Original Article

FREQUENCY OF MACROPROLACTINEMIA IN HYPERPROLACTINEMIC PATIENTS IN THE CLINICAL PRACTICE AT RAWALPINDI

Lubna Ehtizaz, Dilshad Ahmed Khan, Aamir Ijaz

Armed Forces Institute of Pathology, National University of Medical Sciences, Rawalpindi. Pakistan

ABSTRACT

Objective: To determine the frequency of macroprolactinemia in hyperprolactinemic patients, referred for fertility profile to a tertiary care medical setup at Rawalpindi, Pakistan.

Methods: The cross-sectional descriptive study was carried out at the department of chemical pathology and endocrinology, Armed Forces Institute of Pathology (AFIP) Rawalpindi. All adult patients of aged 20-50 years of either sex referred for fertility profile including serum prolactin level (PRL > 360mIu/l in males and > 530mIu/l in females) were consecutively included in the study. The serum of these patients were reanalyzed after PEG precipitation. The prolactin recovery (R% value) was calculated as: (R %) = Prolactin in supernatant / total Prolactin x 100. A recovery (R%) of value < 40 % of prolactin was considered as macroprolactinemia(MPRL).

Results: Total of 187 patients were labeled as hyperprolactinemic patients, having serum PRL levels above the reference range. Thirty-six pregnant females and with a history of drug intake affecting prolactin levels were excluded. Out of 151 hyperprolactinemic patients, 31 (20.5%) patients had MPRL and 120 (79.5%) true hyperprolactinia (THPRL) after PEG precipitation. Macroprolactinemic patients included 25females (81 %) and 6 males (19 %). Most of the female patients belonged to age group 18-30 years (61.3 %). The serum PRL levels mean+ SD were higher in THPRL 1171.1 ± 914.20 as compared with MPRL 857.90 ± 515 (p=0.014).

Conclusion: Total of 187 patients were labeled as hyperprolactinemic patients, having serum PRL levels above the reference range. Thirty-six pregnant females and with a history of drug intake affecting prolactin levels were excluded. Out of 151 hyperprolactinemic patients, 31 (20.5%) patients had MPRL and 120 (79.5%) true hyperprolactinia (THPRL) after PEG precipitation. Macroprolactinemic patients included 25females (81 %) and 6 males (19 %). Most of the female patients belonged to age group 18-30 years (61.3 %). The serum PRL levels mean+ SD were higher in THPRL 1171.1 ± 914.20 as compared with MPRL 857.90 ± 515 (*p*=0.014).

Keywords: Macroprolactenemia; PEG precipitation, Hyperprolactenemia, Prolactin levels.

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INTRODUCTION

Prolactin hormone secretion is pulsatile and it has an inhibitory effect over the gonadotropin secretion, so its hypersecretion can cause oligomenorrhea or amenorrhea in women. In men, the increased prolactin levels can affect the electrolytic balance and cause gynecomastia, galactorrhea, decreased libido and impotence. The sera of healthy individuals contain different forms of PRL, including monomeric prolactin (monoPRL) the size of which is 23 kDa (85%-90% of total prolactin), dimeric PRL of 45-60 kDa (10%-15%) and a portion of mono PRL bound with IgG to form 150–204 kDa big prolactin molecule or macroprolactin (MPRL). Macroprolactin is 'Big" prolactin which due to its large size cannot cross the capillary blood barrier and reach the target tissues therefore it has little biological activity but is immunologically active [1]. Serum PRL concentration varies in males and females based on different immunoassay systems. Hyperprolactinemia is a condition of serum prolactin more than the reference range. It can be true hyperprolactinemia which is one of the commonest endocrine disorders characterized by monomeric PRL levels greater than the normal reference range or pseudo-hyperprolactinemia which is due to the presence of MPRL. MPRL shows varying degree of cross reactivity with currently available immunoassays giving rise to falsely high value of serum PRL [2].

We can prevent unnecessary and expensive pituitary imaging, inappropriate medication and surgical intervention in patients with pseudo-hyperprolactinemia by establishing screening method for detection MPRL in the lab. The gold standard

Correspondence: Dr Dilshad Ahmed Khan, National University of Medical Sciences, Rawalpindi, Pakistan.

