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ORIGINAL ARTICLE

Importance of screening for macroprolactin in all hyperprolactinaemic sera

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Abstract

Introduction: Prolactin (PRL) exists in different forms in human serum. The predominant form is monomeric PRL (molecular mass 23 kDa) with smaller amounts of big PRL (molecular mass 50–60 kDa) and at times macroprolactin (molecular mass 150–170 kDa). Macroprolactin, generally considered to be biologically inactive, accounts for the major part of prolactin in some patients. Different immunoassays for prolactin differ in reactivity with this macromolecular complex. *Aim:* The present study was undertaken to assess the incidence of macroprolactinaemia in our cohort of hyperprolactinemic patients. *Method:* 204 samples with hyperprolactinemia were evaluated for macroprolactinemia by polyethylene glycol (PEG) precipitation and gel filtration chromatography (GFC). Recoveries ≤60% after PEG precipitation were considered to have macroprolactinaemia. *Results:* A total of 43 (21%) of these patients had less than 60% recovery after PEG precipitation. GFC confirmed that in seven of these patients macroprolactin was the major part of the prolactin. Recoveries were < 40% PEG precipitation in these samples. Combined macro and hyperprolactinemia was observed in two samples and the recovery after PEG precipitation was >40% but ⩽50%. The incidence of macroprolactinemia in our cohort of hyperprolactinaemic patients was noted to be 4.4%. *Conclusion:* Macroprolactin is a significant cause of misdiagnosis, unnecessary investigation, and inappropriate treatment and hence it is useful to screen all patients with high PRL levels with PEG precipitation and to apply GFC to samples with recoveries <50%.

Keywords: Macroprolactin, hyperprolactinaemia, PEG precipitation, Gel filtration chromatography

INTRODUCTION

Hyperprolactinemia is a common cause of galactorrhea, amenorrhea, and infertility in women.[1] The diagnosis depends on the measurement of circulating prolactin. Prolactin (PRL) circulates in a variety of forms. In normal sera, monomeric prolactin with a molecular mass of 23 kDa accounts for 85–95% of the prolactin present and a 50-kDa species (big prolactin) makes up <10%. Big big prolactin, or macroprolactin, a prolactin- IgG complex with a molecular mass of ~ 150 kDa, accounts for a small but variable percentage of total prolactin.[2-4] However, because of its high molecular mass, macroprolactin is confined to the vasculature and hence exhibits limited bioactivity *in vivo*. As a consequence, subjects whose hyperprolactinemia can be accounted for by the presence of macroprolactin, may not exhibit the classic signs or symptoms of the hyperprolactinemia.

Even though macroprolactin is considered to be biologically inactive, it is measured by immunoassay for PRL. Macroprolactin is detected to varying degrees by all PRL immunoassays[5-8] and its presence commonly leads to diagnostic confusion and misdiagnosis.[9] Information obtained from both the United Kingdom National External Quality Assurance Scheme (NEQAS) and the College of American Pathologists, external quality control system indicated that throughout the UK and the United States, approximately 5–l5% of hyperprolactinaemic sera may be entirely accounted for by the presence of macroprolactin.[9] The distinction between true hyperprolactinaemia and macroprolactinaemia cannot reliably be made on the basis of clinical

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presentation alone. As a consequence, screening for macroprolactin must be included in the routine investigation of all hyperprolactinaemic patients.

Precipitation with polyethylene glycol (PEG) is widely used to detect the presence of macroprolactin in hyperprolactinaemic samples.[10-13] Recovery of PRL of >60% after precipitation with PEG 6000 indicates that macroprolactin is not present in significant amounts.[14] When recovery is ≤60%, macroprolactin may be present and the lower the recovery the more certain this is, with recoveries, ≤ 40% typically consistent with the presence of substantial quantities of macroprolactin.[13] Gel filtration chromatography (GFC) is regarded as the reference technique and this may be used to investigate cases where the recovery post-PEG is close to the cut-off.[10] The present study was undertaken to examine the incidence of macroprolactinaemia in our cohort of hyperprolactinemic patients

METHODS

ADVIA Centaur ® (Siemens Medical Solutions Diagnostics, USA) immunoassay is routinely used in our laboratory to estimate PRL. 204 sera samples (184 women and 20 men) with PRL levels more than 700 mIU/L were collected over a period of one year. These samples were from a general endocrinology service of teaching hospital and tertiary referral centre. The hospital records of these patients were reviewed retrospectively. Data on presenting symptoms and drug history and MRI findings were noted. These samples were stored at -20^{o} C until further analysis. This study was approved by the Medical Ethics Committee of the hospital.

