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140 Cases of Macroprolactinemia: Selected Clinical and Technical Laboratory Aspects

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ABSTRACT

Background: Macroprolactinemia can be an overlooked cause of hyperprolactinemia. To document the presence of macroprolactin in serum indirectly, an initial precipitation of the complexed prolactin (PRL) using polyethylene glycol (PEG) is followed by measurement of free and total prolactin by immunoassay.
Objectives: Adaptation of the PEG method to our PRL immunoassay to detect cases with predominant macroprolactinemia and to study the short- and long-term changes of the relationship between PRL forms.
Patients and Methods: One hundred forty hyperprolactinemic patients (aged 17-72 years) in whom macroprolactin constituted ≥ 60% of the total PRL were included in our study. The predominance of macroprolactin was measured by adapted PEG procedure, followed by immunoradiometric and chemiluminescence methods.
Long-term observations with repeated serum PRL measurements were made in 20 cases. For another 20 of 41 patients with indications for metoclopramide (MCP) stimulation test, we analyzed short-term alterations in free and complexed PRL levels.
Results: Adjustment of the PEG method by testing samples in dilution minimized the interference of PEG in the immunoassays and let proper detection of predominant macroprolactinemia. During the long-term observations, the ratio of macroprolactin to total PRL remained relatively constant, independent of changes in total PRL levels. During the MCP test, in the majority of patients with macroprolactinemia (except those with associated PRL-secreting adenoma), an acute rise of PRL level followed by a rise in macroprolactin resulted in a short-term decrease in macroprolactin/total PRL ratio.
Conclusions: Confirmation of the predominance in serum of macroprolactin explains the discordance between the raised PRL level and scant of absent symptoms characteristic for hyperprolactinemia. Its proper detection can influence further management.

▸ *Implication for health policy/practice/research/medical education:*
Macroprolactinemia can be an overlooked cause of hyperprolactinemia and this may lead to misdiagnosis and mismanagement. Therefore proper detection of macroprolactin is important for clinical practice.

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1. Background

Nearly 20 years has passed since the fully evidenced cases of macroprolactinemia were reported by Hattori *et al.* (1, 2). According to Hattori (1), the first such case was described 11 years earlier by Whittaker *et al.* (3), likely the first to introduce the term "Big-Big PRL" (BB-PRL). During the last 20 years, many original reports, reviews, and comments on this topic have been published in medical

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journals of which some selected earlier examples can be cited (4-12).

In general, macroprolactin is defined as a complex of monomeric prolactin with anti-PRL IgG. Due to macroprolactin high molecular mass, its bioavailability and bioactivity are decreased, which, with its reduced clearance, may account for the persistence of hyperprolactinemia (7, 12). Despite numerous publications (4-12), our understanding of the macroprolactin problem is still poor, and BB-PRL measurement methods are not broadly accessible (13). The most frequently used procedure for the routine evaluation of the meaningful predominance of macroprolactin in serum is a simple and rapid method of precipitation of the complexed PRL using 25% polyethylene glycol (PEG), followed by the measurement of the free PRL fraction in the supernatant after centrifugation of the precipitated bound PRL fraction and the total PRL in untreated serum. It is generally accepted that a recovery of PRL in the post-PEG supernatant sample of ≤ 40% reflects the predominance of BB-PRL (6, 7, 10-13).

2. Objectives

The aim of our study was to adapt PEG precipitation method to our immunoassays to detect patients with predominant macroprolactinemia among those with hyperprolactinemia and examine the short- and long-term changes in the relationship between PRL forms. To study the short-term changes in total, free, and complexed prolactin levels, the metoclopramide (MCP) stimulation test was appropriate. Metoclopramide (a dopamine receptor antagonist) is useful for testing prolactin secretory reserves, which is usually excessive in patients with functional hyperprolactinemia. In contrast, the response to MCP is markedly diminished or absent in patients with an autonomous PRL-secreting pituitary adenoma (6, 8, 12).

