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Original Article

# Low prevalence of macroprolactinaemia among patients with hyperprolactinaemia screened using polyethylene glycol 8000

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**الملخص**

**أهداف البحث:** فرط بروﻻكتين الدم الجسيم هو سبب حميد معروف لفرط بروﻻكتين الدم. التفريق بين فرط بروﻻكتين الدم الجسيم وفرط بروﻻكتين الدم الحقيقي مهم، لأن الأول لا تطلب أي علاج. تم إجراء هذه الدراسة لتحديد مدى انتشار فرط بروﻻكتين الدم الجسيم بين مرضى فرط بروﻻكتين الدم باستخدام البولي إيثيلين جلايكول ٨٠٠٠.

**طرق البحث:** أجريت دراسة مستعرضة على مرضى تم تشخيصهم بفرط بروﻻكتين الدم بمستشفى سينز ماليزيا الجامعي من عام ٢٠١١م إلى ٢٠١٣م. وقيس البروﻻكتين من مصل المرضى باستخدام كوباس إي ٤١١، وتم علاج نفس المصل باستخدام البولي إيثيلين جلايكول ٨٠٠٠، للتفريق بين فرط بروﻻكتين الدم الحقيقي وفرط بروﻻكتين الدم الجسيم. واستخدم شفاء البروﻻكتين لأقل من ٤٠٪ كمؤشر على وجود فرط بروﻻكتين الدم الجسيم.

**النتائج:** ضمت مجموعة الدراسة ١٣٣ مريضا بفرط بروﻻكتين الدم، منهم ١٢٠ (٩٠٪) إمرأة و١٣ (٩.٨٪) رجلا، أعمارهم ١٨-٦٨ عاما، ومعدل العمر لهم (الانحراف المعياري) ٣٤.٣٧ (١١.٧٥) عاما. وُجد فرط بروﻻكتين الدم الجسيم لدى تسع سيدات بمعدل انتشار ٦.٨ ٪ ( ٩٥٪ فترة ثقة: ٢.٤٪، ١١.١٪).

**الاستنتاجات:** معدل انتشار فرط بروﻻكتين الدم الجسيم الذي تم اكتشافه باستخدام البولي إيثيلين جلايكول ٨٠٠٠ بين المرضى الذين تم تشخيصهم بفرط بروﻻكتين الدم كان منخفضا. وأظهر فحص فرط بروﻻكتين الدم الجسيم باستخدام البولي إيثيلين جلايكول ٨٠٠٠ أن معظم المرضى الذين حضروا بفرط بروﻻكتين الدم في مستشفى سينز ماليزيا الجامعي كان فرط بروﻻكتين الدم الحقيقي.

**الكلمات المفتاحية:** فرط بروﻻكتين الدم؛ فرط بروﻻكتين الدم الجسيم؛ البولي إيثيلين جلايكول ٨٠٠٠

**Abstract**

**Objectives:** Macroprolactinaemia is a known benign cause of hyperprolactinaemia (hyperPRL). Differentiating macroprolactinaemia and hyperPRL is important, as macroprolactinaemia does not require treatment. This study was conducted to determine the prevalence of macroprolactinaemia among hyperPRL patients through the use of polyethylene glycol 8000.

**Methods:** From 2011 to 2013, a cross-sectional study was conducted on patients diagnosed with hyperPRL in Hospital Universiti Sains Malaysia (HUSM). Sera from these patients were measured for PRL using cobas e411 (Roche Diagnostics, Indianapolis, USA) (sandwich principle) and the same sera were treated with polyethylene glycol (PEG) 8000 to differentiate true hyperPRL from macroprolactinaemia. PRL recovery of less than 40% was used as an indicator of the presence of macroprolactin.

**Results:** A total of 133 hyperPRL patients, 120 (90%) women and 13 (9.8%) men, aged 18–68 years, with mean (standard deviation) age 34.37 (11.75) years comprised this study cohort. Nine female patients were found to have macroprolactinaemia with an estimated prevalence of 6.8% (95% CI: 2.4%, 11.1%).

**Conclusions:** The prevalence of macroprolactinaemia detected using PEG 8000 among patients diagnosed as hyperPRL was low. Screening for macroprolactin using

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PEG 8000 indicated that the majority of patients who presented with hyperPRL in HUSM were true hyperPRL.

