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Original Article

Biochemistry Section

Prevalence of Macroprolactinaemia by Polyethylene Glycol Precipitation Method: A Cross-sectional Study

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ABSTRACT

Introduction: Macroprolactin, an immunoreactive molecule resulting from association of monomeric Prolactin (mPRL) and immunoglobulin G is a significant cause of misdiagnosis, unnecessary radiological investigation and treatment for hyperprolactinaemia. Data on its prevalence and clinical manifestation varies regionally. Case presentation can vary with assymptomatic cases to those with galactorrhea and irregular menses.

Aim: To find the prevalence and clinical features associated with macroprolactin in cases of hyperprolactinaemia in hospital patients.

Materials and Methods: A cross-sectional study was conducted in the Department of Biochemistry, Institute of Postgraduate Medical Education and Research and SSKM hospital, Kolkata, India, from November 2018 to April 2019. Serum samples were assayed for serum PRL levels in 1400 subjects by Chemiluminescence immunoassay (Immulite 1000 siemens) based on presenting symptoms of galactorrhoea, amenorrhoea and infertility. Serum PRL samples (n=240) above the manufacturer's reference cut-off level (PRL ≥30 ng/mL) were obtained from patients with or without symptoms of hyperprolactinaemia. Retesting for PRL levels were done following precipitation of macroprolactin using Polyethylene Glycol (PEG, MW: 6000). Fourty cases with physiological causes of PRL excess, hypothyroidism, polycystic ovary syndrome, antidopaminergic drug intake, hepatorenal diseases and chest wall disorders were excluded. The results were expressed in terms of Mean±Standard Deviation (SD) and compared using student t-test.

Results: Prevalence of macroprolactin was 16 (13.3%) out of 120 among true hyperprolactaemic cases (male=2; female, n=14) based on percentage recovery of PRL in post-PEG cases (Recovery Rate (RR) <40%). The mean pre-PEG and post-PEG values were 52.5 ng/mL and 19.2 ng/mL (RR: 36.5%; p-value <0.05), respectively. The mean pre PEG, PRL values were significantly lower in macroprolactaemic cases than those with true hyperprolactinaemia (52.5 ng/mL versus 74.57 ng/mL; p-value 0.038). Some of the Macro-PRL cases reported with complaints of galactorrhoea, menstrual irregularities and infertility.

Conclusion: The results revealed a prevalence rate similar to those reported in other studies worldwide. Clinical features alone are an unreliable tool to distinguish between cases with true high PRL levels and macroprolactinaemia. Macro-PRL cases once diagnosed requires no extended endocrine review and long term management. Hence, in cases with high PRL levels discordant with clinical symptoms/radiological data routine PEG precipitation test is an inexpensive assay for initial screening for presence of macroprolactin and also monitoring of patients already started on dopamine agonists for hyperprolactinaemia of unknown aetiology.

Keywords: Galactorrhoea, Hyperprolactinaemia, Prolactin

INTRODUCTION

Human PRL hormone secreted by the lactotroph cells of anterior pituitary gland is unique amongst the adenohypophyseal hormones. The primary control of its secretion is inhibitory rather than stimulatory. Dopamine is the principal PRL inhibiting factor regulating PRL secretion [1]. Circulating plasma PRL can be categorised into monomeric prolactin (mPRL, Molecular Weight; MW: 23 kDa), big PRL (bPRL, MW:40-65 kDa), and big-big PRL or macroprolactin (bbPRL) with a MW between 150 and 170 kDa [2]. mPRL of MW 23 kDa, is the biologically active form of PRL and contributes more than 80% to the total serum PRL in a majority of normal and hyperprolactinaemic individuals. The Macro-PRL is responsible for the physiological activity and pathological symptoms due to PRL hormone. Macro-PRL or IgG-bound PRL isoforms constituting about 10-15% of serum PRL are thought to possess no clinical importance because they exhibit little biological activity [3]. Although, biologically inactive or minimally active, macro-PRL is immunoreactive in most of the immunoassays used for estimation of serum PRL levels and thus can lead to a misdiagnosis of hyperprolactinaemia [4]. The clinical significance of macroprolactinaemia has been an issue of debate. Moreover, its prevalence varies regionally across the globe (10-46%) [5]. Some reports have associated it with galactorrhoea, menstrual irregularities and infertility, whereas others have suggested that it causes no symptoms [6]. The PEG precipitation test is widely used to detect pseudo hyperprolactinaemia caused by bPRL and/or macro-PRL [4]. Current best practice recommends that all sera with increased total PRL concentrations be sub-fractionated by PEG precipitation [7]. Treating serum samples with PEG allows laboratories to distinguish patients with true hyperprolactinaemia, in which there are supra physiological concentrations of biologically active monomer, from those with macroprolactinaemia, characterised by increased concentrations of macroprolactin and/or bPRL together with normal concentrations of bioactive monomeric PRL. In the absence of PEG screening, misdiagnosis and consequent clinical mismanagement of patients with hyperprolactinaemia can occur. In this perspective the objective of the study is to explore the clinical features associated with macro-PRL in cases of hyperprolactinaemia.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Biochemistry, Institute of Postgraduate and Medical Research

