Research Article

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Assessment of macroprolactinemia rate in a training and research hospital from Turkey

Türkiye'de bir eğitim ve araştırma hastanesinde makroprolaktinemi oranının saptanması

DOI 10.1515/tjb-2016-0156
Received March 17, 2015; accepted May 2, 2016; previously published online December 9, 2016

Abstract

Objective: Macroprolactinemia detection is important to avoid unneccessary tests and overtreatment. High prolactin levels require routine screening and clinicians must be aware of macroprolactinemia frequency encountered with the method in use. In this study we aimed to determine the macroprolactinemia rate in our laboratory.

Methods: Prolactin results of different patients analysed on two different immunoassay systems within two consecutive years were evaluated. Analyses were performed on Beckman Coulter UniCel® DxI800 and Roche Cobas® e601 immunoassay systems. Samples for macroprolactin analysis were precipitated using polyethylene glycol (PEG) 6000. Post-PEG recovery < 40% was defined as positive, 40–60% as gray-zone and > 60% as negative for macroprolactin.

Results: For the samples analysed on DxI800 (n = 14,958) hyperprolactinemia frequency was 8.1% (n = 1208). One of 138 samples submitted for macroprolactin analysis was positive, while three of them were in the gray-zone. For the samples analysed on Cobas® e601 (n = 14,040) hyperprolactinemia frequency was 13.9% (n = 1954). Eighteen of 238 samples submitted for macroprolactin analysis were positive, while 21 of them were in the gray-zone.

Conclusion: A difference was found between two immunoassay systems used in our laboratory in terms of macroprolactinemia rate. However, inability of simultaneous analyses on both systems, lack of evaluation with gel filtration chromatography, and heterophile antibody blocking tube were the limitations.

Keywords: Prolactin; Macroprolactin; Immunoassay; Polyethylene glycol.

Özet

Amaç: Makroprolaktineminin saptanması gereksiz tetkik ve tedavinin önlenmesi açısından önemlidir. Yüksek prolaktin düzeyleri rutin tarama gerektirir ve klinisyenler kullanılan analiz yöntemiyle karşılaşılan makroprolaktinemi sıklığının farkında olmalıdır. Bu çalışmada laboratuvarımızda makroprolaktinemi oranının saptanması amaçlandı.

Yöntemler: Ardışık iki yıl içerisinde farklı hastaların iki farklı immunoassay sisteminde çalışılmış prolaktin sonuçları incelendi. Analizler Beckman Coulter UniCel® DxI800 ve Roche Cobas® e601 immunoassay sistemlerinde yapıldı. Makroprolaktin istemi olan örnekler polietilen glikol (PEG) 6000 ile çöktürüldü. PEG çöktürme sonrası geri elde değeri < %40 makroprolaktin için pozitif, %40–60 gri zon ve > %60 negatif olarak değerlendirildi.

Bulgular: DxI 800'de çalışılmış örneklerde (n = 14.958) hiperprolaktinemi sıklığı%8.1'di (n = 1208). Makroprolaktin istemi olan 138 örneğin biri pozitifken üçü gri zondaydı. Cobas e601'de çalışılmış örneklerde (n = 14.040) hiperprolaktinemi sıklığı%13.9'du (n = 1954). Makroprolaktin istemi olan 238 örneğin onsekizi pozitifken 21 örnek gri zondaydı.

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Sonuç: Laboratuvarımızda kullanılmış iki immunoassay sisteminde makroprolaktinemi sıklığı açısından fark bulunmuştur. Ancak, her iki sistemde analiz edilen örneklerin farklı olması, jel filtrasyon kromatografisi ve heterofil antikor blokan tüp kullanılamamış olması çalışmanın limitasyonlarıdır.

Anahtar Kelimeler: Prolaktin; Makroprolaktin; Immunoassay; Polietilen glikol.

Introduction

Hyperprolactinemia is a common clinical condition characterized by elevated concentration of prolactin (PRL) in the circulation. Prolactin is synthesized and secreted by lactotrophs in the anterior pituitary gland and can exist in the form of three different variants in blood: monomeric PRL (monoPRL), big PRL (bigPRL), and macroprolactin (macroPRL). The major circulatory form monoPRL (23 kDa) accounts for 80–95% of the total PRL and is known to have both in vivo biological and immunological activity. The presence of excess monomeric PRL is associated with the classical symptoms and signs of true hyperprolactinemia such as oligomenorrhea/amenorrhea, galactorrhea, and infertility in women, loss of libido and impotence in men. BigPRL (48–56 kDa) is thought to be a dimer of covalently bound PRL monomers and accounts for 10–15% of PRL immunoreactivity. Another variant of PRL in blood, macroPRL (150–204 kDa), is the conjugate of hormone prolactin with itself and/or with its autoantibody [1–3].

In macroprolactinemia predominating variant in circulation is macro-analyte with non-pathogenic monomeric PRL concentrations. The large molecular size of macroPRL leads to prolonged clearance of plasma from this molecule and falsely elevated prolactin results. MacroPRL is thought not to have any biological activity due to the difficulty in passing through capillary walls. Therefore in hyperprolactinemia attributable to macroPRL the classical symptoms and signs of true hyperprolactinemia are not observed [4].

