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The detection of macroprolactin by precipitation and ultrafiltration methods

Wykrywanie makroprolaktyny za pomocą metody precypitacji i ultrafiltracji

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Abstract

Background: Prolactin (PRL) exists in human blood in several molecular forms. Macroprolactin (MaPRL), which most often consists of monomeric PRL and immunoglobulin G, has the highest molecular weight but no biological activity. Immunoassays do not distinguish MaPRL from monomeric PRL, what can lead to an incorrect diagnosis of hyperprolactinaemia. The most commonly used technique to separate the isoforms of PRL is precipitation with polyethylene glycol (PEG). Another technique — ultrafiltration — seems to be useful in MaPRL detection. The aim of this study was to evaluate the occurrence of MaPRL in hyperprolactinaemic patients and to compare the results obtained by precipitation and ultrafiltration.

Material and methods: The study was conducted on 120 sera obtained from patients hospitalised in the Department of Clinical Endocrinology, Medical University of Lodz, in whom PRL concentration was above 30 ng/mL Of these 120 patients, 25 had pituitary adenoma, 52 had polycystic ovary syndrome (PCOS), and 43 had idiopathic hyperprolactinaemia (HPRL). Macroprolactin was detected using two methods: precipitation with PEG and ultrafiltration. Concentration of PRL was measured by Immulite 1000 immunoassay (Siemens).

Results: We detected a predominance of MaPRL in ten patients (three with macroprolactinoma, three with PCOS and four with HPRL) using precipitation and ultrafiltration. Positive correlation and diagnostic concordance between the results of precipitation and ultrafiltration were noted, especially in the group with functional hyperprolactinaemia. In half of the patients with macroprolactinaemia, and in 12 of the 110 subjects without significant amounts of MaPRL, real PRL concentration was within the reference range.

Conclusions: MaPRL is not a significant clinical problem in the studied population. However, in patients with hyperprolactinaemia, especially non-organic, screening for macroprolactinaemia should be performed. The effectiveness of the precipitation and ultrafiltration methods for detecting MaPRL is comparable in functional hyperprolactinaemia, but the usefulness of ultrafiltration in patients with pituitary adenoma requires further examination. **(Pol J Endocrinol 2011; 62 (6): 529–536)**

Key words: *prolactin, macroprolactin, hyperprolactinaemia, polyethylene glycol, precipitation, ultrafiltration*

Streszczenie

Wstęp: We krwi ludzkiej występuje kilka izoform prolaktyny (PRL). Największą z nich, ale niewykazującą aktywności biologicznej jest makroprolaktyna (MaPRL). Stanowi ona połączenie monomerycznej PRL z immunoglobuliną G. Zestawy diagnostyczne do pomiaru stężenia PRL nie odróżniają MaPRL od postaci monomerycznej, co może prowadzić do błędnego rozpoznania hiperprolaktynemii. Najczęściej stosowaną techniką rozdziału izoform PRL jest precypitacja za pomocą glikolu polietylenowego (PEG). Przydatną w wykrywaniu MaPRL wydaje się też być technika ultrafiltracji. Celem pracy była ocena częstości występowania MaPRL u pacjentów z hiperprolaktynemią oraz porównanie wyników uzyskanych za pomocą metody precypitacji i ultrafiltracji.

Materiał i metody: Surowice do badania uzyskano od 120 osób hospitalizowanych w Klinice Endokrynologii Uniwersytetu Medycznego w Łodzi, u których stężenie PRL było wyższe niż 30 ng/mL — 25 chorych z gruczolakiem przysadki, 52 kobiety z zespołem policystycznych jajników (PCOS) i 43 osoby z hiperprolaktynemią idiopatyczną (HPRL). Do wykrywania MaPRL zastosowano metodę precypitacji PEG-iem i technikę ultrafiltracji. Stężenie PRL oznaczano na analizatorze Immulite 1000 (Siemens).