(IPGME&R) and SSKM Hospital, Kolkata, West Bengal, India, over a period of four months from November 2018 to April 2019. At the onset of research activity, necessary approval from Institutional Ethics Committee for research and Departmental approval has been taken for the handling of endocrine laboratory data (As per memo no. IPGME&R/IEC/2019/480, 09.07.2019). The study principles and procedures adhered to the ethical standards formulated in the (1975, revised in 1983) Helsinki declaration.

Fourteen hundred serum samples were assayed for serum PRL levels based on presenting symptoms of galactorrhoea, amenorrhoea and infertility. The size was calculated based on cumulative prevalence that have shown that the prevalence of hyperprolactinaemia was around 5% in family planning clinic, 9% in women with secondary amenorrhea, and 17% among women with polycystic ovary syndrome [8]. These samples were derived by direct requisition sent on behalf of the physicians both from inpatient and outpatient departments of the hospital for PRL estimation. Retesting for PRL levels were done following precipitation of macroprolactin using polyethylene glycol (PEG, MW: 6000, Merck).

Inclusion criteria: Two hundred and forty serum PRL samples above the manufacturer's reference cutoff level (PRL ≥30 ng/mL; cutoff value, considered irrespective of gender, diagnosis and treatment till then) were obtained from patients with or without symptoms of hyperprolactinaemia.

Exclusion criteria: Forty cases with physiological causes of PRL excess, hypothyroidism, Polycystic Ovary Syndrome (PCOS), on antidopaminergic, antipsychotic drug intake, hepatorenal diseases and chest wall disorders were excluded.

Study Procedure

Serum PRL was assayed by Electro Chemiluminescence Immunoassay (ECLIA) (Immulite 1000 siemens) with intra-assay CV% of 10.66% and inter-assay CV% of 8.45%, respectively. Twenty-five grams of PEG6000 (Merck) was dissolved in 60 mL of distilled water at 18-25°C and mixed in vortex with volume fulfilled till 100 mL of solution (concentration: 25 gm%). A 250 microlitres of that 25% PEG solution was added at room temperature (20-25°C) to an equal volume of patient sera. After vortex mixing and waiting for 30 minutes, the solution was centrifuged at 9500 RPM Xg for 10 minutes. After performing the PEG precipitation test, the PRL level was again estimated in the supernatant by ECLIA. PRL RR was determined by the ratio: Supernatant-PRL/ Initial-PRL x 100, after correction of post-PEG PRL result for PEG dilution factor of 2. When RR was <40%, cases were classified as predominant macro-PRL, 40-60% were classified as indeterminate and RR >60% indicated as monomeric PRL predominance [9,10]. All macro-PRL cases were extensively verified for the presence or absence of clinical symptoms of PRL excess based on data collected from patients' electronic medical records.

STATISTICAL ANALYSIS

Data obtained from the laboratory were statistically using IBM Statistical Package for Social Sciences (SPSS) version 20.0. All values were expressed as mean±SD. Comparison of continuous variables was evaluated using Student's t-test. Probability value p <0.05 was considered to be statistically significant at a confidence limit of 95%.

RESULTS

Biochemical Analysis

The mean age of the population was 35.7±4.5 years, 67% being women. Among the 240 cases of hyperprolactinaemia, 32 cases of macro-PRL excess were detected (RR <40%). The prevalence of macro-PRL was 13.33%. Eighteen cases showed a RR between 40-60% (7.5%). The remaining 190 cases (79.1%) were truly hyperprolactinaemic (RR >60%). The initial pre-PEG, PRL values (mean±SD) were significantly lower in macroprolactaemic cases than those with true hyperprolactinaemia (52.5±12.2 ng/mL and 75±34.6 ng/mL, respectively) (p-value 0.038) [Table/Fig-1].

