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Assessment of macroprolactin after polyethylene glycol precipitation in two *commercial immunoassays*

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ABSTRACT

Hyperprolactinemia leads to several diagnoses, however, the immunoassay used for the prolactin determination and the presence of macroprolactin, could induce a misdiagnosis. The aim of this study was to evaluate the usefulness of separation and detection of macroprolactin by precipitation with polyethylene glycol, using two commercial immunoassays, Axsym Abbott and Advia Centaur Bayer in patients with established diagnosis of hyperprolactinemia. One hundred and seventeen samples were tested. Samples were obtained from female patients aged between 22 to 59 years old. We determined total prolactin (PRL), macroprolactin and free prolactin. In 30 of these samples hyperprolactinemia was detected. PRL recovering percentages of supernatant fraction were from 6.5% to 78.5% and from 26.2% to 75.4% when we compared Advia to Axsym systems initial values, respectively. Based on the PRL recovering percentage, 7 samples with significant macro-PRL presence were observed. The macroprolactin prevalence in these 30 samples, after PEG precipitation, was of 23% and 10% in the Axsym and Advia system respectively. Based upon these findings, we establish that references values need to be determined for both instruments, having applied the polyethylene glycol precipitation for the prolactine determination.

Key words: *Polyethylene glycol, immunoassays, macroprolactin, hyperprolactinemia.*

RESUMEN

La detección de hiperprolactinemia (hiper-PRL) conduce a varios diagnósticos, sin embargo, el tipo de inmunoensayo empleado para la determinación de prolactina y la presencia de macroprolactina (macro-PRL), pueden inducir un mal diagnóstico. Así, el objetivo principal de este estudio fue determinar la utilidad de la separación y medición de macroprolactina por precipitación con polietilenglicol (PEG) usando los inmunoensayos comerciales Axsym Abbott y Advia Centaur Bayer en pacientes con hiperprolactinemia. La población en estudio fueron 117 muestras obtenidas de un laboratorio clínico privado, de pacientes mujeres con edades desde 22 hasta 59 años, obteniendo resultados de prolactina total, macroprolactina y prolactina libre, de las cuales 30 muestras presentaron hiper-PRL. Los porcentajes de recuperación de PRL en el sobrenadante fueron de 6.5% a 78.5% y de 26.2% a 75.4%, comparando los valores iniciales en los sistemas Advia y Axsym, respectivamente. Basados en el porcentaje de recuperación de PRL, se observaron 7 muestras con macroprolactinemia. La prevalencia de macroprolactina en estas muestras, después de la precipitación con PEG, fue del 23% y del 10% en el sistema Axsym y Advia, respectivamente; estableciendo que son necesarios valores de referencia para cada instrumento donde se aplique el tratamiento con PEG, para la determinación de prolactina.

Palabras clave: Polietilenglicol, inmunoensayos, macroprolactina, hiperprolactinemia.

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INTRODUCTION

The clinical syndrome of hyperprolactinemia (hyperPRL) has been characterized extensively; the predominant symptoms are galactorrhoea, oligomenorrhoea or amenorrhoea, infertility or libido reduction in women and erectile dysfunction in men.[1-3]

Prolactin (PRL), is a globular protein consisting of 199 amino acids with three intramolecular disulfide bonds, is synthesized as a pre hormone in the anterior hyphofisis with a molecular weight of 26 KDa. Physiological levels of PRL are higher during pregnancy and lactation than any other stage and mean serum levels are higher in women than men.[2]

Besides the monomeric PRL, which accounts for approximately 85% of the total circulating PRL in most of normal subjects and in those patients with hyperprolactinemia, other molecular weight variants of PRL can be found in serum. Post-translation modification of pituitary PRL generates a variety of PRL species, including glycosilated and phosphorylated variants, along with 14, 16 and 22 KDa proteolysed forms.[4,5] Macroprolactin (macro-PRL), has a molecular weight in the 150-170 KDa range and accounts for a variable percentage of the PRL variants found in serum. In some detection methods, macro-PRL is a common cause of apparent hyperprolactinemia, and because of this, it is essential to introduce screening programs to examine samples with elevated total immunoreactive PRL in order to quantify the presence of macro PRL and the monomeric PRL component which is known to be the one bioactive *in vivo*.[6] Although many patients with macroprolactinemia lack of typical symptoms of an elevated PRL, there are multiple reports of patients with macroprolactinemia who present amenorrhoea, galactorrhoea and infertility[7], and for this reason the correct assessment of the presence of macro-PRL may help define the real etiology in patients with idiopathic hyperprolactinemia.