Wyniki: Za pomocą metod precypitacji i ultrafiltracji znaczące ilości MaPRL wykryto u 10 osób (3 z *macroprolactinoma*, 3 z PCOS i 4 z HPRL). Zaobserwowano dodatnią korelację oraz wysoką zgodność diagnostyczną pomiędzy wynikami obu metod, szczególnie w grupie osób z hiperprolaktynemią czynnościową. U połowy osób ze stwierdzoną makroprolaktynemią oraz u 12 spośród 110 osób bez znaczącej ilości MaPRL we krwi rzeczywiste (po usunięciu makroform hormonu) stężenie PRL mieściło się w zakresie wartości referencyjnych.

Wnioski: Makroprolaktyna nie stanowi znaczącego problemu klinicznego w badanej populacji. Jednakże u osób z hiperprolaktynemią, szczególnie pochodzenia nieorganicznego, powinno się wykonywać badania przesiewowe w kierunku makroprolaktynemii. Skuteczność precypitacji i ultrafiltracji w wykrywaniu MaPRL jest porównywalna u osób z hiperprolaktynemią czynnościową, natomiast do określenia przydatności metody ultrafiltracji u pacjentów z gruczolakiem przysadki potrzebne są dalsze badania.
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Słowa kluczowe: *prolaktyna, makroprolaktyny, hiperprolaktynemia, glikol polietylenowy, precypitacja, ultrafiltracja*

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Introduction

The presence of various forms of prolactin (PRL) in human blood was described more than 30 years ago [1–3]. It was proved that PRL occurs mainly in three molecular forms: a monomeric PRL with molecular weight ~ 23 kDa; a larger form named "big PRL" (~50 kDa) and a "big, big PRL" with a molecular weight over 100 kDa which is also called macroprolactin (MaPRL). In the majority of cases, MaPRL consists of monomeric PRL connected with immunoglobulin; most frequently type G, although other forms which have a very high molecular mass such as conglomerates of glycosylated PRL have also been found in human serum [4–8]. The predominant isoform of PRL in healthy people, and also in most individuals with hyperprolactinaemia, is a monomeric molecule which amounts to more than 85% of circulating hormone. However, in some patients with hyperprolactinaemia, the dominant form becomes MaPRL, which in normal conditions does not exceed 2% of total serum PRL. It has been shown that MaPRL is biologically inactive because the large molecular size of this complex prevents its crossing through the capillary blood barrier and reaching target cells [9, 10]. Moreover, immunoglobulin connecting with specific epitops of PRL molecule may reduce the binding of the hormone to its receptors [11, 12]. On the other hand, most commercial immunoassays used to measure PRL level do not distinguish MaPRL from monomeric PRL, which can lead to an incorrect diagnosis of hyperprolactinaemia and the implementation of unnecessary imaging study and treatment. Cases where MaPRL predominates in human serum are called macroprolactinaemia. The percentage of hyperprolactinaemic patients with macroprolactinaemia has been studied several times, and ranges from 10% to 45% [13–17]. Among methods used to detect MaPRL, gel filtration chromatography (GFC) is acknowledged to be the gold standard, but its highly complex, time-consuming, and expensive procedure prohibits its use in routine screening for MaPRL [18–20].

The most widely applied alternative method is precipitation with polyethylene glycol (PEG). However, this relatively simple, rapid and cheap technique is not perfect because PEG can interfere with some PRL immunoassays and it can precipitate not only MaPRL but partially also monomeric PRL [18, 21, 22]. Therefore, other methods of detecting MaPRL are still being sought. Recently, it has been shown that ultrafiltration based on physical separation of high weight molecules such as "big, big PRL" from the smaller isoforms of hormone may be useful in screening for MaPRL [23–25].

In the present study, we detected MaPRL using the classical method of precipitation with polyethylene glycol, and also the ultrafiltration method. The aim of this study was to evaluate the occurrence of MaPRL in hyperprolactinaemic patients hospitalised in the University Hospital of Lodz and to compare the results obtained by using two MaPRL screening techniques.

