Journal of Endocrinology, Metabolism and Diabetes of South Africa 2023; 28(3):100–104
https://doi.org/10.1080/16089677.2023.2215046

JEMDSA
ISSN 1608-9677 EISSN 2220-1009

RESEARCH ARTICLE

Relevance and therapeutic implication of macroprolactinemia detection using PEG 6000 in women of childbearing age with hyperprolactinemia: experience at a tertiary hospital

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Introduction: Macroprolactin may interfere with hormonal assay and falsely increase serum prolactin levels. Therefore, failure to identify macroprolactinemia can lead to inappropriate investigations and treatment in women already susceptible to anxiety and stress. The aim of this study was to identify macroprolactinemia among women of childbearing age with hyperprolactinemia.
Materials and methods: A cross-sectional study was conducted in a tertiary care setting at the endocrine unit. Study participants were recruited from both endocrine and gynaecological outpatient consultation services. They were women of childbearing age (18 to 49 years) consulting for signs and symptoms of gonadal dysfunction or hyperprolactinemia (PRL > 25 ng/ml). Total prolactin was measured using a Human direct ELISA method. Polyethylene glycol 6000 (PEG 6000) precipitation was used to detect macroprolactin.
Results: A total of 33 women with a mean age of 31 ± 7 years (range 21–48) were enrolled. Twenty-seven (81.8%) participants were symptomatic, the majority (23/27) (69.7%) reported having galactorrhoea, and 21 (63.4%) women reported having an irregular menstrual cycle. The median pre-precipitation prolactinemia reduced significantly after PEG precipitation from 61.2 (IQR 33.2–115.9) ng/ml to 33.8 (IQR 17.9–70.5) ng/ml, $p < 0.001$. After PEG precipitation, five participants had a serum prolactin recovery rate below 60% and, therefore, a prevalence of macroprolactinemia at 15.2%. Four out of five (80%) women with macroprolactinemia presented with the symptoms amenorrhea, oligomenorrhea, and galactorrhoea.
Conclusion: PEG 6000 permitted the detection of macroprolactinemia in women of childbearing age with hyperprolactinemia who otherwise would have been subjected to unnecessary medical investigations and treatment.

Keywords: hyperprolactinemia, macroprolactin, PEG, prolactin, prolactin recovery rate

Introduction

Prolactin (PRL) is a single-chain protein synthesised and released by lactotroph cells of the anterior pituitary gland.[1] Its secretion is regulated by dopamine, which has an inhibitory effect on lactotroph cells.[1] When prolactin secretion increases in the absence of pregnancy, clinical symptoms such as galactorrhoea and irregular menstrual cycles may occur. These menstrual abnormalities include spaniomenorrhoea and amenorrhoea, which may contribute to infertility. Hyperprolactinemia is a well-recognised hormonal aetiology of infertility among women of childbearing age. It affects 30–40% of infertile women and 15–20% of women with menstrual disorders.[2] Impairment of gonadal function and, ultimately, infertility result from suppression of the pulsatile secretion of gonadotrophins.[3] The majority of prolactin molecules present as monomers that are biologically active, but these may also exist as macromolecules (macroPRL) known as big and big-big prolactin, which may interfere with laboratory measurements of the protein.[4] According to Vilar *et al*., in 2019, two Brazilian series reported macroPRL as the third cause of non-physiological hyperprolactinemia after drugs and pituitary adenomas.[5] All three forms of prolactin are indistinguishable by routine laboratory assays.

Prolactin is measured in the biochemistry laboratory using immunometric methods on patient serum. The gold standard method to distinguish macroprolactin (big and big-big forms) from the monomeric form is gel-filtration chromatography, which is an expensive and time-consuming process.[6] Polyethylene glycol (PEG) has been used as a rapid and less costly method to screen for macroprolactinemia during routine analysis in clinical laboratories and research settings.[7] The presence of macroPRL may interfere with hormonal assay and falsely increase prolactin values.[1] As a result, misdiagnosis, unnecessary investigation and prescription of inappropriate treatments can be experienced by these women who are already susceptible to anxiety and stress related to infertility.