Recent studies have indicated that precipitation of macro-PRL with polyethylene glycol (PEG) has been the most widely validated and applied method for most laboratories[8,9] for the detection of macroprolactinemia levels, however, there is a significant variability in the detection of macro-PRL in hyperprolactinemia sera by different PRL immunoassays in routine usage.[10, 11] In this study we examined the detection of macro-PRL in patients with hyperprolactinemia through PEG precipitation in the Axsym and Advia commercial immunoassays.

METHODS

Design of study

An experimental, descriptive transversal study was developed in males and females aged between 22 and 59 years. Patients gave consent to participate in agreement with the 1975 Helsinki declaration and its amendments for 2002. Whole blood samples were collected by venepuncture (Vacutainer tubes of 5 mL with clot activator and gel for serum separation) and allowed to clot. Serum was recovered by centrifugation (Clay Adams model Dinac) to 2,500 rpm, 5 minutes at room temperature (18-25°C), and was aliquoted and stored at 4°C in order to determine PRL afterwards. Patients did not have any considerable physical activity before the sample was extracted.

One hundred and seventeen samples were analyzed using the Axsym Abbott PRL Micro Particle Enzymatic Immunoassay (MEIA) with a sensibility of 0.6 ng/mL. Hyperprolactinemic samples were separated into two aliquots (300 μL). The first sample was taken to the Capermor International Reference Laboratory at Mexico City in order to determine total PRL using Advia Centaur instrument with a sensibility of 0.3 ng/mL. The other aliquot was used in the second part of the study where the PEG precipitation procedure was applied.[4] Both immunoassays were calibrated according to the World Health Organization International Reference Preparation for PRL 84/500.[10]

Treatment with polyethylene glycol procedure

For hyperprolactinemia samples, Merck PEG 6000 was used as 25% solution (m/v).[4] Once prepared, the solution was stored at 4°C for a three-month maximum period. In a separate 12 x 17 mm glass tube, 500 μL of serum was added along with 500 μL PEG 25% solution, obtaining a 12.5% final concentration of PEG. The sample was mixed for a minute in vortex and centrifuged to 2800 rpm 30 minutes at 4°C.[4] The supernatant fraction was separated into two aliquots; the first aliquot of the post-treatment PRL was taken to the Carpermor Reference Laboratory in order to determine prolactin in it. And the second one was analyzed using the Axsym instrument through MEIA procedure.

Determining of post-treatment PRL

In order to develop the post-treatment prolactin determination through the Axsym system, calibration

was necessary and was made using treated PEG standards. Determination of post-treatment PRL using Advia instrument was developed under the same conditions as the ones used for samples without PEG treatment. The precipitation method with PEG in this system does not cause any interference. It is validated and does not require calibration with treated PEG standards.

Free-PRL

Once the results of post-treatment PRL were obtained with PEG, the macro-PRL was determined using the following calculation:[12]

$$macroPRL\% = \frac{\left(PRL_{serum} - PRL_{\sup erna\ \tan t}\right)}{PRL_{serum}} X100$$

Free-PRL recovering less than 40% = significant presence of macro-PRL, free-PRL recovering that exceeded 50% = no significant presence of macro-PRL. The samples with free-PRL recovering between 40 and 50% were considered indeterminate or at the gray zone.[12-14]

Statistical analysis

Descriptive statistics are means ± standard deviation (SD). A Student's t-test for dependent samples was applied to data using Statistica software V. 6.0. A p-value < 0.05 was considered significant.

RESULTS

Total-PRL determination was made to 117 samples through the MEIA Axsym immunoassay; 30 samples resulted hyperprolactinemic due to a PRL value that exceeded 24.2 ng/mL (upper reference limit suggested by the manufacturer), these samples corresponded to female patients ranked between 22 and 59 years (mean 34 years).