Email: **dakhan@cpsp.edu.pk**

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method for detecting MPRL is gel filtration chromatography (GFC), a procedure that allows for quantification of all three variants of PRL [3]. Unfortunately, this method is labor intensive and not suitable for performance in clinical laboratories. Moreover, it is not available at most of the diagnostic centers. In contrast, precipitation with polyethylene glycol (PEG) is a widely-used screening test for MPRL and is easily performed in clinical laboratories. Pretreatment of the patient serum with polyethylene glycol (PEG) is the method used to detect MPRL in serum, which precipitates large molecular forms of PRL leaving the monomeric form in the supernatant. As recommended by Fahie-Wilson [4], the samples are classified into truly hyperprolactinemic (recovery >60%), probably macroprolactinemic (recovery 40–60%) and macroprolactinemic (recovery <40%) on the basis of monoPRL recovery from the original sample.

The prevalence of macroprolactinemia in hyperprolactinemic population varies from 4% to 46%, depending on the population studied and the assay used for PRL measurement. Highest prevalence of macroprolactinemia has been reported as 46% in Brazilian population [5]. The prevalence of macroprolactinemia in hyperprolactinemic patients reported in different population include; 10% in France [6], 26% in Ireland [7], 17% in Belgium [8], 6.4% in Saudi Arabia (2) and 17% in Iran [9]. This suggests that macroprolactinemia is overlooked in diagnosis as a cause of hyperprolactinemia whose frequency is often underestimated [10]. In India, the prevalence of only 11% in hyperprolactinemic subjects is reported [11]. Pituitary production of prolactin in patients with hyperprolactinemia due to macroprolactin is different from that in women with monomeric hyperprolactinemia [12]. The present study looks into the frequency of macroprolactinemia in hyperprolactinemic patients at a tertiary care medical setup at Rawalpindi. This will enable better understanding of hyperprolactinemia in our patients avoiding unnecessary expensive investigations by them and also reduce the stress associated with it.

MATERIALS & METHODS

The cross sectional descriptive study was carried out in the Chemical Pathology Department of Armed Forces Institute of Pathology (AFIP), Rawalpindi, after approval from the institutional ethical review committee. A total of 187 subjects of both genders, aged from 20-50 years and having serum PRL levels above the reference ranges (> 360mIu/l in males and > 530mIu/l in females) were labelled as hyperprolactinemic. Medical history and examination of each patient was carried out. Thirty-six pregnant females and with a history of drug intake affecting prolactin levels were excluded.

A total of 151 subjects included in the study after informed consent consisting of 25 females and 6 males. The age ranged from 20 to 50 years. Blood samples (5ml) were drawn from antecubital vein using aseptic techniques, in plain serum tubes from each subject for serum prolactin. Serum was separated by centrifugation at 1500 g for 10 minutes. Basal prolactin levels were determined on Beckman Access II Immunoassay analyzer. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (5-4240mIU/L).

The samples of 151 hyperprolactinemic patients were reanalyzed on Beckman Access II Immunoassay analyzer after PEG precipitation (13) using the Fahie-Wilson method [4]. Briefly PRL concentration in the patients' sera were reanalyzed after PEG precipitation. The serum sample (200µL) was mixed with an equal volume of 25% PEG solution. The mixture was thoroughly vortexed and allowed to stand for 20 minutes. After centrifugation at 2500 × g for 15 minutes, the supernatant was aspirated and reanalyzed for PRL. Ratio of the PRL

in the supernatant to total PRL in serum was calculated. The Prolactin recovery (R% value) was calculated as: Prolactin Recovery (R %) = Prolactin in supernatant / total Prolactin x 100. A recovery (R%) of value < 40 % of prolactin was considered as macroprolactinemia [9]. The hyperprolactinemic patients were classified into truly hyperprolactinemic (recovery >60%), probably macroprolactinemic (recovery 40–60%) and macroprolactinemic (recovery <40%) on the basis of mono PRL recovery from the original sample.

STATISTICAL ANALYSIS

Statistical analysis of data was done by using Statistical Programme for Social Sciences (SPSS) version 21. Patients age and PRL values was reported as mean and standard deviation (SD). Frequency of macroprolactinemia was calculated on the basis of recovery method by the formula: Prolactin in supernatant / total Prolactin x 100. A value less than 40% was considered positive for macroprolactinaemia in this study. A p-value of 0.05 was considered ass Significant.