Considering the psychological and financial impact these diagnostic errors may have on patient management and prognosis, we sought to determine the prevalence and effect of macroprolactinemia among Cameroonian women of childbearing age

who present with signs of gonadal dysfunction using the PEG precipitation method, which is an affordable and easily reproducible method used in clinical biology laboratories.

Materials and methods

Study design, setting and population

We undertook a cross-sectional study at the endocrine unit of the Yaoundé Central Hospital, a tertiary hospital in Cameroon, over 12 months (June 2020 to June 2021). Our study participants were drawn from outpatient consultations in the endocrinology unit of the above-mentioned hospital. This included all consecutive women of childbearing age (18 to 49 years) consulting for gonadal dysfunction (galactorrhoea, spaniomenorrhoea and/or amenorrhoea) or hyperprolactinemia (PRL > 25 ng/ml).[8] Pregnant or nursing mothers were not included in the study.

Clinical and biological sample collection

We provided all the information related to decision-making as well as additional concerns on consent and information forms. After this, we obtained written informed consent from each individual who agreed to participate in the study. The participants were interviewed on the basis of a pre-established questionnaire. The clinical evaluation included: anthropometric parameters, vital parameters, gynaecologic and pharmacologic history, medical history and breast examination. After clinical examination, we collected a five millilitres venous blood sample in a dry tube after ensuring asepsis. The sample was left to clot at room temperature and then centrifuged at 1 500 revolutions/minute for 10 minutes. The resulting serum was collected and stored at −20°C for prolactin and macroprolactin assay.

Prolactin assay

Prolactin was measured from serum samples through the direct ELISA method using reagents supplied by Human (Human ELISA test for quantitative determination of prolactin KIT® REF 53030 pack 21002; Human, Wiesbaden, Germany) according to the manufacturer's procedure. Reagents for the assay are ready-to-use and pre-dispensed in sealed reagent strips. All of the assay steps were performed manually by the investigator.

Macroprolactin measurement

Macroprolactin was measured following precipitation with 25% polyethylene glycol 6000[9] (Polyethylenglykol 6000, ROTIPURAN® Ph.Eur. Carl Roth GmbH, Karlsruhe, Germany).

PEG precipitation procedure

Four hundred microlitres (400 µl) of 25% PEG solution were added to 400 µl of patient serum at room temperature. After mixing and stabilising at room temperature for 30 minutes, this tube was centrifuged at 1 800 revolutions per minute for 30 minutes at 20°C. Then, the supernatant was isolated for PRL analysis as previously described. After correction for dilution, the results were compared with those obtained from unprecipitated serum. The PRL recovery rate (RR) was calculated using the formula:

$$\text{Recovery rate (RR\%)} = \frac{2*\text{PRL level post} - \text{PEG precipitation}}{(\text{PRL level pre} - \text{PEG precipitation})} *100$$

Prolactin recovery rate of less than 60% was considered a significant level of macroprolactin, while a recovery rate equal to or above 60% indicated a non-significant level of macroprolactin. Laboratory analysis was performed at the CIAB EXACT laboratory, a private laboratory located in the same town as the hospital.

Statistical analysis

Data were analysed using SPSS software version 21.0 (IBM Corp, Armonk, NY, USA). Quantitative variables were reported as means and standard deviations or median and interquartile range, depending on the distribution of data, whereas qualitative variables were expressed in terms of frequencies and proportions. The results of the prolactin analysis were expressed as a percentage of PRL recovery after precipitation.

Ethical considerations

The research protocol was submitted to the Regional Ethics Committee of Human Research of the Center Region (Clearance number: 1591/CRERSHC/2020). Our study was carried out in strict accordance with the ethical principles of the Declaration of Helsinki. Results were disclosed to participants by the investigators at the end of the study.

Results

During the study period, 77 women were eligible for participation in our study. We did not include 41 due to normal serum prolactin or failure to give their informed consent. We later excluded 3 participants due to incomplete data, giving a final participant number of 33 participants. The results that follow are those of the 33 participants who completed the study (Figure 1).

Clinical characteristics of the study population

Overall, the women in our study population had a mean age of 31.7 ± 6.9 years (range 21–48). The majority of the participants reported symptoms of hyperprolactinemia. As regards the symptoms that the women reported, 23 (69.7%) had galactorrhoea while 6 (18.2%) did not have any symptoms. Other symptoms reported were spaniomenorrhoea, amenorrhea and infertility (Figure 2).

