Macroprolactinemia and the new look for the diagnosis of hyperprolactinemia

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Abstract

Serum prolactin were collected from 33 patients with hyperprolactinemia and measured with both PEG precipitation and GFC methods. The result of this study showed a pseudohyperprolactinemia in 28 patients in which their prolactin level retain to the reference range while the remaining 5 patients showed a true hyperprolactinemia because the presence of big and big big molecule of prolactin in their serum caused a pseudohyperprolactinemic state and even in appropriate treatment.

Introduction

Hyperprolactinemia is defined as hyper secretion of prolactin from lactotroph cells in pituitary gland. The cause of this conditions either physiologic or pathologic condition. True hyperprolactinemia caused by biologically active prolactin and associated with the suppression of gonadotropin secretion and gonadal activity. Individuals found to have macroprolactinemia have been reported to have non-pathogenic gonadotropin and gonadal activities. The symptoms of hyperprolactinemia however, are relatively common and nonspecific and, therefore, are likely to occur coincidentally in same patients with macroprolactinmeia, as has been reported[(1,2)].

Nevertheless despite an expensive clinical, hormonal and neuroradiological investigation, no cause can be found in some patients whose serum prolactin concentration remain elevated for many years[(3)]. These patients might have idiopathic hyperprolactinemia which can not detected by curve imaging techniques. This have been described by Jackson et al.[(3,4)] as macroprolactinemia[(3)].

The origin of macroprolactin is still poorly understood. Some authors described the occurrence of a prolactin autoantibody and it is possible that such an antibody causes hyperprolactinemia. Other authors described big big prolactin in the absence of prolactin autoantibody as a polymer of monomeric prolactin bound by disulfide bridges, noncovalent partially glycosylated aggregates of monomeric prolactin or prolactin linked with IgG by disulfide bridges[(5)].

Prolactin circulates in serum in three major molecular size identifiable by gel-filtration chromatographic monomeric prolactin (23 KDa), big prolactin (45-60 KDa), and big big prolactin or macroprolactin (150-170 KDa)[(1,6,7)].

The frequency and clinical consequences of macroprolactinemia have not been clearly established mainly because of difficulty in identifying those patients biochemically. This previously required the use of gel filtration chromatography, which could not be used routinely because it need, intensive and expensive technique. Consequently laboratories have not differentiated routinely between the different forms of prolactin, this has meant that published experience of this condition consist of case reports and of small group of patients[(8,9)].

Recently, a screening test using polyethylene glycol (PEG) has been used to identify macroprolactin in serum. Macroprolactin if present in serum, is precipitated by PEG leaving reduced level in the supernatant. This is a simple inexpensive test that can easily be integrated into laboratory practice[(10)].

The aim of this study is to identified the macroprolactine, which has reduced bioactivity but can be the cause of high prolactin values in patient samples, which can help resolve diagnostic confusion and avoid expensive investigation and inappropriate treatment.

Statistical analysis

Comparison of clinical and biochemical characteristics between true hyperprolactinaemic and macroprolactinemic individuals was performed by the X^2 test for categorical variables and the student unpaired t-test for continuous variables. Results are expressed as mean (SE), SD and statistical significance was set at an X level of 0.05. Z test between two proportions was also used in this study.

Patients and methods

Study participants

Post pubertal female individuals older than 18 years and hyperprolactinemic (serum prolactin > 28 ng/ml) were included in the study. Information of symptoms and signs, imaging investigation, diagnoses and treatment used was obtained from these individuals. Prolactin was measured in all participants at the time of presentation. Macroprolactin was measured in archived sera stored at -20 °C.

We identified 33 individuals with macroprolactinemic whom prolactin concentration fell within the reference range after PEG precipitation, 5 individuals of them classified as true hyperprolactinemia.

Assay methodology

Serum estradiol, follicular-stimulating hormone (FSH), and lutenizing hormone (LH) were measured by the use of minividas. In order to estimate the concentration of macroprolactin presented in hyper prolactinemic individuals with mild or no symptoms, specimens were tested for prolactin after treatment with PEG by gel filtration chromatography . Normal sera (0.5 ml) were subjected to gel filtration chromatography over sephadex G200 (25 × 40) cm in phosphate buffered saline (137 mmol/L sodium chloride, 10 mmol/L sodium phosphate) pH 7.4 at a rate of 0.5 ml/min. The column was calibrated with blue dextran, insulin, bovine serum albumin, egg albumin, α-amylase, pepsin and tryptophan as standard proteins. Eluted protein was quantified by its absorbance at 280 nm. Prolactin concentration in the fractions (1.5 ml) were determined by Radioimmunoassay with the monomeric and macroprolactin values derived from the relative areas under the peaks.