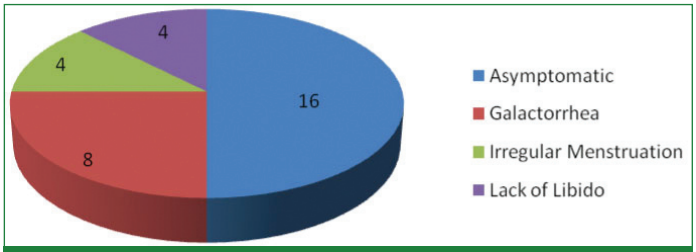
PEG recovery rate (%)	Macro-PRL group	Intermediate group	True hyper-PRL group
<40	32 (13.3%)	-	-
40-60	-	18 (7.5%)	-
>60	-	-	190 (79.1%)

[Table/Fig-1]: Distribution of 240 cases after PEG precipitation test.

The post-PEG values for macro-PRL cases were 19.73±7.73 ng/mL.

Clinical Manifestations

Of the 32 cases of macro-PRL, 24 were women and the remaining eight were men. Eight women had symptoms of galactorrhoea (25%). Four cases of irregular menstrual cycle were noted (12.5%). Lack of libido was the chief complaint in men (n=4). Sixteen remaining cases screened primarily for infertility in both sex and were otherwise asymptomatic [Table/Fig-2]. True PRL excess cases presented with chief complaints of galactorrhoea in 135 (71%) cases, menstrual irregularities in 30 (15.9%) and reproductive dysfunction in 25 cases (13.1%).



[Table/Fig-2]: Symptomatic distribution of macro-PRL cases.

DISCUSSION

Screening for macro-PRL in serum samples is necessary only when the initial analysis indicates an increased total PRL concentration in the absence of history of intake of offending drugs, hormonal or systemic illness. Under these specific circumstances, two outcomes are possible. This abnormal biologically inactive form of PRL shall cross-react in the assay but when corrected for macroprolactin, the bioactive monomeric PRL concentration in the serum after PEG precipitation may still remain high above the normal reference range. In the second case when corrected for macroprolactin, the biologically active PR monomer concentration in this serum may fall within the normal PRL reference range. PEG precipitation has its own drawbacks and gel filtration chromatography remains the gold standard for macro-PRL detection [11]. Nevertheless, PEG precipitation remains to be the most standardised practical method available for routine screening for macroprolactinaemia. Though, slightly more expensive method of PEG precipitation which is less susceptible to the aggregate form is a better representative of the biologically active form [12]. However, instead of reporting results as a percentage of macroprolactin employing a 40% cutoff, it may be advantageous to express results in terms of absolute post-PEG PRL concentration, together with an appropriate reference interval. In differentiating between true hyper-PRL and macro-PRL in serum, this approach might serve to facilitate better interpretation of results because from a clinical point of view the determination of excess monomeric PRL value is of overriding concern rather than simply to estimate the percentage of macro-PRL present in serum [3]. Some investigators had evaluated their reports according to an alternative cutoff (50%) for post-PEG recovery and have observed a difference in the macroprolactinaemia prevalence in comparison to the conventional cutoff ranges used in this study [13].

The prevalence of macroprolactin among hyper-PRL cases may depend on the nature of the samples chosen for analysis. The fact

that all hyperprolactinaemic samples were collected independently of the presence or absence of pituitary prolactinomas the presence of true adenoma cases in the study sample may affect the final outcome for the prevalence of macro-PRL. In the present study the prevalence of macro-PRL was 13.33%. Similar study all across the world have shown a prevalence rate ranging from 10-46% [14,15]. However, studies conducted by Silva AM et al., have shown a low prevalence rate of 2.1% for macro-PRL in 96 enrolled cases of PRL excess [16].

The clinical significance of macroprolactinaemia and its management has been an issue of debate for many years. Some have reported symptoms of galactorrhoea, menstrual irregularities and infertility associated with it while others have suggested that it to be asymptomatic. Macro-PRL has been hypothesised to be biologically inactive and hence macroprolactinaemia cases are generally considered to be clinically asymptomatic. In contrast, Alfonso A et al., have reported menstrual irregularities in 56% of macro prolactinaemic females [17]. We have compared the two groups with true hyper-PRL and macro-PRL and found that the clinical features as expected were more prevalent and overt in the former. Similarly, it was observed that pre-PEG values in macro-PRL cases were much lower than those with true hyper-PRL cases [Table/Fig-2]. Study by Hattori N et al., had two cases of galactorrhoea among 15 cases with macro-PRL [18].