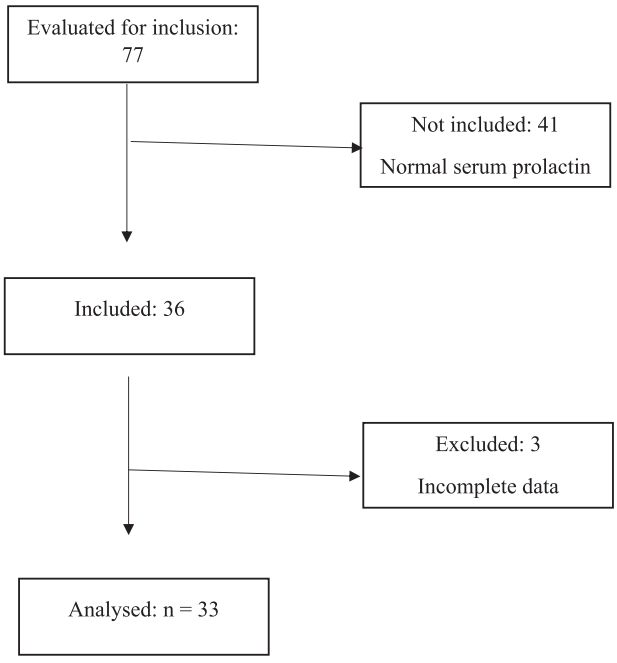

Figure 1: Flowchart of participants.

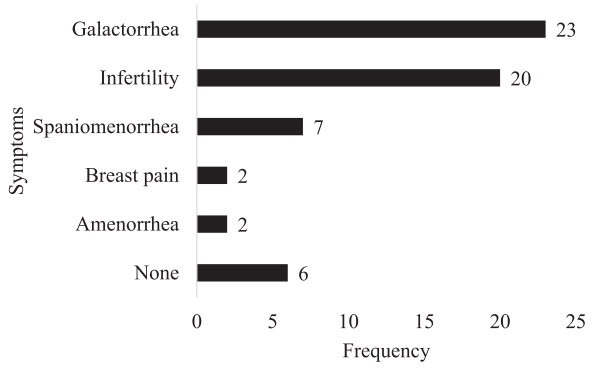

Figure 2: Distribution of hyperprolactinemia-related symptoms in the study population.

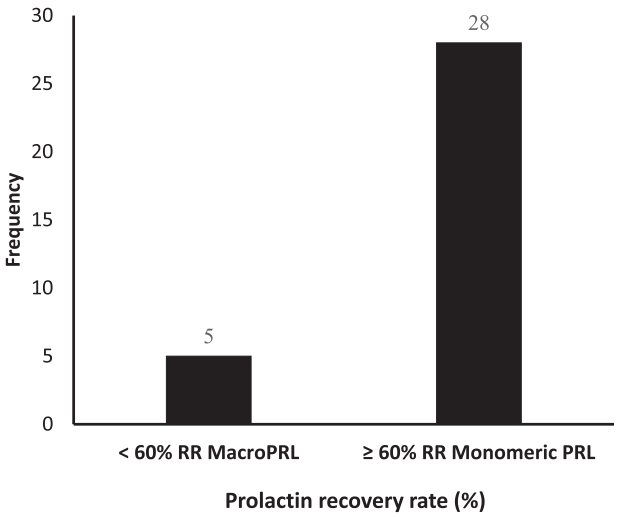

Figure 4: Prevalence of macroprolactinemia in the study population.

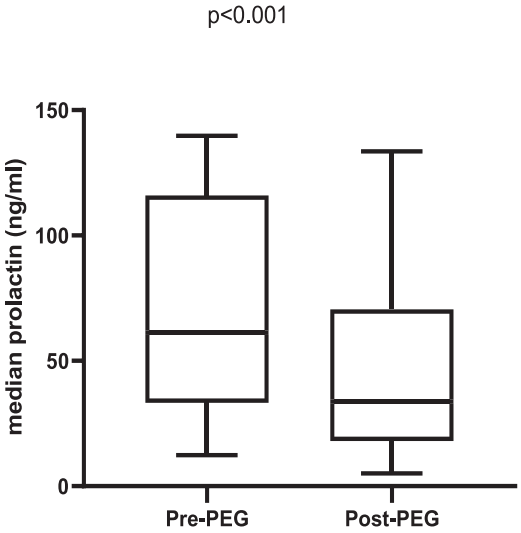

Figure 3: Prolactin levels before and after PEG precipitation.

