Original Article

Detection of macroprolactinemia in hyperprolactinemic patients by PEG precipitation using Elecsys 2010 immunoanalyser

Hassan Taghipour[1], Heydar Ali Esmaili[2], Esmaeilnaeimi[3]

ABSTRACT

Objective: Macroprolactinemia is one of the important and benign causes of hyperprolactinemia. Recognition of this benign condition can avoid unnecessary diagnostic procedures and inappropriate treatments. The aim of our study was to evaluate the effectiveness of polyethylene glycol (PEG) precipitation test in determining the macroprolactinemia in hyperprolactinemic subjects using Elecsys 2010 immunoanalyser.

Methodology: We performed the PEG precipitation test in 188 hyperprolactinemic subjects with prolactin levels >750 mIU/L. The results of PEG test were expressed as prolactin recovery (R% value).We also performed PEG precipitation test on 100 sera from normoprolactinemic subjects for determination of PEG precipitation test reference interval.

Results: According to the recovery percentage (R%) interpretative criterion, the prevalence of macroprolactinemia was 17% (R% <40%), whereas 80.8% of subjects showed a true hyperprolactinemia (R% >60%).We also estimated the prevalence of macroprolactinemia by comparing the PEG test results with PEG test reference interval. By this new interpretative method, the prevalence of macroprolactinemia was 18.6%, while 81.4% showed true hyperprolactinemia.

Conclusion: We recommend PEG precipitation test as a simple, convenient and rapid screening method for macroprolactinemia, specially for users of Elecsys 2010 immunoanalysers, because this system has high reactivity to macroprolactin.

KEY WORDS: Prolactin, Macroprolactin, Hyperprolactinemia, Macroprolactinemia, Polyethylene glycol.

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1. Dr. Hassan Taghipour, MD,
Assistant Professor of Pathology, Department of Pathology,
Sina Hospital, Tabriz University of Medical Science, Tabriz, Iran
2. Dr. Heydar Ali Esmaili, MD,
Associate Professor of Pathology, Department of Pathology,
Alzahra Hospital, Tabriz University of Medical Science, Tabriz, Iran.
3. Dr. Esmaeilnaeimi, MD,
Mehr Pathology Laboratory, Gorgan, Iran.

Correspondence:

Dr. Hassan Taghipour, MD,
Assistant Professor, Department of Pathology,
Sina Hospital, Azadi Avenue, Tabriz, Iran.
E-mail: taghipourh@yahoo.com
taghipourh@tbzmed.ac.ir

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INTRODUCTION

Prolactin is a polypeptide hormone secreted by the anterior pituitary gland. The function of this hormone is the initiation and maintenance of lactation. Prolactin is secreted in a pulsatile, sleep dependant rhythm, with the highest levels being attained during sleep. Prolactin measurements are most commonly performed in the evaluation of reproductive disorders, in either men or women and in the assessment of anterior pituitary function. The important sign and symptoms of hyperprolactinemia include galactorrhea, oligomenorrhea, amenorrhea, infertility, loss of libido and impotence. There are three major molecular forms of prolactin in the circulation: monomeric prolactin

(23 KDa), dimeric prolactin (45-50KDa) and macroprolactin (150-170 KDa).[1,2]

Monomeric prolactin, which is biologically active form, accounts for approximately 85-95% of the total prolactin present in the most of normal individuals and in those patients with hyperprolactinemia.[3] The dimeric prolactin accounts for less than 10% of circulating prolactin in normal population. Macroprolactin is a complex of IgG and monomeric prolactin,[4,5] which accounts a small proportion (less than 1%) of total prolactin in normal subjects.[1,2] Macroprolactin has low renal clearance due to its large molecular structure and hence accumulates in the circulation. In some instances(mostly in hyperprolactinemic subjects), the relative amounts of circulating forms can be quite different, and macroprolactin may account for even 90% of total prolactin.[6] It is generally believed that macroprolactin has a low or lacks biological activity in vivo, because due to its large molecular size, it cannot cross the endothelium and reach the cell surface receptors.[7,8] Laboratory prolactin assays vary in their reactivity to macroprolactin, but almost all of currently available immunoassays will detect macroprolactin to some extent.

The presence of macroprolactin in circulation can lead to overestimation of prolactin and this situation can cause diagnostic confusion, unnecessary pituitary imaging and inappropriate surgical and medical treatments.[9,10] Therefore, it is essential that all laboratories make a screening method for macroprolactin in all sera from hyperprolactinemic patients.[11]

The most common method used to detect macroprolactin is pretreatment of the patient serum with polyethylene glycol (PEG), which precipitates large molecular forms of prolactin and leaving the monomeric form in the supernatant. The PEG-treated sample reassayed and the residual prolactin activity or recovery can be reported.