Prolactin autoantibodies were identified with the method described by Hattori et al[(11)]. to be correlated with clinical and laboratory findings, mainly with big big prolactin bioactivity in vitro. Summering up, 100 µl serum and 50 µl [I^{125}] PRL incubated for 1 hour at 37 °C, 150 µl of 25% PEG were added, and the reaction volume was vortexed and centrifuged at 4500 rpm for 30 min. The pellet was washed

with 12.5% PEG and the radioactivity measured with 8-counter.

Results

Serum from 33 females participant were analyzed for prolactin before and after treatment with PEG mean ± SD for prolactin in untreated sera for these subjects was (34.32 ± 14.05 mg/ml). Treatment of these samples with PEG and reanalysis makes the subjects with two groups: the first one deals with a decrease in prolactin values in 28 samples and bring its values to reference range, mean ± SD was 29.48 ± 6.98 ng/ml before treated the sera with PEG and 11.05 ± 2.97 ng/ml after treated the sera, while the second group deals with the remaining 5 samples with mean ± SD 61.44 ± 12.89 ng/ml which remain with hyperprolactin after treated the sera with PEG despite its little decrease in its values, mean ± SD after treated sera with PEG was 57.20 ± 10.92 ng/ml considered as true hyperolactinemic subjects (see Fig. 1).

Prolactin and macroprolactin concentrations in normal sera after gel filtration chromatography

Measurement of the relative amounts of macroprolactin and monomeric prolactin in a subset of 10 randomly selected normal sera by gel filtration chromatography revealed that macroprolactin makes up 3-9% of the total prolactin present. In contrast, 58-67% recovery of prolactin was mentioned when applying the PEG immunoprecipitation method to the same 10 sera.

Comparison of hormone concentrations between true and macroprolactine individuals

In table 1, we summarize the biochemical data of individuals identified as having either confirmed macroprolactinea. Total prolactin was similar in both groups in which macroprolactin was not measured at the time of diagnosis. After treatment with PEG, serum prolactin decreased from (29.48 to 11.05 ng/ml) in macroprolactinemic individuals, and from 61.44 to 57.20 in true hyperprolactinemic individuals ($P < 0.000$), see Table 1.

All the samples were exhibited a decrease in its values after treatment with PEG, although the effect was not as dramatic cohort, see Fig. 1.

Serum FSH did not differ in its values between the two groups of subject with mean ± SD 5.8 ± 1.69 in group 1 and (6.7 ± 2.27) $P > 0.05$, while estradiol and LH showing a higher values with the macroprolactinemic group individuals with mean ± SD in the two groups 152.72 ± 19.34 ng/ml for macroprolactinemic individuals and 39.96 ± 26.06 ng/ml for the second group, 11.39 ± 3.09 ng/ml for first group and 2.14 ± 0.66 for the second group respectively.

Comparison between true hyperprolactinemia and macroprolactinemia in clinical signs and investigation

In true hyperprolactinemia there was a significant relation in both oligomenorrhea or amenorrhea and galactorrhea ($P < 0.001$), while in those of macroprolactin a significant relation observed only with oligomenorrhea or amenorrhea ($P < 0.001$), there was no differences in frequency of headache or infertility between the two groups.

Discussion

The clinical implication of macroprolactinemia has remained a confusing area for many years, with some reports documenting associated galactorrhea and menstral disturbances, and other suggesting that patients remain asymptomatic despite marked macroprolactinemia[8,12].

There is good evidence that macroprolactin does not effect the control of pituitary prolactin secretion via the short loop feed back mechanism or the secretion of gonadotropins as does monomeric prolactin in case of prolactinoma. In case of hyperprolactinemia attributable to macroprolactin, the response of pituitary secretion of monomeric prolactin and thyroid stimulating hormone to dopamine antagonist[11,13] are normal and the frequency distribution of the concentration of serum monomeric prolactin is similar to that of total prolactin in the whole population. Serum estradiol and lutenizing hormone were significantly higher in the group with hyperprolactinemia attributable to macroprolactin that in the group with increased monomeric prolactin.

The predominance of serum prolactin in this study[15,16] is in the macroprolactinemic form (84.9%), in which the biologically active prolactin-IgG complex which cleared more slowly than monomeric prolactin and the big and big big prolactin is biologically inactive which cannot cross the blood barrier to reach the target tissue and have lower affinity for the specific receptors, in which the both caused pseudohyperprolactinemia and asymptomatic clinical condition[5,17-20].