Total-PRL determination

Values of total-PRL determination in the hyperprolactinemic sera obtained from Axsym system presented a variation ranging from 27 to 122.01 ng/mL, with a mean value of 55.02 ± 25.2 ng/mL. Levels of total-PRL obtained from Advia system presented a variation ranging from 13 to 114.4 ng/mL with a mean value of 41.5 ± 24.51 ng/mL. These values are represented in *figure 1*. Samples 6, 8, 11, 14, 23 and 24 had the higher variation values for each instrument showing lower values when determined through Advia system. This finding shows a significant statistical difference (p = 0.003).

In Advia instrument, direct PRL determination allows to observe in 7 samples (10, 14, 15, 16, 20, 22 and 23) that PRL values were less than 24.2 ng/mL, that is the normal upper limit applied to this study. All of these samples were considered normoprolactinemic in this system. Values ranging from 0.0 – 24.42 ng/mL and 24.2 – 48.4 ng/mL were considered normoprolactinemic and hyperprolactinemic, respectively.

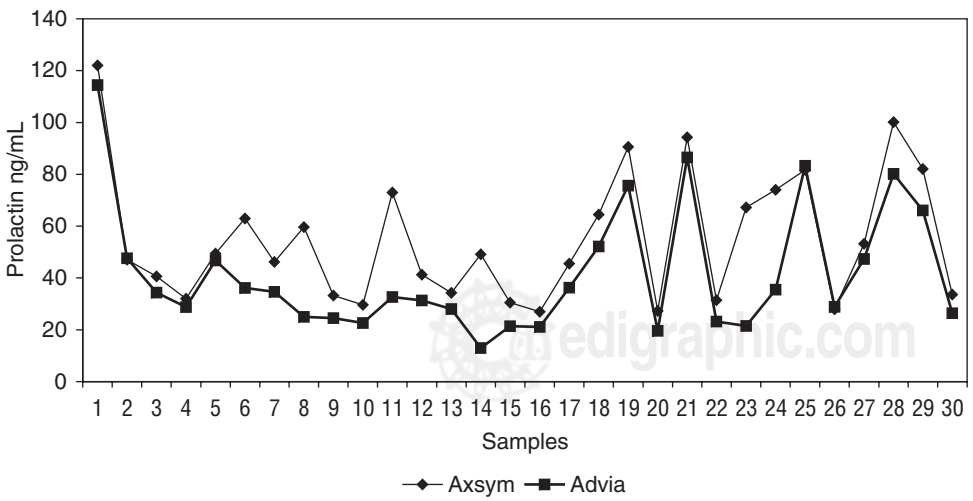

Figure 1. Values of total prolactin determination in both methods. Samples 6, 8, 11, 14, 23, and 24 have statistically significant differences, with a p = 0.003, paired t test.

Axsym Post-Treatment PRL

After the PEG precipitation treatment was applied to the samples, ranges of post-treatment PRL levels detected were 3.18 to 95.7 ng/mL, mean 30.14 ± 21.45 ng/mL. Direct PRL determination and post-treatment PRL determinations through Axsym instrument are shown in *figure 2a*, where diminution of post-treatment determined values in each sample are shown.

There are 17 samples that are specially noted in the *figure 2a* due to their determinate post-treatment values were less than 24.2 ng/mL, and 7 of these samples had a decrease of 50% in relation to the other samples.

Advia post-treatment PRL

After the PEG treatment, the PRL determination in Advia system was done. Figure 2b shows the results, and diminution in the post-treatment values is detected, range 3.4 and 81.4 ng/mL; mean 24.18 ng/mL ± 17.16 ng/mL. Fourteen of these samples show values below 24.2 ng/mL and 7 samples have a 50% diminution or more in the post-treatment determination referring to direct determination in Advia system.

Free-PRL after PEG precipitation

PRL recovering percentages of supernatant fraction were 6.5% to 78.5% and 26.2% to 75.4% when we compared to Advia to Axsym systems initial values, respectively. Based upon the PRL recovering percentage, and the threshold established for Axsym immunoassay in this study, 7 samples with significant macro-PRL presence were observed. Using same threshold in the Advia immunoassay, 4 Axsym-macro-PRL positive samples (6, 7, 8 and 11) were not considered with a significant macro-PRL presence, because these samples, prolactin recovering values were less of 40%.