A volume of 250 μL of the patient sera with PRL levels of ≥ 700 mU/L were treated with 250 μL of PEG 6000 (25% w/v) solution per the protocol described by Suliman *et al*.[10] The mixtures were subjected to mixing for a minute with a vortex and centrifuged at 3000 rpm for 30 minutes. PRL was measured both in untreated serum and in the supernatant, and results were expressed as percentage of PRL recovery. The recovery of PRL post-PEG was calculated as (post-PEG PRL ÷ pre-PEG PRL) x 100%. All samples with ≤60% recovery of PRL were further tested by GFC for confirmation of presence or absence of macroprolactin. Any sample with PEG recovery of >60% was excluded of macroprolactin. Reproducibility of the PEG precipitation test was evaluated in three different serum samples, studied five times each, on different days. Intra-assay and inter-assay coefficient variations were ± 3·74% and ±7 %, respectively. Samples with recovery of ≤60% were further analysed by gel filtration chromatography.[11] Gel filtration chromatography was performed on Sephadex G-100 optimized to separate macro and monomer PRL. Up to 1 mL of serum was applied to the column and eluted with Tris-buffered saline, pH 7.4. Fractions (1.0 mL) were collected and prolactin was measured in ADVIA Centaur. Macro and monomeric prolactin were determined from the area under the peaks (AUC) using GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

RESULTS

Prolactin levels in the 204 samples studied ranged from 733 -35000 mIU/L. Forty-three samples (21%) had post-PEG recovery level of ≤ 60%. Using the most commonly published cutoff of < 40% PRL recovery, 3.43% (7/204) samples were suspected of having macroprolactin predominantly. The remaining 36 samples had recovery >40% but less than 60%.

Macroprolactin was the predominant form in all the samples which had post-PEG recovery of <40% (75 to 99% macroprolactin) except one sample with PRL level of 5932 mIU/L which had elevated levels of both macro and monomeric forms (60% macro and 40% monomeric PRL) as shown in Figures 1 and 2. Of the 36 samples which had post PEG recovery of between 40-60%, only two samples had low to moderate amount of macroprolactin 25 and 35% respectively. Both of these had post PEG recovery between 40% and 50%. In the remaining 34 hyperprolactinemic samples the monomeric PRL was the major circulating form (Figure 3). However, subjects positive for macroprolactin also had elevated monomeric prolactin. We also noted that the samples with >50% post-PEG recovery contained predominantly monomeric prolactin.

GFC confirmed the presence of macroprolactin in 9 samples (≥20% macroprolactin). Therefore the prevalence of macroprolactinaemia in our study population was 4.4% (9/204). The results of gel filtration chromatography showed that PEG recovery of <40% was 100% sensitive for detecting the presence of significant macroprolactinaemia while values >50% indicated absence of this condition. Values of

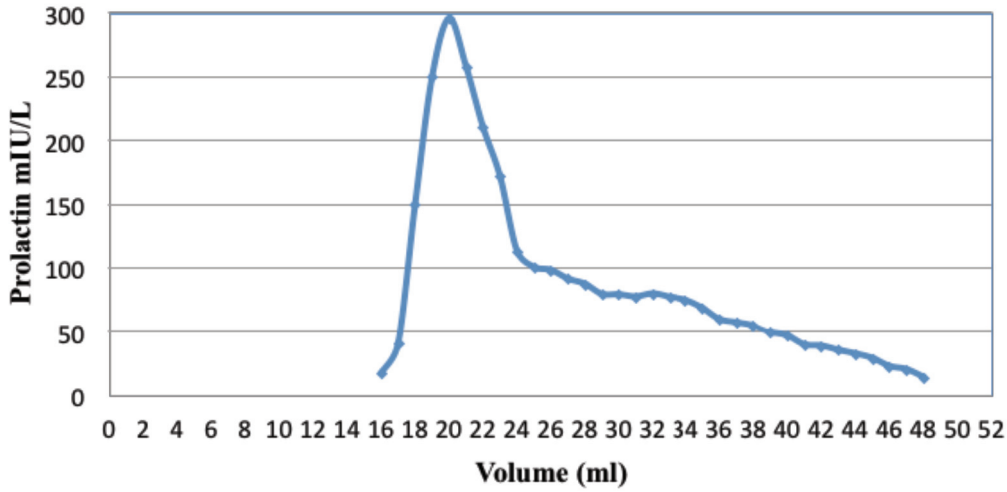

FIG. 1: Gel filtration chromatography showing the presence of predominantly macroprolactin in a sample with a PEG recovery of less than 40%