Measurement of the recovery of serum prolactin after precipitation with PEG has been most extensively used for the detection of macroprolactin in cases with hyperprolactinemia, but it has become clear that macroprolactin may be present in substantial quantities in conjunction with increased monomeric prolactin from a prolactinemia or other cause, it is therefore necessary not only to detect the presence of macroprolactin but also to determine the concentration of the monomeric prolactin component[21,22]. It has been suggested that because recovery of prolactin after PEG precipitation correlates with the quantity of macroprolactin present, an estimate of monomeric prolactin may be obtained by determining recovery after PEG precipitation and interpolation from the correlation[15].

Laboratories screening for macroprolactin routinely rely on prolactin recoveries of < 40% after treatment of sera with PEG to distinguish between true hyperprolactinemia and macroprolactinemia. The 40% threshold routinely used, however, is arbitrarily defined with little scientific basis. In certain cases recoveries < 40% may be consistent with true hyperprolactinemia[2].

Polyethylene glycol precipitation and GFC give different estimates of monomeric prolactin because some monomeric prolactin is coprecipitation with serum proteins by PEG. It is likely that PEG also precipitated big prolactin to some extent.

Table (1): Clinical and laboratory information in both true hyperprolactinemic and macroprolactinemic groups

Characteristics	Hyperprolactinemia (n=5)		Macroprolactinemia (n=28)	
	Mean	SE	Mean	SE
Total prolactin (ng/ml)	61.44	5.77	29.48	1.32
Prolactin after PEG precipitation (ng/ml)	57.20	4.88	11.05	0.56
FSH (mlu/ml)	6.70	1.02	5.80	0.32
LH (mlu/ml)	2.14	0.29	11.39	0.58
Estradiol (pg/ml)	39.96	11.66	152.72	3.65
Clinical features	No.	%		
Oligomenorrhea or amenorrhea	5	100.0	9	32.1
Galactorrhea	5	100.0	19	67.9
Infertility	5	100.0	28	100.0
Headache	3	60.0	19	67.9

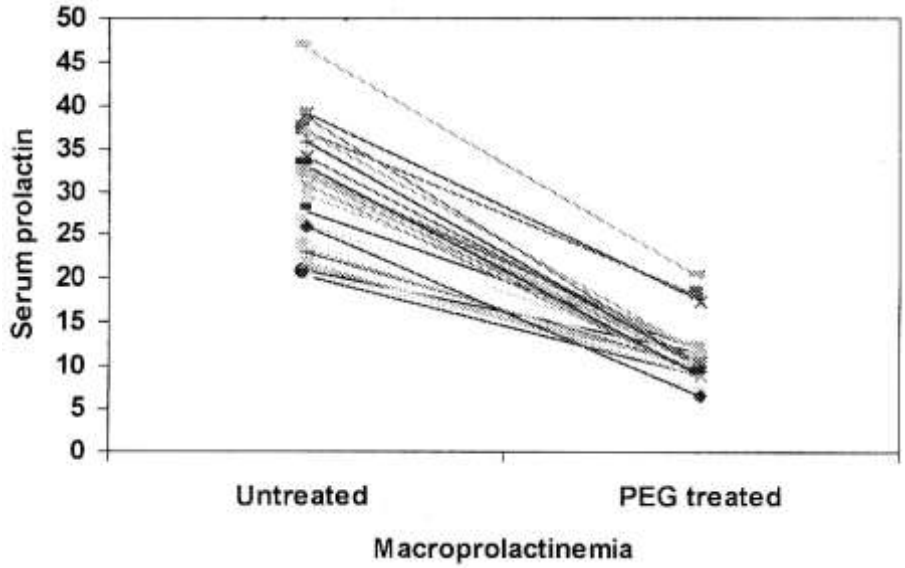

Fig. (1): Serum prolactin concentrations before and after treatment with PEG in patients with either macroprolactinemia or true hyperprolactinemia

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الملخص

تم قياس هرمون الحليب في مصل الدم لـ ٣٣ امراة مصابة بارتفاع هذا الهرمون، حيث قيس هذا الهرمون بطريقة الترسيب بـ PEG وكذلك بكروموتوكرافيا الترشيح الهلامي. حيث اظهرت نتائج هذه الدراسة رجوع ٢٨ حالة من المصابات بارتفاع هذا الهرمون الى المستوى الطبيعي بعد ترسيبه واعادة قياسه، واشارت العينات الخمسة الباقية فقط بارتفاع حقيقي لهذا الهرمون. ان وجود الجزيئات ذات الوزن الجزيئي العالي من هرمون الحليب في مصل الدم تؤدي الى حالة تشخيص بارتفاع هرمون حليب كاذبة مما يؤدي الى وصفات دوائية لا داعي لها بسبب التشخيص الخاطئ لهذه الحال