Reproducibility

The reproducibility of the precipitation treatment with PEG was evaluated using serum containing different free-PRL and macro-PRL concentrations. Coefficients of variation (CV) obtained were 4.0% for serum with a mean of total-PRL of 122.4 ng/mL and free-PRL of 101.28 ng/mL (83% free-PRL) without significant presence of macro-PRL (n = 20); CV were 5.9% for serum with a mean of total-PRL of 45.8 ng/mL and free-PRL of 6.76 ng/mL (14% free-PRL) with a significant presence of macro-PRL (n = 20), and CV were 3.7% serum with mean of total-PRL of 56.9 ng/mL and free-PRL of 19.38 ng/mL (34% free-PRL) with significant presence of macro-PRL (n = 20).

Clinical data

The prevalence of symptoms associated to hyperprolactinemic syndrome was referred by patients which samples were classified like true hyper-PRL, 13 patients (43.3%) referred cephalea, 11 patients (36.6%) referred depression, 9 patients (30%) referred anxiety and weight gain, 8 patients (26.5%) referred fatigue, 7 patients (23.3%) referred amenorrhoea, 4 patients (13.3%) galactorrhoea, 3 patients (10%) referred anovulation, 2 patients (6.6%) presenting infertility and 1 patient (3.3%) referred opsomenorrhoea (menstrual periods up to 35 days). Additional-

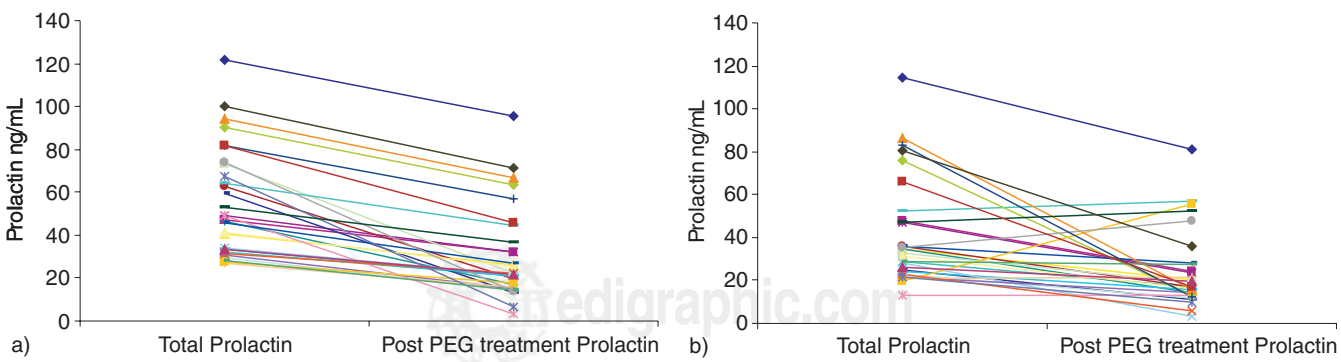

Figure 2. Total and post PEG treatment prolactin determination. **a)** Axsym total and post-treatment prolactin determination. **b)** Advia total and post-treatment determination. In both cases a decrease in the detected PRL after precipitation with PEG of macro-PRL in relation to direct PRL are observed.

ly, 2 out of 7 patients with macro-PRL presented galactorrhoea.

DISCUSSION

Results show that precipitation of macro-PRL by centrifugation with PEG to recover free-PRL is an effective, reproducible, sensitive, simple and cheap method for the detection of macro-PRL, and this concur with data already reported.[15]

Axsym immunoassay rendered very different results in direct and post-treatment PRL determinations, fact that confirms that macro-PRL interferes with the direct PRL detection in this system. This information is not mentioned by the manufacturer.