In this study we evaluated the detection of macroprolactin in patients with hyperprolactinemia by PEG-precipitation in the Roche-Elecsys immunoassay system and determined the prevalence of macroprolactinemia in hyperprolactinemic subjects in our center.

METHODOLOGY

Over an 18 months period from June 2008 to December 2009, 2240 patients were referred to our centre for measurement of serum prolactin. Whole blood samples were collected by venepuncture and serum was separated by centrifugation. Prolactin measurement was performed and hyperprolactinemic subjects were identified. The PEG precipitation test was performed on all hyperprolactinemic subjects and also on one hundred randomly selected normoprolactinemic subjects.

Prolactin assay: Serum prolactin was measured by Elecsys 2010 immunoanalyser. The method used by Elecsys 2010 for prolactin measurement is a non-competitive heterogenous immunoassay. The analytical sensitivity (lower detection limit) of method is 1mIU/L or 0.047µg/L.The prolactin reference range is 21-530 mIU/L (1-25 µg/L) for females and 21-425 mIU/L (1-20 µg/L) for males. PEG does not interfere with Elecsys2010 immunoanalyser which uses electro chemiluminescent immunoassay method.

PEG precipitation test: The PEG precipitation test was performed according to Fahie-Wilson method. Three hundred microliters of a 25%(w/v) solution of PEG6000 was added to equal volume of patient serum, obtaining a 12.5% final concentration of PEG. The sample was mixed for one minute in vortex and centrifuged at 2000*g for 30 minutes. Prolactin was measured in the supernatant using Elecsys 2010 and the results were compared with those obtained from untreated serum. The results of PEG test were expressed as prolactin recovery (R% value).

$$\text{Prolactin recovery (R\% value)} = \frac{\text{prolactin in supernatant (PLR post)}}{\text{total prolactin}} \times 100$$

Interpretation criteria: Based on the data published in literature,[12,13] a R% value< 40 was considered as indicative of macroprolactinemia, and a R% value > 60 was considered as indicative of true hyperprolactinemia, and a R% value between 40-60% was considered as "gray zone".

We also performed PEG precipitation test on 100 sera from normoprolactinemic subjects, and based on the results obtained from normal subjects, the PEG precipitation test reference interval was determined. This new reference interval (18 – 440 mIU/L) was used for interpretation of PEG test results of hyperprolactinemic patients. (Suliman criterion) According to this criterion, PEG test results of hyperprolactinemic subjects are compared with PEG test reference interval, and patients with PEG test results within the reference range are considered as having macroprolactinemia.[14]

RESULTS

From 2240 patients referred to our centre, 188 (8.4%) cases were hyperprolactinemic with prolactin levels more than 750 mIU/L. One hundred sixty two (162) of them were women (86.2%) and 26 of them were men (13.8%). Thirty two (17%) cases of

hyperprolactinemic patients had R% values less than 40%, and 152(80.8%) cases had R% values greater than 60%, and 4 of them had R% values between 40-60%. Furthermore, according to R% interpretative criterion the prevalence of macroprolactinemia in hyperprolactinemic patients was 17%, whereas (80.8%) of patients had true hyperprolactinemia, and 2.2% were in "gray zone" of PEG test.

Because recently, many studies and researches propose that the priority for the laboratory should be to determine the elevation of monomeric prolactin rather than the simply detect the presence of macroprolactin, and it has been revealed that interpretation of results based on percentage recovery can be misleading when there is coexistence of macroprolactin and elevated levels of monomeric prolactin, for this reason, we applied a new criterion (Suliman criterion) for interpretation of results, and compared the PEG precipitation test results of hyperprolactinemic patients with PEG precipitation test reference interval .

The PEG precipitation test reference interval which was calculated from PEG test results of one hundred normoprolactinemic subjects was 18 – 440 mIU/L. According to suliman criterion, after PEG precipitation, 35(18.6%) cases of hyperprolactinemic subjects showed a normal monomeric prolactin (PRL_{post} < 440 mIU/L) and these subjects considered as having macroprolactinemia, where 153(81.4%) cases had elevated levels (PRL_{post} > 440 mIU/L) of monomeric prolactin after PEG precipitation (true hyperprolactinemia). The results are summarised in Table-I.