RESULTS

A total of 151 hyperprolactimic subjects were included in the study consisting of t consisting of 25 females and 6 males. The mean age was 31.32 ranging from 16 to 47 years. Demographic characteristics and clinical finding at time of investigation are shown in Table-1. Menstrual irregularity and infertility were common presenting complaint in the female patients with THPRL (p=<0.01). The frequency of MPRL in different age group of hyperprolactinemic patients' is shown in table-2. Most of the female patients belonged to age group 18-30 years (61.3 %). Out of the 31 patients with macroprolactenemia, 25 (81%) were females and 6 (19%) were males (Fig-1).

Out of 151 hyperprolactinemic patients, 31 patients had recovery of monomeric PRLR % (mean +SD) <40 (32.8 + 4.7 %) diagnosed as MPRL (Fig-2). One hundred twenty had R % (mean + SD) > 40 (56.7 + 8.9) labeled as THPRL. The serum PRL levels mean+ SD were higher in THPRL 1171.1 ± 914.20 as compared with MPRL 857.90 ± 515 (p=0.014) as shown in. Figure-3. Mean difference of PRL values between THPRL and MPRL patients is 313(95% CI=65.40-561.0).

Table-1: Demographic and clinical feature of Hyperprolactinemic patients (n=151)

Variable	THPRL (*n* = 120)	MPRL (*n* = 31)	*p*- value
Age, years, mean ± SD	31.02 ± 8.17	31.32 ± 8.24	0.85[a]
Male n (%) Female n (%)	17(14) 103(86%)	6 (19) 25(81)	0.47[c]
Menstrual Irregularities n (%)	78(65%)	13(42%)	0.012
Men libido n (%)	9(52%)	4(24%)	0.337
Female Infertility n (%)	38(32%)	5(16%)	0.00001

THPRL: True Hyperprolactinaemia; MPRL Macroprolactinaemia

[a] student's*t*-test, [c] χ^2 test

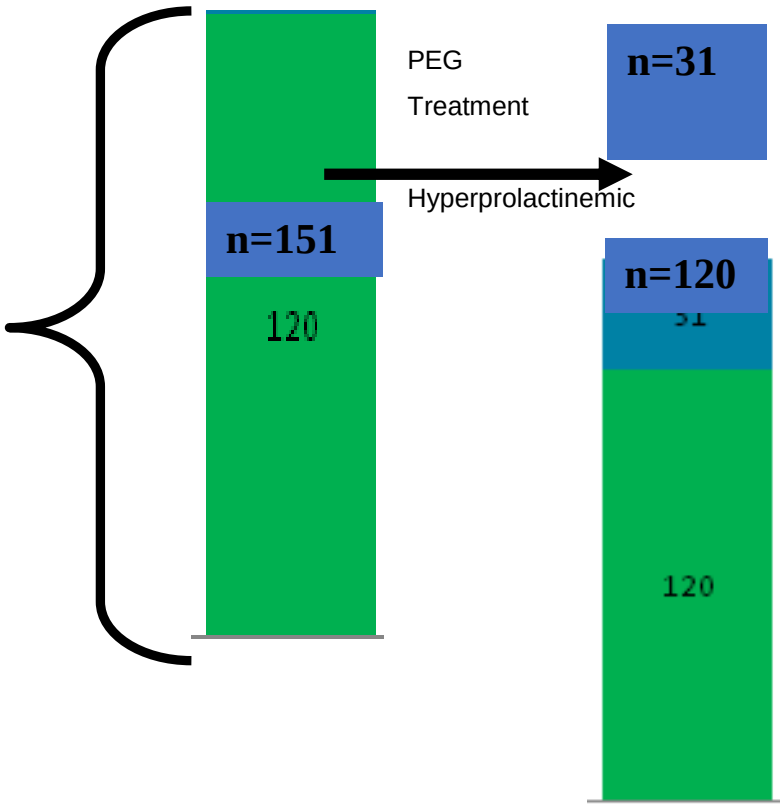

Figure-1: Frequency of macroprolactinemic and true hyperprolactinemic after treatment with PEG.