**Keywords:** Hyperprolactinemia; Macroprolactin; Macroprolactinemia; PEG 8000



## Introduction

Prolactin (PRL) is a polypeptide hormone, consisting of 199 amino acids that is synthesized in and secreted by lactotroph, which are specialized cells of the anterior pituitary gland. PRL is secreted episodically by the anterior pituitary and is primarily under tonic inhibitory control of the hypothalamus.[1]

PRL is synthesized as a prehormone with a molecular weight of 26 kDa.[2] When the prehormone is proteolytically cleaved, the resulting mature polypeptide has a molecular mass of 23 kDa, and this monomeric form accounts for the majority (85%) of total PRL in the serum of normal subjects.[1] In addition to monomeric PRL, other molecular mass variants of PRL can be demonstrated in serum. Big PRL has a molecular mass in the 50 kDa range and is thought to be a covalently bound dimer of PRL, accounting for approximately 10–15%. Big big PRL or macroprolactin, which has a molecular mass of more than 150 kDa, usually contributes a small, though variable amount to circulating levels.[2] Moreover, post-translational modification of pituitary PRL generates a variety of additional species, including glycosylated and phosphorylated variants, together with 14, 16 and 22 kDa proteolysed forms,[1] and has three intramolecular disulphide bonds.[3]

Physiological levels of PRL are higher during pregnancy and lactation than otherwise, and mean serum levels are higher in women than in men.[2] HyperPRL is a condition of excess monomeric PRL. The clinical syndromes of hyperPRL are galactorrhoea, oligomenorrhoea, amenorrhoea, and infertility in women; and reduced libido, oligospermia, impotence and galactorrhoea in men.[4] HyperPRL has an estimated prevalence of 15% in women with secondary amenorrhoea, a condition that affects at least 3% of women of reproductive age.[5]

In general, macroprolactin is a non-bioactive prolactin isoform, usually composed of a PRL monomer and an IgG molecule that has a prolonged clearance rate similar to that of immunoglobulins. This isoform is clinically non-reactive but interferes with immunological assays used for the detection of PRL.[6] When the serum of a patient with hyperPRL contains mostly big big PRL, the condition is termed macroprolactinaemia. Because certain laboratories fail to screen hyperprolactinaemic sera for macroprolactin, this may lead to misdiagnosis and unnecessary medical and surgical intervention[7] or delayed diagnosis and inappropriate treatment.[8,9]

Screening hyperprolactinaemic sera for the presence of misleading concentrations of macroprolactin is readily performed in biochemistry laboratories, although the procedures are not automated. The most widely employed method is to treat the hyperprolactinaemic serum with PEG, which precipitates out high-molecular weight constituents including immunoglobulins. Re-assay of the serum for PRL will then identify those sera, which yield values within the relevant normal range indicative of macroprolactinaemia, and not true hyperPRL.[10]

## Materials and Methods

A cross-sectional study was conducted in 2013 involving patients aged ≥18 years old, in Kelantan, who were investigated for hyperPRL, and who attended Endocrine Clinic HUSM from 2010 to 2013. PRL levels which were above the reference range for our hospital for age and sex (female-non pregnant: 0.21–1.0 nmol/L, male: 0.17–0.65 nmol/L) were included in this study. These interval values were used, as hyperPRL was defined as a level of PRL above the upper limit of normal PRL level, in a single measurement, as described by Melmed et al. 2011.[11]

The number of serum samples required to determine the prevalence of patients with macroprolactinaemia was calculated using the one-sample proportion formula, and with the Type I error and study precision at 5%, the number of serum samples required was from 196 subjects.

Inclusion criteria in this study were: patient with PRL level of >0.65 nmol/L for male patients and >1.0 nmol/L for female patients. We excluded patients with inadequate or missing serum samples and patients with more than 30% missing data. After applying inclusion and exclusion criteria, only 133 serum samples were available. Since the calculated sample size was larger than the number of serum samples available, no sampling method was applied and all serum samples were included in the study. These serum samples were stored at −20° C until further analysis.

Data on presenting symptoms and medical history were extracted from hospital medical records. This study was approved by the Human Research Ethics Committee of USM (HREC) USMKK/PPP/JEPeM [263.4(1.4)]. All aspects of this study comply with the Declaration of Helsinki.

A volume of 250 μL of the patient's serum was treated with 250 μL of PEG 8000 (25% w/v) solution. The mixtures were mixed for approximately ten seconds in a rotating shaker and centrifuged between 1500 and 10,000 g for five minutes. The supernatant was measured for PRL and results were expressed as percentage of PRL recovery. The recovery of PRL post-PEG was calculated using the following formula: % Recovery post PEG precipitation = (PRL value post PEG precipitation/PRL value in pre PEG) × 2 × 100%.

Data were entered and analysed using SPSS version 21. Exploratory data analysis was conducted to determine the distribution of numerical data and frequency of categorical data. Numerical data with normal distribution are presented as the mean and standard deviation (SD), whereas for skewed data, data are presented as median and interquartile range (IQR). The prevalence of patients with macroprolactinaemia and 95% CI was calculated.