Material and methods

Patients

The study was conducted on 120 patients (110 women and 10 men) hospitalised in the Department of Clinical Endocrinology, Medical University of Lodz. In all the subjects, the concentration of PRL measured in a fasting state was above 30 ng/mL. Based on the clinical data and laboratory results, the following diagnoses were made: 25 cases of prolactinoma (12 macroadenomas and 13 microadenomas); and 95 patients with functional hyperprolactinaemia (52 women with polycystic ovary syndrome and 43 cases of idiopathic hyperprolactinaemia). Some of the patients with prolactinoma were treated pharmacologically before the examination using dopamine agonists (13 subjects) and three persons also underwent neurosurgical therapy.

PRL immunoassay

Prolactin concentration was measured by enzyme-amplified chemiluminescent immunoassay (Immulite 1000, Siemens). Analytical sensitivity of the assay is 0.5 ng/mL. Intraassay coefficients of variation (CV) at the PRL concentration of 16.3 ng/mL is 6.1% and interassay CV at the concentration of 14.1 ng/mL is 9.6%. Reference ranges are 1.9–25.0 ng/mL for women and 2.5–17.0 ng/mL for men.

Precipitation with PEG

Precipitation with PEG was performed according to the method proposed by Olukoga and Kane [18] and also followed a protocol recommended by the Diagnostic Products Corporation. Equal volumes of PEG (Sigma) and serum were mixed. The obtained solution was incubated at room temperature for 10 minutes and then a sample was centrifuged at 3,000 rpm for 30 minutes. Prolactin concentration was measured in 10-fold diluted, untreated serum (PRL_{total}) and also in 10-fold diluted supernatant obtained after PEG precipitation (PRL_{PEG}). The percentage ratio PRL_{PEG}/PRL_{total} was calculated. In accordance with most literature data [18, 13, 26], a result equal to or below 40% was assumed to be predominance of MaPRL in the serum sample (macroprolactinaemic subjects).

Ultrafiltration

Ultrafiltration process was performed according to the procedure described by Kavanagh-Wright [21, 27]. The

Microcon YM-100 ultrafilter (Millipore; membrane with cut-off 100 kDa of molecular mass) was used to separate macroforms of PRL. The serum samples (25 µl) were mixed with 475µl phosphate buffer saline (PBS) and were centrifuged at 3,000 rpm for 45 minutes. Prolactin concentration was measured in filterable fraction of serum and in the original serum sample. Recovery hormone after ultrafiltration was calculated by dividing the PRL concentration of ultrafiltrate by the PRL concentration of untreated serum.

Statistical analysis

The data obtained from the experiment was recorded on Excel (MS Office 2007) worksheets. Basic descriptive statistics (mean, SE) were calculated. A statistical analysis was performed using one-way ANOVA followed by Fisher's t-test (LSD — least significant difference) method according to Statistica 9 computer program (licensed to the Medical University of Lodz). In the case of the analysis of data measured before and after PRL forms separation (precipitation and ultrafiltration methods), a pairwise test was applied. Additionally, the Pearson linear correlation coefficient (r) was determined, in the case of their statistical significance (p), and the equation of regression was calculated ($y = ax + b$). Statistical differences between the tested values were at a significance level of $p < 0.05$. The sensitivity, specificity and ROC curve were used as methods determining the precision of the diagnostic test.

Results

The clinical data of the patients and the mean of PRL levels (total, after PEG precipitation, and after ultrafiltration) are presented in Table I. As had been anticipated, the patients with prolactinomas had higher levels of total PRL than women with PCOS and HPRL. After the removal of PRL macroforms by precipitation or ultrafiltration methods, the values of the hormone in the group with pituitary adenomas remained the highest, and repeatedly exceeded the reference range. The means of PRL level in PCOS and HPRL groups were significantly lower, but were also above the reference range. The means of PRL recovery after PEG precipitation were significantly higher than the values of the hormone after ultrafiltration in most investigated groups.