Gel filtration chromatography (GFC) which is an expensive and labor intensive method is known to be the gold standard for the determination of macroPRL [5]. Alternatively macroPRL may be detected by reanalysis after polyethylene glycol (PEG) precipitation and estimating the recovery which is a relatively more convenient and cheaper method, therefore an appropriate way for the routine screening of macroprolactinemia [6].

In routine practice screening all hyperprolactinemic sera for the presence of macroPRL is essential for the differential diagnosis of hyperprolactinemia [7, 8]. Otherwise clinicians focus on unnecessary further research and follow-up studies which give rise to unfavorable consequences such as additional costs, consumption of time, labor loss and emotional stress. Identification of macro variant is thought to reduce unnecessary treatment as well as the number of idiopathic cases [9]. Today automated immunoassay systems and relevant reagents are widely used in clinical laboratories for prolactin analysis [10]. Like every analytical method, immunoassays also suffer from interference sources such as serum constituents, cross-reactants, endogenous antibodies and macro-analytes. MacroPRL is known to interfere with prolactin assays to various degrees and produce false positive results with differing frequencies [11, 12]. In previous studies macroprolactinemia has been reported to occur in 15–46% of the hyperprolactinemic specimens with differences mainly depending on the immunoassay system used [9, 13–15]. Routine screening of macroprolactinemia in all hyperprolactinemic sera and interactions with clinicians to increase the awareness about the frequency of this issue with the method used in laboratory is of great importance. In this study, we therefore aimed to determine the macroprolactinemia rate within two consecutive years retrospectively considering two different immunoassay systems used in our laboratory.

Materials and methods

Study design

In this study data were collected retrospectively by analyzing the records of laboratory information system. Accordingly a total of 14,958 PRL analyses were performed on Beckman Coulter UniCel® DxI800 Immunoassay System and 14,040 PRL analyses were performed on Roche Cobas® e601 Immunoassay System within two consecutive years. All the samples with an order of macroPRL on UniCel® DxI800 (n = 138) and Cobas® e601 (n = 238) were included in the study. The study was approved by ethics committee of Ankara Numune Training and Research Hospital.

Laboratory analyses

Prolactin assay

PRL analyses were performed on Beckman Coulter UniCel® DxI800 (Beckman Coulter Inc., USA) and Roche

Cobas® e601 (Roche Diagnostics, Mannheim, Germany) immunoassay systems by chemiluminescence and electrochemiluminescence, respectively. PRL assay on UniCel® DxI800 had an imprecision (% CV) of 4.7% and 3.9% at concentrations of 6.10 µg/L and 18.5 µg/L, respectively. And % CV values of 3.3% and 3.37% at PRL concentrations of 11.46 µg/L and 40.95 µg/L were achieved on Cobas® e601.

Upper reference limits (URLs) for the Access Prolactin test on UniCel® DxI800 Immunoassay System were accepted as 13.12 µg/L and 26.72 µg/L for males and females, respectively, according to the manufacturer's package insert. For the Elecsys Prolactin II test on Cobas® e601 Immunoassay System URLs for males and females were as follows; 15.2 µg/L and 23.3 µg/L according to the manufacturer's package insert.

PEG-precipitation

PEG-precipitation was performed by adding 150 µL of serum to an equal volume of 25% (w/v) PEG6000 (Fisher Scientific, USA) dissolved in distilled water and followed by centrifugation at 3000 × *g* for 30 min PRL analysis was performed in the supernatant. The post-PEG precipitation PRL concentration was determined by multiplying the PRL result by 2 to correct for dilution with PEG. PRL recovery was calculated by dividing the post-PEG PRL result by the initial PR L result [16]. PEG-precipitation ratio greater than 60% (recovery less than 40%) was used as the cutoff value for the diagnosis of macroprolactinaemia. Post-PEG recovery < 40% was accepted as positive for macroPRL, 40–60% as gray-zone and > 60% as negative [17].

Results

For the samples analyzed on DxI800 (n = 14,958) hyperprolactinemia frequency was 8.1% (n = 1208). 138 (11.4%) of them were analyzed for the presence of macroPRL. The frequency of samples with hyperprolactinemia for the samples analyzed on Cobase601 (n = 14,040) was 13.9% (n = 1954). 238 (12.2%) of them were analyzed for the presence of macroPRL according to the clinicians' request (Table 1).

On DxI800 one of 138 samples submitted for macroPRL analysis was positive while three of them were in the gray-zone. And eighteen of 238 samples submitted for macroPRL analysis on Cobase601 were positive while 21 of them were in the gray-zone (Table 2).

Table 1: Distribution of different sample cohorts analysed on two systems.

Immunoassay system	Samples [n (%)] with		
	PRL analysis	Hyperprolactinemia	MacroPRL analysis
DxI 800	14,958	1208 (8.1%)	138 (11.4%)
Cobas e601	14,040	1954 (13.9%)	238 (12.2%)

Table 2: Results of post-PEG recovery for individuals with hyperprolactinemia.