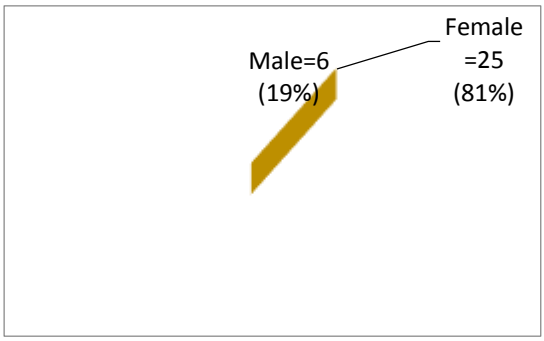

Figure-2: Frequency of macroprolactinemic in female and male subjects

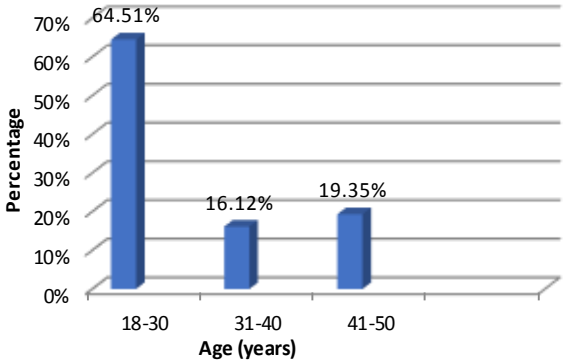

Figure-3: Age distribution of hyperprolactinemia reported at AFIP Rawalpindi**.**

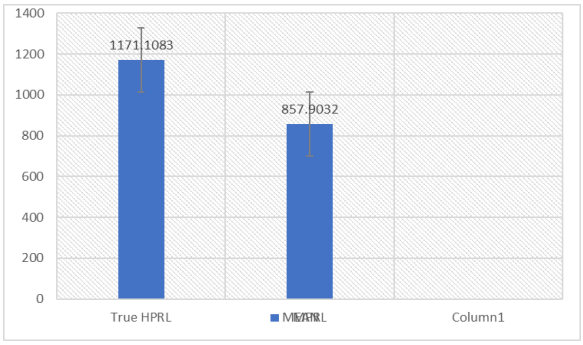

Figure-4: Comparison of basal PRL (mIU/L) in (MPRL) and true hyperprolactinemics macroprolactinemics (THPRL) patients (*p=0 .014)

DISCUSSION

Measurement of serum PRL levels is frequently prescribed during the evaluation of patients with reproductive disorders. Elevated PRL levels in serum give rise to hyperprolactinemia which is a common cause of infertility in women. Our study group included 16% MPRL in contrast to 32% in True HPRL patients with referred for infertility investigations including fertility profile. Macroprolactinemic work up should be done to exclude as a cause of infertility in patients with hyperprolactinemia which is consistent with [14]. We

observed no significant difference in serum prolactin levels between MPRL and THPR patients with PCOS which is an observation similar to a study conducted by [15]. In another study conducted on women with PCOS prevalence of macroprolactinemia was reported as 5.8% showing that there might be other causes of hyperprolactinemia in polycystic ovarian syndrome. Macroprolactinemia is hyperprolactinemia with MPRL as the predominant form of PRL [16]. We need to differentiate between hyperprolactinemia due to benign clinical condition of MPRL and true hyperprolactinemia due to increased levels of monoPRL which requires treatment. Considerable reliance is placed on the laboratory results, due to the overlapping symptoms of HPRL for the decision of medical treatment and/or surgical management especially in case of prolactinomas.

The statement that macroprolactinemic patients cannot be distinguished from True hyperprolactinemic patients on the basis of clinical features alone was later confirmed by [15] and [17] who were of the opinion that there was no difference in frequency of menstrual irregularity and infertility between the macroprolactinemic and hyperprolactinemic subjects.