3. Patients and Methods

Of 175 detected cases with predominant macroprolactinemia, we obtained sufficient data for 140 subjects for inclusion into our study. Of these 140 patients, there were 136 females and 4 males (aged 17-72 years). The majority of our hyperprolactinemic patients was investigated for the presence of BB-PRL, because they were either asymptomatic or poorly symptomatic in relation to PRL levels. In 41 of 140 cases, with indications for initial testing of PRL secretory reserve, the oral metoclopramide (MCP) stimulation test was performed, in 20 of whom we analyzed short-term alterations in total, free, and complexed PRL levels, induced by 10 mg MCP administered orally. MRI or CT imaging was usually performed in patients in whom the PRL level was higher than 100 μg/L and sometimes with a PRL of 50–100 μg/L if there were other reasons to do so. Twenty patients were investigated for the presence of BB-PRL during a longer observation period, ranging from 6 to 120 months (median length 36 months). To precipitate the complexed PRL in serum, we used 25% polyethylene glycol (PEG-6000, Fluka W/V), and after vigorous vortexing, followed by centrifugation for 15 minutes at 3000 rpm (1500 g), the appropriate dilutions of post-PEG supernatant and untreated serum were made in a zero calibrator or a designated PRL solvent. Both PRL measurements (the total and free PRL) were made at the same final dilution (x10) and in the same run, initially by immunoradiometric methods (PRL-IRMA, CIS, France and PRL-IRMA, Immunotech, France) and later by chemiluminescence (CLIA) on an Immulite 2000 (Siemens, Germany). The sensitivity of the IRMA assays was 0.65 μg/L, the range of calibrators was 0.5-180 μg/L, and the intra- and inter-CV % was 3-5% and 6-10%, respectively. Same data for the PRL CLIA Immulite 2000 were: 0.5 μg/L, 0.5-150 μg/L, 2.8-3.3% and 4.0-5.3%, respectively.

4. Results

Among 140 hyperprolactinemic patients with predominant macroprolactinemia, we noted 7 with pituitary hypertrophy and 6 with pituitary adenoma; the remaining 127 patients were diagnosed with idiopathic hyperprolactinemia. The presence or absence of specific clinical symptoms of hyperprolactinemia is presented in *Table 1*.

The total and free PRL concentrations (range, median, and Mean ± SD), the calculated percentages of BB-PRL, and the ranges of the observed responses to MCP stimulation (expressed as percentage in relation to basal level) are presented in *Table 2*.

Table 1. The incidence of Symptoms Characteristic for Hyperprolactinaemia in 3 Groups of Patients With Predominant Macroprolactinemia

	Quantity	Absent	Mild	Present
Idiopathic hyperprolactinaemia	127	96	24	7
Pituitary hypertrophy	7	6	1	0
Pituitary adenoma	6	1	2	3

Table 2. The Total and Free PRL Concentrations, and the Calculated Percent of BB-PRL, as Well as the Response to MCP Stimulation in 3 Groups of Patients With Predominant Macroprolactinemia

	PRL Conc., μg/L			
Diagnosis	Total	Free	Calculated BB-PRL, %	Response to MCP stimulation, %
IH [a], (n=127)				
range	26 - 305	0.2 - 34	60 - 100	210-960
Mean ± SD	78 ± 45	9 ± 7	85 ± 12	313
Median	62	8	87	255
PH [a], (n=7)				
Range	57 - 220	1 - 11	92 - 100	200 -295
Mean	125	7	96	250
PA [a], (n=6)				
Range	70 - 700	4 - 75	86 - 94	11 - 30
Mean	310	31	90	15

[a] Abbreviations: IH , Idiopathic Hyperprolactinaemia; PH, Pituitary Hypertrophy, PA, Pituitary Adenoma

In long term-observations in 20 patients (*Tables 3 , 4*), the percentage ratio of BB-PRL remained stable despite the moderate changes in total PRL concentration that were caused by the treatment with dopamine agonists or cessation of this treatment, This ratio was not stable during pregnancy.

Table 3. Serum PRL Concentrations (Total & Free) With the Calculated Percentage of BB-PRL in 15 Hyperprolactinaemic Females With Predominant Macroprolactinemia, Measured During Long-Term Observation Lasting 6 to 120 Months, While Being on or Off Treatment.