The recoveries of PRL after PEG precipitation, and after ultrafiltration, were compared by linear regression. We noted a positive correlation between the two methods for the whole studied group — patients with and without macroprolactinaemia together (Figure 1) and in the group without macroprolactinaemia ($r = 0.3409$, $p < 0.001$) but no correlation of PRL recoveries was observed in the group with macroprolactinaemia ($r = –0.0489$, $p > 0.05$).

In accordance with most literature data, the recovery of PRL after PEG treatment equal to or less than 40% indicates a prevalence of MaPRL in the examined serum sample. The limit of recovery PRL after ultrafiltration

Table I. *Clinical data and serum levels of PRL in subjects*
Tabela I. *Dane kliniczne i stężenia PRL we krwi badanych osób*

Clinical diagnosis		Prolactinoma (n = 25)				HPRL (n = 43)	PCOS (n = 52)
		Macroadenoma (n = 12)		Microadenoma (n = 13)			
Mean values		No treatment	Treatment	No treatment	Treatment		
Age [years] Min–max		40 ± 6.2 22–52	36 ± 2.8 24–45	34 ± 3.5 20–48	42 ± 4.6 23–54	28 ±1.2 17–60	26 ± 0.8 17–43
PRL [ng/mL]		3,322 ± 1,891.2	206 ± 94.9	148 ±16.9	86.2 ± 19.5	60 ± 3.1	57 ± 3.2
PRL min–max [ng/mL]		426 – 10,540	33 – 751	99 – 211	40 – 144	32 – 113	31 – 143
Recovery of PRL after PEG (%)		62 ± 10	97 ± 11.9	78 ± 2.4	85 ± 5.6	69 ± 1.9	68 ± 1.7
Recovery of PRL after UF [%]		43 ± 4.8	69 ± 9.1	60 ± 7.5	66 ± 8.8	60 ± 2.2	64 ± 2.0
PRL_{PEG} [ng/mL]		1,421 ± 484.4	201 ± 102.7	121 ± 12.3	68 ± 17	43 ± 3.0	40 ± 2.7
PRL_{UF} [ng/mL]		1,424 ± 784.0	131 ± 68.0	97 ± 15.7	47 ± 9.0	36 ± 1.6	36 ± 1.7
Number of pts having PRL conc. [ng/mL]	30–49.9	0	2	0	2	16	26
	50–99.9	0	1	1	2	23	22
	≥ 100	5	4	6	2	4	4

HPRL — idiopathic hyperprolactinaemia; PCOS — polycystic ovary syndrome; PRL_{PEG} — prolactin level after PEG precipitation; PRL_{UF} — prolactin level after ultrafiltration

