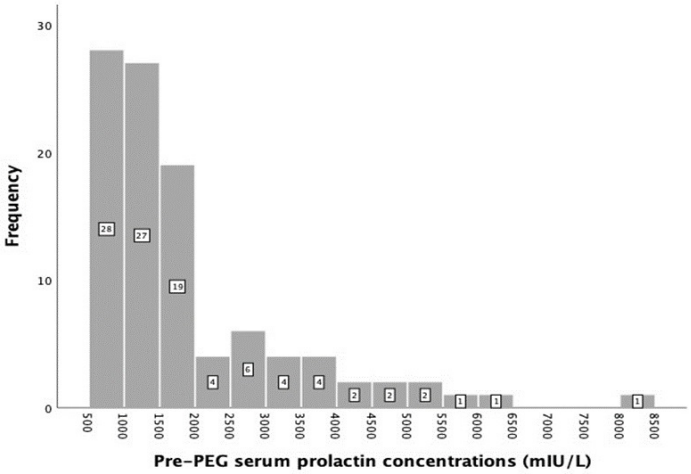

Figure 1 : Frequency distribution of serum prolactin concentration before PEG precipitation.

post-PEG. Only four (4%) samples had a percentage recovery <40%, indicating macroprolactinaemia. The samples were from two subjects with an underlying diagnosis of schizophrenia, and one each for microprolactinoma and investigation for infertility. The majority (88.1%) had a percentage recovery > 60% (61.7- 95.6%) and were considered to be negative for macroprolactinaemia. Results for eight (7.9%) samples were, however, indeterminate. All four subjects with macroprolactinaemia had raised monomeric prolactin levels. Similarly, all subjects in the indeterminate group and those with percentage recovery >60% had raised monomeric prolactin levels or true hyperprolactinaemia (Table II).

DISCUSSION

Macroprolactinaemia was detected in 4% of subjects in HTAR, similar to other studies in Malaysia (15,16). A Malaysian study which also used the Advia Centaur (Siemens Healthineers) prolactin assay and a similar protocol of screening all prolactin samples ≥700 mIU/L, found that 3.4% of their cases have macroprolactinaemia (15). Another study in Malaysia reported a macroprolactinaemia frequency of 6.8% (16). Their gender-specific prolactin assay upper reference interval was used as cut-offs for screening samples for macroprolactinaemia with prolactin measured using the Elecsys II (Roche Diagnostics) assay (16). At the moment, there are no uniform prolactin cut-off levels that indicate which samples should undergo PEG precipitation (17). Some laboratories screen all samples with hyperprolactinaemia (prolactin level above the upper limit of reference interval) whilst others specify a prolactin cut-off level, above which samples undergo screening for macroprolactinaemia irrespective of the clinical history (11, 17). Others excluded those with known physiological and pharmacological causes of hyperprolactinaemia and those with macroprolactinoma as the finding of macroprolactinaemia in such cases will unlikely change the patient's management. The Pituitary Society recommends testing in patients with moderately elevated prolactin (500–3000 mIU/l) levels and atypical symptoms (e.g., headaches and diminished libido in the presence of regular menses), whereas the Endocrine Society recommends screening in asymptomatic patients with hyperprolactinaemia (8,18). However, clinical features are not necessarily reliable as most of the symptoms are relatively non-specific and can also be attributed to other causes (2,7,17). Hence, many laboratories have moved towards universal screening for all cases of hyperprolactinaemia (5,17).

As mentioned, there are recommendations to report the monomeric prolactin levels post-PEG together with percentage recovery as it may detect cases where elevated monomeric prolactin co-exists with macroprolactinaemia, as demonstrated in our study as well as others (7, 16, 21). Jasam et al 2009, noted that nine out of 16 (56.3%) subjects with macroprolactinaemia also had elevated monomeric prolactin (21). Although the proportion of those with macroprolactinaemia was shown to decreased with increasing pre-PEG prolactin levels, there were cases where macroprolactinaemia existed in samples with a relatively high pre-PEG prolactin, even with levels above 4500 mIU/L (7). This reiterate the importance of reporting both percentage recovery and monomeric prolactin levels post-PEG.

The monomeric prolactin levels post-PEG for specific prolactin assays have been reported in the literature (14, 21). Each laboratory, however, must ensure that the monomeric prolactin reference intervals suit its population, as different reference intervals were obtained from different studies despite using the same assay and PEG protocol. For example, Jassam et al. 2009 established a significantly higher post-PEG upper reference limit (38% higher) despite using the same protocol and immunoassay system (Advia Centaur) as Beltran et al. 2008 (14, 21). Relying on the Beltran et al. reference limit would have misclassified five of their patients as not having macroprolactinaemia.

Our study also noted eight (7.9%) subjects with indeterminate percentage recovery, which was similar to previous studies (6, 21). In another study, four out of 22 subjects with intermediate percentage recovery showed evidence of macroprolactinaemia on analysis with GFC (21). Unfortunately, GFC was not performed in these samples, which is considered a limitation of our study. Another limitation was the use of an assay and gender-specific monomeric reference interval obtained from another study, as ideally the laboratory's own reference interval should be established.

Based on the current study, we recommend our centre to routinely screen macroprolactin in samples with prolactin level ≥700 mIU/L using the PEG precipitation technique. In addition, both the percentage recovery

and the monomeric prolactin levels post-PEG should be reported as the former classifies the presence or absence of macroprolactinaemia whist the latter would be able to detect cases where elevated monomeric prolactin co-exists with macroprolactinaemia.

CONCLUSION

In HTAR, macroprolactinaemia was detected in 4.0% of hyperprolactinaemia cases (prolactin ≥700 mIU/L), all of which also had raised monomeric prolactin levels. Screening for macroprolactinaemia should be recommended as a routine practice in cases of hyperprolactinaemia. Both the percentage recovery and the monomeric prolactin levels should be reported to provide a clearer interpretation of the PEG precipitation results.

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