% recovery in the range > 40% but ≤ 50% represented a grey area with 6 % of samples being positive and 94 % negative. (Table 1)

The clinical indications for prolactin measurements in patients who had post-PEG recovery of <60% are shown in Table 1**.** The causes were infertility, polycystic ovarian syndrome (PCOS), menstrual irregularities, microprolactinoma and antipsychotic treatment. In our study, we noted that four patients were on psychiatric follow-up. Five patients had either a computed tomography (CT) scan or magnetic resonance imaging (MRI) and three were diagnosed to have microadenoma. These patients were prescribed bromocriptine or cabergoline.

DISCUSSION

Measurement of PRL is one of the most commonly undertaken hormonal investigations in evaluating patients with reproductive disorders. Individuals whose hyperprolactinemia can be accounted for by the presence of macroprolactin, may not exhibit the classic signs or symptoms of the hyperprolactinemic syndrome. However,

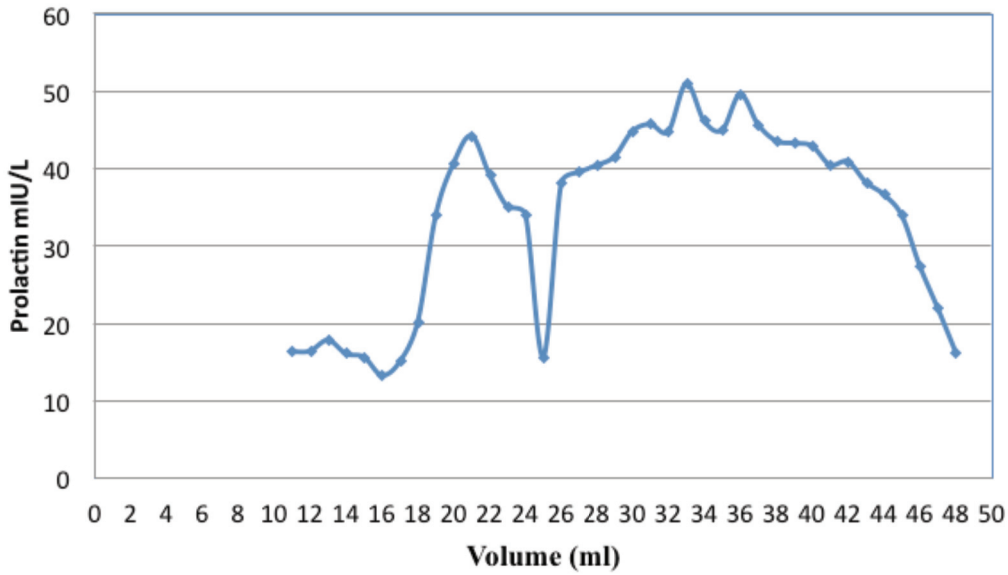

FIG. 2: Gel filtration chromatography showing the presence of both macro and monomeric prolactin in a sample with a PEG recovery of less than 40%