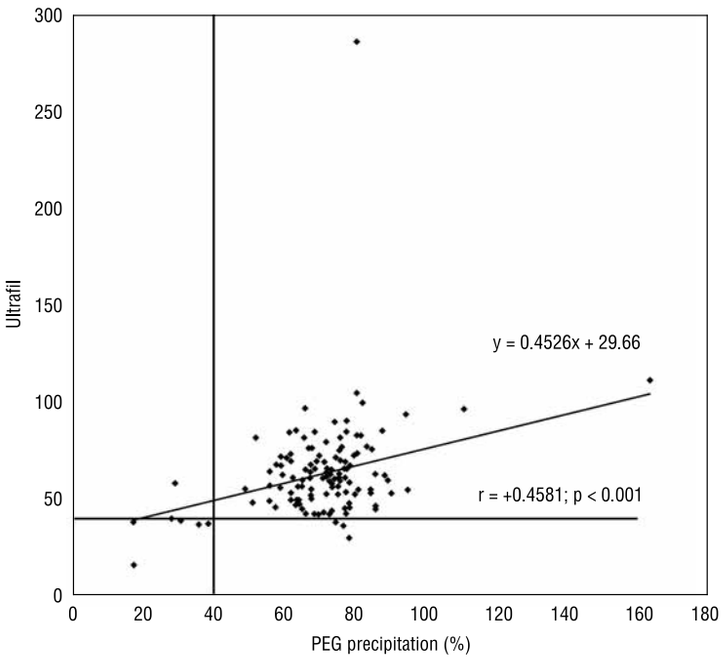

Figure 1. *Correlation between recoveries of PRL (%) after PEG precipitation and ultrafiltration methods. PEG — polyethylene glycol*
Rycina 1. *Współzależność odzysku PRL po precypitacji PEG-iem i odzysku po ultrafiltracji; PEG — glikol polietylenowy*

has not been quantified exactly in the literature [23–25]. In order to determine the precision of both diagnostic techniques for detecting MaPRL, i.e. PEG precipitation and ultrafiltration methods, the area under the ROC curve (AUC ROC) was used. The ROC curve describes the relationship between the true positive and the false positive results of examined methods (precipitation and ultrafiltration) for the different possible cut-off points of a diagnostic test (in our study — ultrafiltration method). The area under the ROC curve indicates the precision of the examined method (UF). The size of the area under the ROC curve indicates the power of a diagnostic test. It is generally accepted that the significant power of a diagnostic test is represented as a volume of AUC ROC of between 0.80–0.95. In our experiment, AUC ROC amounted to 0.91. The highest value of the swelling of the ROC curve has been accepted as a cut-off point — in our study the range of cut-off points of ultrafiltration was 38–40% (Figure 2). Additionally, the sensitivity curve and the specificity curve for UF were plotted. The cut-off point of UF was chosen near the intersection value of the sensitivity and specificity curves and demonstrated the high precision of the test (97%). In the used test, the cut-off point for ultrafiltration method was also in the range of 38–40% and those values were very similar to the cut-off point for PEG precipitation — 40% (Figure 3).

We performed a diagnostic concordance test, sometimes known as 'test effectiveness'. It defines the percentage of truly positive and truly negative results in relation to all performed tests. It is assumed that a test with effectiveness of less than 80% is of no diagnostic usefulness. We found that the agreement between positive and negative results of precipitation and ultrafiltration methods was high and reached almost 97% (Table II).

Table II. *Number of MaPRL "positive" or "negative" results basing on 40% criterion for both methods*
Tabela II. *Liczba wyników „pozytywnych" lub „negatywnych" dla MaPRL na podstawie 40% kryterium dla obu metod*

Ultrafiltration (n = 120)	Precipitation (n = 120)	
	Positive results (PEG ≤ 40)	Negative results (PEG > 40)
Positive results (UF ≤ 40)	6 (5.0%)	3 (2.5%)
Negative results (UF > 40)	1 (0.8%)	110 (91.7%)
Diagnostic concordance	96.7%	

Patients with macroprolactinaemia

Using the 40% limit for PEG precipitation and ultrafiltration, significant amounts of MaPRL were detected in ten patients among the 120 hyperprolactinaemic subjects (8.3%). Macroprolactin was found in 12% of patients with prolactinoma, 5.8% of women with