Taghavi M and Sedigheh F have also confirmed presence of symptoms of galactorrhoea and oligomenorrhoea in infertile women with macro-PRL [19]. However, the pituitary images were normal in all these cases [17]. A prevalence of macro-PRL (21%) in 102 hyper-PRL cases with associated reproductive dysfunctions was reported by other researchers [20]. In contrast to the presence of macroprolactinaemia is suspected cases of mild hyperprolactinaemia without any pathological pituitary findings, recent reports of a few cases of macroprolactinaemia with prolactinomas and typical hyperprolactinaemic symptoms have come into notice. Since, the symptoms of hyperprolactinaemia attenuated following treatment with dopamine agonists, biological activity of the high-molecular isoform has been proved to exist. These were similar to that of elevated monomeric PRL levels. Consequently, in these rare cases of macroprolactinaemia pituitary diagnostic imaging, medical treatment, and prolonged follow-up is required [21].

Gel filtration chromatography revealed the existence of multiple isoforms of PRL in circulation. Monomeric PRL (mPRL) is in equilibrium with IgG-bound PRL (bbPRL). The mPRL circulates along with bbPRL in these patients and could be the product of dissociation of bbPRL in vivo, i.e., bbPRL here is thought to act like a reservoir of biologically active PRL [22]. The dissociation of PRL from low affinity, high-capacity IgG antibodies could lead to increased bioavailability of monomeric PRL and serve as a plausible explanation for the clinical manifestations of hyperprolactinaemia in these studies as well as in the cases we have studied [23]. The findings are in agreement to this particular work which approves the functionality of a macro-PRL molecule in circulation.

However, there is another aspect that requires consideration during PEG precipitation of samples. Ram S et al., have reported that monomeric PRL is coprecipitation with serum globulins by PEG [23]. In fact, an increased amount of serum globulin concentrations can lead to increased precipitation of monomeric PRL. So, a false impression of macro-PRL presence warrants caution in PEG test interpretation in cases of IgG myeloma and polyclonal hypergammaglobulinaemia as in HIV infection [24]. These considerations thus should be kept in mind while drawing conclusion about a PEG precipitation result.

Macroprolactinaemia is not known to require specific treatment although beneficial effects have been described by use of dopamine agonist in patients who present with symptoms. Most importantly, spontaneous remission of symptoms may also occur in these cases [25]. Reports from a 10 year follow-up of macro-PRL cases have concluded it to be benign condition apart from subtle symptoms of oligomenorrhoea and galactorrhoea in some and is not related to infertility. Once identified it requires no further endocrine investigation or follow-up [2]. Screening for macro-PRL is also important in clinical practice in clarifying hyperprolactinaemia in apparently healthy asymptomatic individuals to avoid incorrect diagnosis and unnecessary investigations. Moreover, PEG test may be useful in follow-up of cases already on dopamine agonist for hyperprolactinaemia where PRL levels remain elevated even after clinical remission of symptoms. Validated normative reference interval for post-PEG PRL in male and female have been established on most commonly used immunoassay platform and are mostly in concordance with post-PEG PRL values obtained via GFC [26].

Limitation(s)

The study is limited to its application in a hospital based population and requires a wider multicentric study area. The study tries to correlate clinical symptoms with immunological alterations in PRL values only in a point in time. Hence, progressive study needs to be designed to estimate serial change in macro-PRL levels in future along with discernible symptoms among hyper PRL cases. Moreover, the genuine hyper PRL detection rate and PRL RR using latest diluted serum PEG calibration solution precipitation method were significantly higher than those obtained with the macro PRL screening method used in this study.

CONCLUSION(S)

The study provides opportunity for assessment of the PRL molecule. Some changes in PRL molecule like phosphorylation may increase the antigenicity leading to the production of anti-PRL autoantibodies and their class switch in conditions like chronic exposure to allergens or in presence of other autoimmune disorders like Graves disease and Hashimoto's thyroiditis. More follow-up studies however are required to characterise the biochemical character of macro-PRL and its relation to the development of clinical symptoms.

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