PRL reacts differently in immunoassays and the prevalence of macroprolactinemia in hyperprolactinemic sera differs according to the assay used. Most of the current immunoassays are interfered by MPRL. Reference ranges of serum PRL levels are assay dependent and vary according to differences in the reactivity of the assay antibodies towards the different isoforms of PRL. Depending on the extent of reactivity of this antibody currently used prolactin Immunoassays are categorized into low, medium and high reactivity groups [18]. The sole method of screening of hyperprolactinemic sera is to re-assay these sera for PRL after MPRL removal. Gel Filtration Chromatography is the gold standard for the estimation of various isoforms of PRL but this technique is time consuming, expensive and not ideal for routine laboratory use [19]. Treatment with PEG is currently the most popular procedure used for removing MPRL from hyperprolactinemic sera preceding immunoassay analysis. Healthcare workers must be aware of the extent to which the assay system may detect MPRL. Thus, recognition of MPRL in sera is dependent on the assay system deployed. Fahie-Wilson and Smith [18] stressed the need for a reliable assay of serum PRL which can reflect the exact in vivo bioactivity of the hormone.

PRL was measured by Access 2 Immunoassay analyzer, which has low reactivity towards MPRL in our laboratory and we undertook this to study the frequency of macroprolactinemia in hyperprolactinaemic samples at our setup in Rawalpindi. Following the criteria of Fahie Wilson we used the cut-off value of 40% recovery in PEG precipitation for detecting MPRL in hyperprolactinemic sera. According to this criterion 31 (20.5%) out of total 151 hyperprolactinemic patients had Macroprolactinemia. True Hyperprolactinemia was revealed in 120 (79.5%) cases. Hassan Taghipour in Iran observed frequency of 17% MPRL in HPRL patients [9]. They analyzed the sera on Elecsys 2010 Immunoassay analyzer, PEG precipitation was performed on all HPRL sera and results interpreted according to Suleiman criterion. The low frequency of MPRL in HPRL patients as compared to our study may be due to difference in the criterion used and the method of analysis. Assim Alfadda carried out a study to determine the prevalence of Macroprolactinemia in patients with hyperprolactinemia [2]. Out of 156 subjects only ten (6.4%) were reported having macroprolactinemia. This low frequency of macroprolactinemia in HPRL patients may be due to low reactivity of the analytical assay (Roche Elecsys Prolactin assay, Prolactin II) used. In a study conducted in Poland a frequency of 9.3% MPRL was

established in patients with HPRL [19]. One of the reasons for low frequency reported in this study as compared to similar studies conducted there was interference of PEG with the immunoassay (Immulite 1000) used.

A frequency of 20.5% MPRL in HPRL patients in both sexes in our setup seems to be higher as compared to study conducted in India where a frequency of only 11% in hyperprolactinaemic females was reported [11]. In our study the frequency of MPRL in hyperprolactinemic females is 19.5%. Study shows that the rate of hyperprolactinemia is higher in women than in men but only before the age of 65 years [20]. Our study shows that MPRL is more prevalent in elderly males in our setup as compared to females who have increased frequency of MPRL in younger age group (18-30 years). The gender discrimination is of importance because Macroprolactinomas in males mainly present with symptoms of mass effects, as opposed to females who present with symptoms of hypogonadism [21]. Sera of 1330 subjects in Japan was analysed and macroprolactinemia was reported in 49 (3.68%) subjects [22]. We suggest that screening for MPRL should be performed in all hyperprolactinemic sera. This is because the diagnosis of macroprolactinemia has major implications as neither pituitary MRI needs to be performed in these cases nor treatment or follow-up of patients with macroprolactinemia is necessary [23]. Moreover, contrary to multidimensional impairment of sexual function in women with elevated monomeric prolactin, macroprolactinemia only seems to alter sexual desire [24].

PEG precipitation is the technique that has proven acceptable and reliable since 1997 and still not implemented in most of the clinical laboratories (Fahie Wilson et al., 2013). Screening for MPRL by using PEG precipitation is cost effective because it avoids unnecessary imaging investigation and treatment. Manufacturers should strive to minimize MPRL interference in assays so the laboratory should be able to provide a result that is clinically relevant.

CONCLUSION

The frequency of macroprolactin was 20.5% among hyperprolactinemic patients mainly young female in the tertiary care clinical setup at Rawalpindi. The clinical significance of macroprolactinemia is very high. If not recognized, it will result in misdiagnosis, unnecessary imaging, inappropriate surgical or pharmacological treatment, waste of healthcare resources and cause unnecessary concern for both clinician and patient

AUTHORS CONTRIBUTION

Lubna Ehtizaz: Carried out laboratory work

Dilshad Ahmed Khan: Conceived the idea

Aamir Ijaz: Supervised clinical samples collection and manuscript writing

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