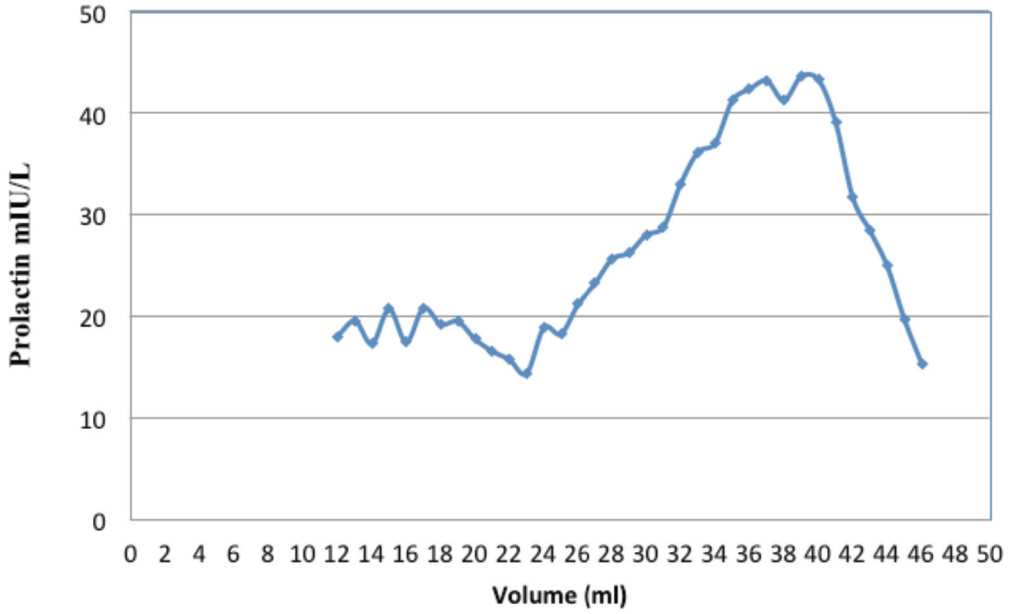

FIG. 3: Gel filtration chromatography showing the presence predominantly monomeric prolactin in a sample with a PEG recovery of more than 40% but less than 60%

some individuals with macroprolactin as the major circulating form may have these symptoms. It is evident from our study that clinical suspicion alone is not a satisfactory approach to indicate the presence of macroprolactin in patients with hyperprolactinaemia. It is therefore important to distinguish such individuals from those with true hyperprolactinemia to avoid unnecessary biochemical and imaging investigations and misleading diagnosis and thus prevent inappropriate drug and surgical treatment. The extent to which the presence of macroprolactin is recognized in sera is assay system dependent. Prolactin measurement by Advia Centaur has been shown to be affected to a lesser degree by macroprolactin compared to other immunoassay systems.[5] In our laboratory PRL is measured by ADVIA centaur. Hence we undertook this to study the incidence of macroprolactin in hyperprolactinaemic samples.

Many studies have suggested that the diagnosis of macroprolactinemia should be indicated when PRL recovery is less than 40% following treatment of serum with PEG.[11-16] We observed similar findings in the seven samples with PEG recovery of <40%, which were confirmed by GFC. PEG recovery of <40% was 100% sensitive for detecting macroprolactin. In our study one sample which had a PEG recovery of <40%, noted to be both macro and monomeric prolactinaemia. PRL level of this sample was 5932 mIU/L. The macro

TABLE 1: Indications for investigations in patients with post-PEG prolactin recovery of ≤ 60%

Patient No	Age in years/ Gender	Non PEG treated mIU/L	PEG treated mIU/L	% PEG recovery	GFC (Macro) %	Clinical information
1	41/F	1067	106	10	99	Follow-up inpsychiatric clinic
2	33/F	833	140	34	90	Investigated for infertility
3	45/F	2028	129	13	88	Microprolactinoma
4	35/F	737	130	18	78	Menstrual irregularities
5	44/F	753	214	28	77	PCOS
6	44/F	812	275	34	75	Microprolactinoma
7	36/F	5932	1444	24	60	Follow-up in psychiatric clinic
8	24/F	1076	462.68	43	35	Infertility
9	36/F	3255	1660	50	25	Psychiatric follow-up

and monomeric prolactin level were 60% and 40% respectively (monomeric PRL level 2372 mIU/L). Although macroprolactin could account for the major circulating form, the biologically active monomeric PRL could still be present in significant excess. Similar findings have been reported by Olukoga & Kane.[17]

Most studies identify a gray area with recovery between 40 and 50% necessitating gel filtration chromatography to confirm or refute the presence of macroprolactin.[18]Only 2/36 subjects with intermediate PEG precipitation showed evidence of macroPRL on GFC. However, these patients positive for macro PRL also had elevated monomeric prolactin. Macroprolactin levels was ranging from 0 to 5% in samples with post PEG recovery of >50% but <60%. Based on GFC, the prevalence of macroprolactinaemia was 4.4% in our study population, similar to the findings of Jassam *et al.*[18]

We observed a good correlation in samples with post-PEG recovery of less than 40% and GFC, PEG precipitation can be used as a method of screening for macroprolactin, thus confirming suggestions in previous studies.[16, 17] Therefore, from our study, a cut-off recovery of less than 40% should mean that patients are classified as having macroprolactinemia and samples with recovery of >40% but $\leqslant$ 50% should undergo further chromatographic work-up. It is not feasible nor necessary to use gel filtration chromatography in the routine laboratory to screen for macroprolactin as the system is technically demanding and expensive.

From a clinical point of view, the presence of excess monomeric PRL is of major concern, and a diagnosis of macroprolactinemia in this setting is misleading and inappropriate. Alternative method has been suggested to overcome this problem is establishing absolute method specific reference intervals for prolactin in PEG-treated sera.[19] Ideally, laboratories should establish the reference interval and should report the prolactin level obtained together with the reference range. This is the further scope of this study.

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