Patient No., Age, y	Month of Blood Collection	Total PRL Conc., µg/L	Free PRL Conc. , µg/L	BB-PRL, %
1, 48	0	134	10	93
	12	68	9	87
	20	62	10	84
2, 23	0	128	7	95
	12	36	1,7	96
	24	112	3,7	97
	36	120	4	97
3, 42	0	71	18	75
	12	83	7	92
	23	55	5	90
4, 28	0	110	10	91
	12	130	7	95
	36	56	4	93
	44	60	4	93
5, 43	0	91	26	72
	16	59	13	78
	36	47	6	87
	66	92	23	75
	90	45	5	89
	120	40	7	82
6, 27	0	44	8	82
	33	33	6	82
	44	60	4	93
7, 24	0	110	1,5	100
	19 [a]	290	52	82
	44	103	3	97
	50	85	1	99
8, 27	0	235	21	91
	54	21	0,2	90
	66	32	2	94
9, 42	0	83	7	92
	6	50	6	88
10, 36	0	36	10	72
	44	41	11	73
	74	39	6	85
11, 30	0	50	8	84
	10	47	8	83
12, 46	0	136	34	75
	10	42	16	62
13, 41	0	86	32	63
	24	73	1	86
	36	76	1	87
14, 45	0	120	3	97
	16	100	3	97
15, 36	0	70	17	76
	32	120	17	86
	36	130	17	87

[a] During: P, Pregnancy

Table 4. Serum PRL Concentrations (Total & Free) With the Calculated Percentage of BB-PRL in 5 Patients With Pituitary Adenoma and Concomitant Predominant Macroprolactinemia Measured During Long-Term Observation Lasting 6 – 72 Months, While Being on or Off Treatment.

Patient No., Age, y	Month of Blood Collection	Total PRL Conc. µg/L	Free PRL Conc. µg/L	BB-PRL, %
Female				
1, 30	0	540	50	91
	19 [a]	510	75	85
	28	250	25	90
	43	40	4	90
2, 32	0	700	75	89
	18 [a]	247	45	82
	42	58	5	91
	62 [a]	280	100	64
	72	41	1	97
3, 25	0	130	38	71
	16	115	14	88
	36	132	14	89
	48	130	12	90
	60	120	14	88
	70	240	40	83
Male				
4, 21	0	100	4	96
	17	76	3	96
5, 72	0	70	4	94
	6	25	1	96

[a] During: Pregnancy

5. Discussion

Macroprolactinemia can be an overlooked cause of hyperprolactinemia, primarily because a significant portion of patients with predominant macroprolactinemia do not present symptoms that are commonly associated with hyperprolactinemia. Therefore, such a situation may lead to misdiagnosis and mismanagement (8, 10, 14). We must also be aware that macroprolactinemia can be associated with other causes of hyperprolactinemia, such as pituitary adenoma and pituitary hypertrophy, and although such coincidence happens rarely, it warrants special attention and full diagnostic workup (7, 11, 14-18),. The special attention concers mainly patients who, in addition to predominant macroprolactinemia, have significantly elevated free PRL. In our experience and those of other groups, a lack of or a markedly decreased response to stimulation with metoclopramide (MCP) in patients with high basal levels of PRL may be indicative of autonomous PRL-secreting pituitary adenoma (6, 8, 12, 17), On the other hand, the majority of patients with predominant BB-PRL shows regular or excessive responses to stimulation with MCP, similar to patients with idiopathic hyperprolactinemia and those who present with pituitary hypertrophy (9). Like other groups, we were able to show that in patients with BB-PRL, MCP caused an initial acute rise in free PRL levels, followed by a slower rise in BB-PRL (4, 6, 15). Further, in 20 patients who were observed for longer periods (ranging from 6 to 120 months), the ratio of BB-PRL to total PRL remained constant, despite marked changes in

Table 5. Serum PRL Concentrations (Total & Free) With the Calculated Percentage of BB-PRL During 1-2 h Metoclopramide Stimulation Test (10 mg *per os*) in 20 Females With Idiopathic Hyperprolactinaemia in Whom Macroprolactin Was a Dominant Form.