Basal prolactinemia, post-PEG prolactinemia and prolactin recovery rate

Median pre-PEG precipitation prolactinemia was 61.2 (IQR 33.2–115.9) ng/ml. After PEG precipitation, prolactin level decreased significantly from 61.2 (IQR 33.2–115.9) ng/ml to 33.8 (IQR 17.9–70.5) ng/ml ($p < 0.001$) (Figure 3). After PEG precipitation, five participants had a prolactin recovery rate below 60%, representing those with significant macroprolactinemia (Figure 4). Thus, the prevalence of macroprolactinemia in our study population was 15.2%.

To identify factors that could be related to the presence of macroprolactin, we subdivided the study population into two groups according to the PRL RR: a macroprolactinemia group (RR < 60%, n = 5) and a true hyperprolactinemia group (RR > 60%, n = 28). As shown in Table 1, the different hyperprolactinemia-related symptoms and clinical parameters were comparable between the two groups. Moreover, four out of the five women with macroprolactinemia presented with symptoms of hyperprolactinemia.

Table 1: Clinical characteristics, past history and symptoms of study participants with macroprolactinemia and true hyperprolactinemia

Factor	Parameters Macro-prolactin (%) n = 5	Prolactin Monomeric prolactin (%) n = 28
Amenorrhea	0	2 (7.1)
Galactorrhoea	4 (80)	19 (67.9)
Spaniomenorrhoea	0	7 (25)
Infertility	3 (60)	17 (60.7)
No symptoms	1 (20)	5 (17.9)
Irregular menstrual cycle	2 (40)	19 (67.9)
Hormonal contraception	0	2 (7.1)
Obstetric history	4 (80)	13 (46.4)
Desire to conceive	3 (60)	17 (60.7)
Pathology of the female genital tract	0	12 (42.9)
Age (years)*	33 ± 5.15	31.5 ± 7.28
DBP (mmHg)*	82 ± 16	76 ± 6
SBP (mmHg)*	114 ± 20	110 ± 10
BMI (kg/m^2)*	25.0 ± 2.89	28.48 ± 5.6

* Data are mean ± standard deviation; BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure.

Discussion

The aim of this study was to detect macroprolactinemia using PEG 6000 among a group of Cameroonian women of childbearing age with hyperprolactinemia. Several methods have been described in the literature for the diagnosis of macroprolactinemia. These methods include the polyethylene-glycol (PEG) precipitation method for screening, and the confirmative and qualitative examinations include gel chromatography (GFC), protein A/G column and ^{125}I-PRL binding studies.[10] Despite the fact that PEG is not highly specific, comparison with other methods showed that PEG precipitation was superior and showed the best concordance with GFC. Moreover, PEG is widely used because it is simple and inexpensive. For these reasons, PEG was validated against gel filtration chromatography, which is a gold standard for the diagnosis of macroprolactinemia, and it appears to be a cost-effective screening method that is affordable to use in a resource-limited setting. A value of less than 40% recovery after PEG precipitation of prolactin has been validated for the diagnosis of macroprolactinemia. With this cut-off, there is a 100% sensitivity in picking up macroprolactin.[11]

We found that 5 out of 33 women included in this study had a prolactin recovery rate of less than 60%, given that the prevalence of macroprolactinemia is 15.2%.

Consistent with this finding, Soh and colleagues recently pooled data from 67 studies (n = 3 770) and reported an overall prevalence of 18.9% for macroprolactinemia in patients with hyperprolactinemia.[12] However, the prevalence reported in this study is lower compared to the prevalence reported in other settings. Fahie-Wilson *et al.* reported a prevalence of macroprolactin at 20% among English hyperprolactinemic patients,[1] while this prevalence ranged from 15% to 35% in Croatian hyperprolactinemic populations[13] and from 8% to 42% in 9 European series.[6] All these showed that macroprolactin is not uncommon in studied populations and should be screened. Overall, the prevalence of macroprolactinemia in patients with hyperprolactinemia varies across the world's regions and countries from 8% to 66% in respect of the cut-off value for hyperprolactinemia used and also according to study designs.[12] Few studies are available in Africa and in the sub-Saharan region in particular. Thus, this study is among the first that sought to determine the prevalence of macroprolactinemia in a cohort of hyperprolactinemic patients from sub-Saharan Africa.