Direct sample determination in both instruments clearly shows that Axsym instrument has higher reactivity to macro-PRL than Advia. These data has been reported by several authors and attributed to etiologic differences reported of macro-PRL,[10-13] The differences in values of each sample in both instruments were notable. Some results even fall within normal range in the Advia determinations. This may be explained by its lower reactivity towards macro-PRL. This data could change completely a patient diagnosis and therefore the way she is treated and also the way that the follow up is done. With all these observations, PRL values in samples with macro-PRL are methods and samples sensitive.[14,16,17]

Advia immunoassay confirms lower reactivity towards macro-PRL, and along with the lack of instrument calibration requirement with pre-treated PEG standards, reassures it as a more efficient method than Axsym in macro-PRL detection from hyperprolactinemic samples.[9,12]

Different pre-treatment values obtained from macroprolactinemic samples 6, 7, 8, 11, 14, 23, and 24 from both instruments, support the premises that wide differences in PRL determination from the same sample tested through two different immunoassays, implies a great possibility that the sample contains macro-PRL; or if during the post-treatment assay the value falls below the upper limit of the immunoassay, it is possible to consider the presence of macroprolactinemia in that sample, as shown in samples 14, 23, and 24 detected in the Axsym system and in the Advia system as well.

The samples located in the gray zone could not be assigned to any of the well-differentiated groups, the group with macroprolactinemia or true hyperprolactinemia. This could be because of the limitations of the PEG method to recognize every possible etiology or dimers presence (macro-PRL), or higher proportion of glycolasilated prolactin.[4,17] For the evaluation of these samples, some authors recommend Gel Filtration Chromatography analysis in order to obtain a conclusive differentiation.[8,18] However, this method is not available for many laboratories due to its high cost up to 27 times more per test than the regular ones.[19]

The analysis of symptoms also presents variability; however, in patients with macroprolactinemia the following was determined: 2 patients (6.6%) had 4 of the symptoms associated with the hyperprolactinemic syndrome, 1 patient (3.3%) had 3 symptoms and other 3 patients with macroprolactinemia presented only one symptom, and one more patient did not had any associated symptom, however 2 of the 7 patients presented galactorrhoea (28.5%). These data support that macro-PRL presence may be interpreted as benign, but it is important to mention that macroprolactinemia presence has been reported in cases of true hyperprolactinemia such as in a pituitarian prolactin secretor tumor or prolactinoma[4,18-20] although in these cases macro-PRL participation has not yet been clarified. We consider that the presence of hyper-PRL symptoms in patients with macroprolactinemia is in agreement with some other reports that indicate a 60% of some populations presenting symptoms.[18,20,21]

On the other hand, looking at the real hyperprolactinemic patients we confirmed the presence of these pathology symptoms because in the studied population those symptoms presented in the following percentages: cephalea 43.3%, depression 36.7%, anxiety and weight gain 30.0%, fatigue 26.7%, amenhorrea 23.3%, and in a minor extent galactorrhoea 13.3% and anovulation 10.0%. It is important to mention that the presence of galactorrhoea has always been attributed to PRL elevation; however, some reports indicate the presence of galactorrhoea in even a 45% of healthy normal PRL producer females. Even the lack of symptoms in 3 patients (10%) was considered true hyperprolactinemic because PEG treatment does not detect all others PRL isoforms reported.[17, 21]

In conclusion we identified the interference of macro-PRL in the Asxym system using the microparticle enzyme immunoassays (MEIA). Seven samples were macroprolactinemic and had considerable variation regarding direct detection of macro-PRL in both immunoassays, and this confirms that the ratio of macro-PRL interference is sample and method sensitive. We also find 4 samples positive to macro-PRL in Ax-

sym and negative in Advia, and this indicates that the thresholds currently in use in both instruments need to be established considering the recovery percentages of PRL in normal controls and in every instrument.

The precipitation with PEG still have limitations in the identification of the different etiologies of macro-PRL, so the implementation of gel filtration chromatography is needed so the hyperprolactinemia diagnostic could be complemented detecting all the molecular forms of PRL presents in the sample. However, if macro-PRL presence in patients causes only interference or have a clinical significance remains unclear.