DISCUSSION

Macroprolactinemia is one of the benign etiologies of hyperprolactinemia and failing to diagnose this benign condition can lead to unnecessary imaging studies and inappropriate treatments. Based on the data published in the literature, the prevalence of macroprolactinemia in hyperprolactinemic patients ranges from 15 to 26%[15-18] and also in some studies the prevalence of macroprolactinemia in hyperprolactinemic patient is reported from 9 to 42%.[19-21]

According to R% criterion, the prevalence of macroprolactinemia in hyperprolactinemicpatients in our study is 17%, which agrees with data reported by other studies. The prevalence of macroprolactinemia

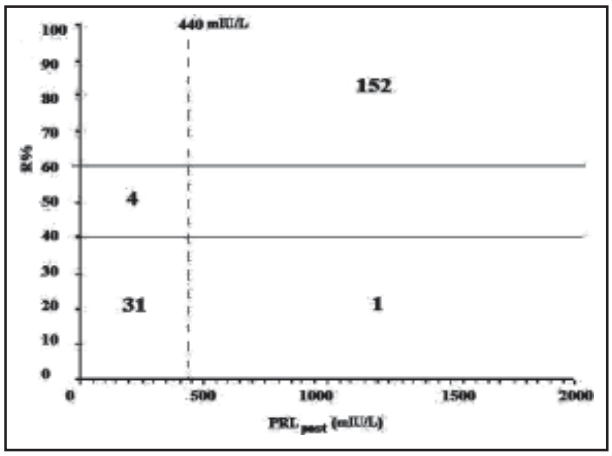

Figure-1: Comparison of R% and Sulimancritera.

in hyperprolactinemic patients according to Suliman criterion is 18.6%, which agrees with the results obtained by R% criterion.

From 32 subjects which had macroprolactinemia according to R% criterion(R%<40), 31 cases showed PRL_{post}<440mIU/L, and only one of the cases had macroprolactinemia along with true hyperprolactinemia (coexistence of macroprolactinemia and true hyperprolactinemia). All of the 152 cases which had true hyperprolactinemia on the basis of R% criterion, showed elevated levels of PRL_{post}(PRL_{post} > 440 mIU/L) and classified as true hyperprolactinemic by suliman criterion, and 4 cases which were within the "gray zone" of R% criterion, showed PRL_{post} levels less than 440mIU/L and classified as macroprolactinemic by Suliman criterion. (Figure 1) As reported in the literature,[21] the prevalence of macroprolactinemia in normoprolactinemic subjects is less than 1%, and in our study all of the one hundred normoprolactinemic subjects had R% values more than 60%, furthermore there were no macroprolactinemic cases among them. In one hundred normoprolactinemic subjects, the R% mean value was 78.3%,and this result confirm that a constant amount of monomeric prolactin (about 15-20%) precipitates with PEG.[22,23]

Based on the degree of reactivity with macroprolactin, commonly used prolactin immunoassays are classified into low, medium and high reactivity groups. The instrument used in our study, Elecsys 2010 immunoanalyser, belongs to high

Table-I: Prevalence of macroprolactinemia in hyperprolactinemic subjects.

	macroprolactinemic	*True hyperprolactinemic*	*Gray zone*	*Total*
R% criterion	32(17%)	152(80.8%)	4(2.2%)	188
Suliman Criterion	35(18.6%)	153(81.4%)	-	188

reactivity group.[24,25] For this reason, screening for presence of macroprolactin is necessary for all medical laboratories, specially for users of Elecsys 2010.

As mentioned before, the samples with R% value between 40-60% are considered as "gray zone", these samples could not be assigned to any of the well-differentiated groups (macroprolactinemic or true hyperprolactinemic). For the evaluation of these samples, some authors recommend Gel filtration chromatography (GFC) in order to make a definite diagnosis.[25] However, this method is too time-consuming, labor intensive and expensive for routine use.

CONCLUSION

Macroprolactinemia is one of the important and benign causes of hyperprolactinemia, and recognition of this benign condition can avoid unnecessary diagnostic procedures and inappropriate treatments. This condition (macroprolactinemia) specially should be considered if hyperprolactinemia is identified in an individual who lacks any of the typical manifestations of hyperprolactinemia. Almost all currently available immunoassays can detect macroprolactin to some extent, and based on the reactivity to macroprolactin, these methods can be categorized into low, medium and high reactivity groups. Therefore, it is essential that the all clinical laboratories should have screening procedures for the detection of macroprolactin.

PEG precipitation test is a convenient, simple, rapid and inexpensive procedure for detection of macroprolactin. We recommend this procedure as a simple screen method for macroprolactinemia, specially for users of Elecsys 2010 immunoanalysers, because this system has high reactivity to macroprolactin.

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