Patient No., Age, y	MCP Test Time, min	Total PRL Conc., µg/L	Free PRL Conc. µg/L	BB – PRL, %
1, 46	0	136	34	75
	60	410	200	52
	120	320	130	60
2, 48	0	68	9	87
	60	200	60	70
	120	150	34	78
3, 40	0	50	8	84
	60	133	66	50
	120	120	42	65
4, 38	0	74	6	92
	60	160	52	67
	120	147	42	72
5, 57	0	62	0	100
	60	107	44	41
	120	98	43	44
6, 24	0	110	1,5	99
	60	250	95	62
7, 23	0	92	7	93
	60	238	77	68
8, 43	0	91	26	72
	60	237	200	16
9, 28	0	116	10	92
	60	264	117	56
10, 42	0	71	18	75
	60	146	93	37
11, 40	0	55	8	86
	60	220	80	64
12, 48	0	51	10	72
	60	170	99	21
13., 35	0	36	10	80
	60	232	184	42
14, 22	0	103	12	88
	60	200	60	70
15, 24	0	63	1,5	98
	60	180	100	45
16, 30	0	61	7	89
	60	300	90	70
17, 28	0	67	13	81
	60	200	100	50
18, 33	0	27	11	60
	60	180	100	45
19, 38	0	60	1,2	98
	60	196	52	73
20, 27	0	32	6	82
	60	120	40	67

total PRL concentrations, caused by treatment with dopamine agonists or cessation of this treatment. Similar results were recently reported by Hattori *et al.* (19). An important methodological problem in evaluating patients with macroprolactinemia is that methods of PRL estimation have variable degrees of reactivity with BB-PRL, and in some cases, the difference in outcome is 2.7–7.2-fold (20). Therefore, each laboratory should examine this matter, determine whether the presence of anti-PRL in serum causes any distortion in its PRL assay, and adapt the method of separating free from complexed PRL to be compatible with the immunoassay. The classical method, gel filtration chromatography (GFC), is time-consuming and too expensive for routine use. Therefore, the most widely used technique has become measuring PRL recovery after serum BB-PRL precipitation with polyethylene glycol. This method, which initially was proposed to detect insulin autoantibodies (2), has been validated by other groups (6, 10, 11, 20-23). It appears that this PEG method, although it is not specific or quantitative, shows the best correlation with GFC (22, 23). Difficulties in BB-PRL measurements arise with the variable influence of the final (12.5%) PEG concentration in the sample on the immunological reaction in immunoassay systems (7, 15, 21). Therefore, some groups that have used the PRL-IRMA assay have proposed treating all calibrators and control samples with 25% PEG solution. Such a procedure is not convenient and is not applicable for automatic platforms. For the IRMA assay, we propose performing both PRL measurements (total and free PRL) in the 10-times final dilution prepared in the zero calibrator (9,17) ; in the case of automatic methods, such dilutions can be made in the appropriate diluent for each immunoassay platform. In our opinion, diluting samples with PBS or distilled water may cause greater distortions between the PRL result in undiluted samples and the recalculated result of the measurement in diluted samples due to the matrix effect. This could explain why Beltram *et al.* (21) did not recommend routine dilution of samples, although it decreases PEG interference. Parallel testing of free and total PRL in diluted samples was introduced recently by Hattori *et al.* (19). Centrifugal ultrafiltration, proposed by Prazeres *et al.*, is a potentially useful method for separating high-molecular-mass forms of PRL (24). This method, defined on physical principles, should not interfere in the PRL assay system and could be useful in assays in which such interference of PEG was noted (13). In practice, however, according to Gibney *et al.* (7) and Kavanagh *et al.* (22), this method can not be recommended as a suitably precise and reliable method for routine use.

The PEG method remains the most useful technique for indirectly measuring the presence of BB-PRL as the predominant form of serum prolactin, but its routine use should be tested for compatibility with a particular PRL immunoassay. Confirmation of the predominance of BB-PRL explains the common discordance between increased PRL levels and scant or absent symptoms that are characteristic for hyperprolactinemia. Incidental coexistence of macroprolactinemia and pituitary adenoma or pituitary hypertrophy

demands special attention, thus, full diagnostic procedures should be undertaken, including the MCP stimulation test.

During the oral MCP test, an acute rise in free PRL levels, followed by a slower rise in BB-PRL, resulted in a short-term decrease in BB-PRL/total PRL ratio in the majority of patients with macroprolactinemia (except those with the associated PRL-secreting adenoma). During long-term observation, the BB-PRL/total PRL ratio remains relatively constant, independent of changes in total PRL levels that are induced by the specific treatment or its cessation.

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