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REFERENCES

1. Gibney J, Smith PT, Mackenna TJ. Clinical relevance of macroprolactin. *Clin Endocrinol* 2005; 62: 633-643.
2. Biller BM. Hyperprolactinaemia. *Int J Fertil Womens Med* 1999; 44: 74-77.
3. Zacur H. Indications for surgery in the treatment of hyperprolactinaemia. *J Reprod Med* 1999; 44: 1127-1131.
4. Hattori N. The Frequency of macroprolactinaemia in pregnant woman and the heterogeneity of its etiologies. *J Clin Endocrinol Metab* 1996; 81: 586-590.
5. Diver MJ, Ewins DL, Worth R, Bowles S, Ahlquist J, Fahie-Wilson MN. An unusual form of big big (macro) prolactin in a pregnant patient. *Clin Chem* 2001; 47: 346-348.
6. John R, McDowell IFW, Scanlon MF, Ellis AR. Macroprolactin reactivities in prolactin assays: an issue for clinical laboratories and equipment manufacturers. *Clin Chem* 2000; 46: 884-885.
7. Olukaga AO, Kane JW. Macroprolactinaemia: validation and application of the polyethylene glycol precipitation test and clinical characterization of the condition. *Clin Endocrinol* 1999; 51: 119-126.
8. Leslie H, Courtney CH, Bell PM, Hadden DR, McCane DR, Elis PK. Laboratory and clinical experience in 55 Patients with macroprolactinaemia identified by a simple polyethylene glycol precipitation method. *J Clin Endocrinol Metab* 2001; 86: 2743-2746.
9. Fahie-Wilson MN, Brunsden P, Surrey J, Everitt A. Macroprolactin and the Roche elecsys prolactin assay: characteristics of the reaction and detection by precipitation with polyethylene glycol. *Clin Chem* 2000; 46: 1993-1995.
10. Smith PT, Sulliman MA, Fahie-Wilson NM, McKenna TJ. Gross variability in the detection of prolactin in sera containing big prolactin (macroprolactin) by commercial immunoassays. *J Clin Endocrinol Metab* 2002; 87: 5410-5415.
11. Schneider W, Marcovitz S, Al-Shammari S, Yago S, Chevalier S. Reactivity of macroprolactin in common automated immunoassays. *Ann Clin Biochem* 2001; 34: 469-473.
12. Pascoe LD, Duran RG, Contreras HI, Manuel AL, Blanco FF, Leaños MA. Frequency of macroprolactinaemia due to antibodies against prolactin in pregnant woman. *J Clin Endocrinol Metab* 2001; 86: 924-929.
13. Gilson G, Schmit P, Thix J, Hoffman J, Humbel R. Prolactin results for samples containing macroprolactin are method and sample dependant. *Clin Chem* 2001; 47: 331-333.
14. Schlechte JA. The macroprolactin problem. *J Clin Endocrinol Metab* 2002; 87: 5408-5409.
15. Guitelman MA. Macroprolactinaemia (macro-PRL) a través del método de precipitación con polietileglicol (PEG). *Rev End Gin Rep* 2002; 9: 7-19.
16. Fahie-Wilson MN. Detection of macroprolactin causing hyperprolactinaemia in commercial assays for prolactin. *Clin Chem* 2000; 46: 2022-2023.
17. Vallette KS, Morange RI, Selim A, Gunz G, Mornage S, Enjalbert A. Macroprolactinaemia revisited: a study on 106 patients. *J Clin Endocrinol Metab* 2002; 87: 581-588.
18. Sapin R, Simon CH. False hyperprolactinaemia corrected by the use of heterofilic antibody-blocking agent. *Clin Chem* 2001; 47: 2184-2185.
19. García ML, Diez HA, Ciriza C, Delgado GM, Orejas GA, Fernández EA. Macroprolactin as etiology of hyperproactinemia. Method for detection and clinical characterization of the entity in 39 patients. *Rev Clin Esp* 2003; 203: 459-464.
20. Fahie-Wilson M. In hyperprolactinaemia testing for macroprolactin is essential. *Clin Chem* 2003; 49: 1434-1436.
21. Serri O, Chik CL, Ur E, Ezzat S. Diagnosis and management of hyperprolactinaemia. *Can Med Assoc J* 2003; 169: 575-581.