It was argued that macroprolactin is confined to the vascular system and has limited access to the prolactin receptor of target organs, resulting in limited bioactivity in vivo and asymptomatic hyperprolactinemia.[13] Consistent with this hypothesis, there was no association between macroprolactinemia and common symptoms of hyperprolactinemia in our study population. Macroprolactinemia is usually suspected when hyperprolactinemic patients do not present with symptoms of hyperprolactinemia. However, some patients present with symptoms such as menstrual disorders, galactorrhoea or signs of hypogonadism, which are thought to reveal concomitant pathologies such as polycystic ovarian disease (PCOS), monomeric prolactinemia or psychogenic erectile dysfunction in men.[6] Isik *et al.* in 2012, among others, reported a prevalence of hyperprolactinemia symptoms in up to 45% of patients with macroprolactinemia.[6,12] In addition, Olukoga *et al.* (2002) suggested that the macroprolactin complex may dissociate in vivo in some cases, releasing bioactive, monomeric prolactin that causes the symptoms in these patients. This release of monomeric PRL, which may result from intermittent dissociation from the low-affinity, high-capacity IgG antibody to which it is bound in macroprolactin, may contribute to the development of symptoms of hyperprolactinemia.[11]

Prolactin is measured in clinical laboratories using plasma and serum, with the aid of automated immunoassays. These are techniques that either use labels or do not. Labels such as enzymes, luminescent substances, radioisotopes and fluorescent substances may be used in a homogeneous or heterogeneous procedure. Heterogeneous immunoassays request bound/free separation steps in order to determine the concentration of analytes.

Current methods include automated immunoassays, which use a two-site immunometric or sandwich principle. Prolactin in the sample reacts with the capture antibody and the labelled detection antibody. The signal generated by the labelled antibodies is directly proportional to the sample prolactin concentration. Radioimmunoassay, immunoradiometric assay and enzyme assays give a wide range of formats for PRL measurement.

Most prolactin assays have as a setback cross-reactivity with other related molecules, such as growth hormone, human placenta lactogen and interference from macroprolactin.[2]

However, modern immunometric assays are elaborated, taking these setbacks into account. Firstly, they are free from cross-reactivity of growth hormone and placental lactogen and, secondly, they are optimised with blocking agents to avoid interference from heterophilic antibodies. Moreover, most prolactin assays are standardised to the World Health Organization's third international prolactin standard 84/500, which contains 23 kDa PRL derived from human pituitaries.[2]

Unfortunately, we did not have direct ELISA-specific prolactin reference intervals for PEG-treated serum samples to interpret median prolactin reduction. These could have been provided by a similar analysis of normoprolactinemic participants to determine their post-PEG prolactin ranges, as described by Gibney *et al.*, who proposed the use of absolute post-PEG prolactin values instead of the prolactin recovery ratio.[5] Furthermore, we used direct ELISA for prolactin assay, which also presents some disadvantages. Antigen immobilisation is not specific, resulting in potentially high background interference. There is no signal amplification and immunoreactivity of the primary antibody may be adversely affected by labelling with enzymes.

Conclusion

PEG 6000 has identified macroprolactinemia in our studied population, with a prevalence of 15.2%. This allowed us to avoid extensive investigations and excessive treatment of hyperprolactinemia. Thus, screening for macroprolactin should be included in the routine investigation of all hyperprolactinemic patients when they are asymptomatic. PEG appears to be cost-effective for the detection of macroprolactin in a low-resource setting.

Abbreviations

MacroPRL: Macroprolactin
PEG 6000: Polyethylene glycol 6000
PRL: Prolactin

Disclosure statement – No potential conflict of interest was reported by the authors.

Funding – The authors reported there is no funding associated with the work featured in this article.

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Received: 03-03-2023 Accepted: 15-05-2023