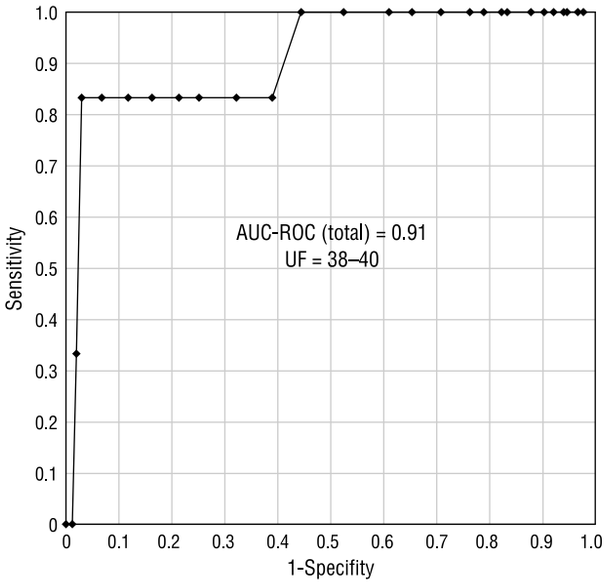

Figure 2. *ROC curve for precipitation and ultrafiltration methods*

Rycina 2. *Krzywa ROC dla metody precypitacji i ultrafiltracji*

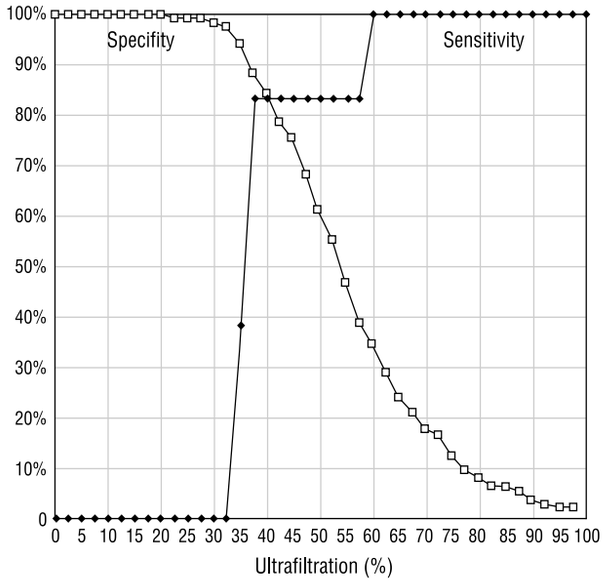

Figure 3. *Sensitivity and specificity curves for ultrafiltration (regarding PEG precipitation cut-off point — 40%)*

Rycina 3. *Krzywe czułości i specyficzności dla ultrafiltracji (zgodnie z punktem odcięcia dla precypitacji PEG-iem — 40%)*

PCOS, and 9.3% of subjects with HPRL (Table III). In six patients (women with PCOS and HPRL), MaPRL was detected by both precipitation and ultrafiltration methods. In four of these cases, the recoveries of PRL after PEG treatment and after UF were very similar. In four other subjects (three men with adenoma and one woman with HPRL), we noted a disagreement between the results obtained by the precipitation and ultrafiltration methods: three patients were macroprolactinaemic according to UF, and (another) one according to PEG. Moreover, three women with HPRL did not show clinical symptoms of increased PRL level (Table III).

After separating macroforms of PRL according to PEG precipitation results, the hormone concentration turned out to be in the reference range (established by the manufacturer) in half of the macroprolactinaemic patients (only women with PCOS and HPRL — patients no. 4, 5, 8, 9 and10 — Table III).

Table III. *Characteristics of patients with macroprolactinaemia*

Tabela III. *Charakterystyka pacjentów z makroprolatynemią*

No.	Sex/age	Clinical diagnosis	Clinical symptoms of HPRL	PRL [ng/mL]	Post-PEG recovery (%)	Post-UF recovery (%)	PRL_{PEG} [ng/mL]	PRL_{UF} [ng/mL]	Treatment
1.	M/22	Macroprolactinoma	Loss of libido	1,0540	29	58	3100	6087	brc
2.	M/28	Macroprolactinoma	Loss of libido	554	75	38	413	208	brc
3.	M/52	Macroprolactinoma	Loss of libido	1,097	79	29	861	319	brc
4.	F/22	PCOS	Oligomenorrhea	57	39	37	22	21	brc
5.	F/20	PCOS	Oligomenorrhea	80	29	40	23	32	brc
6.	F/37	PCOS	Oligomenorrhea	170	38	36	64	62	brc
7.	F/30	HPRL	Infertility	115	77	36	87	41	brc
8.	F/18	HPRL	–	129	18	17	23	20	brc
9.	F/31	HPRL	–	87	18	38	16	33	brc
10.	F/29	HPRL	–	80	31	38	25	30	brc