Post-PEG Recovery (%)	< 40% (positive)	40–60% (gray-zone)	> 60% (negative)
DxI 800	1	3	134
Cobas e601	18	21	199

Discussion

Immunoassays used for prolactin determination have been previously classified into three groups by United Kingdom National Quality Assessment Scheme depending on the interaction with macroPRL: (1) low, (2) moderately and (3) highly interacting assays. Accordingly, Roche Elecsys was supposed to be in the highly, DPC Immulite 2000 was in the moderately, and Bayer ACS-180 was in the lowly interacting group [2].

When we evaluated the macroprolactinemia rate according to the analyzers separately, we found that 7.6% and 8.8% of the results were positive and in the gray zone respectively on Cobas® e601. And 0.7% and 2.2% of analyses performed on UniCel® DxI800 belonged to positive and gray zone, respectively. This was compatible with the previous reports.

Variations in results of prolactin assay among different immunoassay systems may be attributed to several factors. The degree of immunoreactivity between the reagent antibody and macroPRL is supposed to be one of these factors. Autoantibody component of the macroPRL complex may cause interference via cross-reaction. Interference related with endogenous antibodies in immunoassay testing is both variable and unpredictable and thought to be specific to individuals' sample. Endogenous antibodies may cause interference via binding to other antigenic epitopes or paratopes on reagent antibodies, with varying affinities [18]. Besides mechanism and degree of the effect of macro-analyte in each PRL assay is not always exactly the same for the samples containing this molecule. Therefore, macroPRL related interaction

is thought to be dependent on the immunoassay system used as well as the sample [10, 11, 19, 20].

In a study by Byrne et al. [21] results of 317 hyperprolactinemic samples analysed for prolactin on Beckman DxI 800 were compared with those determined with the PEG screening technique on the Wallac AutoDELFIA. Any sample with a discordance exceeding 25% was reanalyzed using GFC in order to obtain a definitive result. Results of the study revealed that prolactin results can be reported directly from the DxI and when results discordant with the clinical presentation are met, prolactin should be analyzed using GFC [21]. Since data belonging to our routine practice was assessed retrospectively, our study lacked an evaluation with GFC. On the other hand, PEG precipitation is accepted as most tightly correlating method with GFC. And GFC is supposed to be infeasible to use in routine laboratory practice as it is too expensive and technically difficult [5, 6].

Sánchez-Eixerés et al. [22] reported that macroprolactinemia prevalence was higher on Elecsys 2010 than ACS Centaur in a study including 956 consecutive routine patients. Almost 9% of sera with increased levels of prolactin were detected to be related with macroPRL on Elecsys 2010 after PEG precipitation method. These samples had normal or slightly increased concentrations of prolactin on ACS Centaur which was reported to have a much lower rate of macroprolactinemia [22].

In a study by Jassam et al. [23] the prevalence of macroPRL among 409 hyperprolactinemic samples was reported to be 4%. In the study data over a 3-year period were collected retrospectively from the laboratory information system of a hospital in UK. PRL analyses were performed on Advia Centaur (Siemens Healthcare Diagnostics Ltd) [23].

Don-Wauchope et al. reported that the prevalence of macroprolactinemia in a routine South African laboratory was 28% (forty-eight of 170 samples with raised serum prolactin). In the study prolactin measurements were performed on Bayer Advia Centaur (Siemens Healthcare Diagnostics Deerfield, IL, USA) and macroprolactinemia was detected using PEG precipitation procedure [24].

Vilar et al. [25] evaluated the prevalence of macroprolactinemia in 115 consecutive patients with hyperprolactinemia. Among them, 19 (16.5%) was detected to have macroprolactinemia after treatment with PEG. It was therefore concluded that macroprolactinemia was a common condition and routine screening for macroprolactinemia in patients with hyperprolactinemia was recommended [25].

In a study by Hattori et al. [26] the prevalence of macroprolactinemia was determined among a total of 1330 hospital workers at a hospital in Japan. Screening was done using PEG precipitation method and accordingly 49 (3.68%) of the subjects had macroprolactinemia and of 44 hyperprolactinemias found, 15 (34.1%) was detected to have macroprolactinemia. Subsequent examination by binding studies for possible aetiologies revealed that antiprolactin autoantibodies forming complexes of prolactin-IgG were the main cause of macroprolactinemia [26].

Our study had some limitations. Firstly, we could not analyze each sample on Beckman Coulter UniCel® DxI800 and Roche Cobas® e601 simultaneously. Secondly, we could not use GFC, as a reference method, immune precipitation methods or heterophile antibody blocking tube for PRL analysis.

Macroprolactinemia is a well-known source of interference in the clinical laboratory. We found a difference between two different immunoassay systems used in our laboratory in terms of macroprolactinemia rate. Studies performed in laboratories regarding this issue are crucial in order to increase the awareness of clinicians about the features of analytical method used for prolactin measurement. Close interactions between laboratories and clinics should be ensured to elucidate the effect of interference on incompatible test results and improve the efficiency in laboratory testing.

Conflict of interest statement: There are no conflicts of interest among the authors.

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