HPRL — idiopathic hyperprolactinaemia; PCOS — polycystic ovary syndrome; post-PEG recovery — recovery of PRL after PEG precipitation; post-UF recovery — recovery of PRL after ultrafiltration; PRL_{PEG} — PRL level after PEG precipitation; PRL_{UF} — PRL level after ultrafiltration; brc — bromocriptine

Table IV. *Characteristics of patients who have real PRL concentration in reference range*
Tabela IV. *Charakterystyka pacjentów, u których rzeczywiste stężenie PRL mieściło się w zakresie wartości referencyjnych*

No.	Sex/age	Clinical diagnosis	Clinical symptoms of HPRL	PRL [ng/mL]	Post-PEG recovery (%)	Post-UF recovery (%)	PRL_{PEG} [ng/mL]	PRL_{UF} [ng/mL]	Treatment
1.	F/35	PCOS	Oligomenorrhea	37	58	67	22	25	brc
2.	F/32	PCOS	Oligomenorrhea	36	61	71	22	25	brc
3.	F/18	PCOS	Oligomenorrhea	39	76	59	29	23	brc
4.	F/20	PCOS	Oligomenorrhea	31	77	76	24	24	brc
5.	F/24	PCOS	Oligomenorrhea	35	70	72	24	25	brc
6.	F/25	PCOS	Oligomenorrhea	51	49	55	25	28	brc
7.	F/24	PCOS	Gallactorrhea	40	62	84	24	33	brc
8.	F/29	PCOS	_	36	60	62	21	22	brc
9.	F/28	PCOS	_	50	64	48	32	24	brc
10.	F/13	HPRL	_	32	66	82	21	26	brc
11.	F/20	HPRL	_	35	78	65	28	23	brc
12.	F/24	HPRL	_	48	56	49	27	24	brc

HPRL — idiopathic hyperprolactinaemia; PCOS — polycystic ovary syndrome; PRL — concentration of PRL after using precipitation or ultrafiltration method; post-PEG recovery — recovery of PRL after PEG precipitation; post-UF recovery — recovery of PRL after ultrafiltration; PRL_{PEG} — PRL level after PEG precipitation; PRL_{UF} — PRL level after ultrafiltration; brc — bromocriptine

In the group without significant amounts of MaPRL (110 subjects), we noted that after the removal of the macroforms of the hormone, the level of PRL was within the reference range in 12 patients (nine cases of PCOS and three subjects with HPRL). However, five of these subjects (42%) did not have clinical symptoms of hyperprolactinaemia (Table IV).

Discussion

Macroprolactinaemia occurs only sporadically in a healthy population [28, 29]. However, significant amounts of MaPRL have been found in hyperprolactinaemic subjects [9, 16, 20, 30–32]. The incidence of macroprolactinaemia depends on the examined population and the method of MaPRL detection. It has been documented that in the United States about 10% of hyperprolactinaemic patients have MaPRL as a predominant form of PRL [33, 34]. Most of the studies conducted in Europe have shown that the frequency of macroprolactinaemia is usually above 20% [13, 14, 35, 36]. In the present study, significant amounts of MaPRL were detected in 8% by evaluating with both methods and in only 5.8 % confirmed by PEG precipitation alone, which seems to be a rather low rate in comparison with the results mentioned above. In comparison with the results of other Polish investigators, we have found a lower frequency of macroprolactinaemia in subjects with idiopathic hyperprolactinaemia (9.3% *vs.* 25% and 34%) [37, 38]. We have used a similar precipitation procedure but different immunoassay (Immulite 1000 *vs.* Immunotech) and we noticed that in patients with HPRL in our group, the basal concentration of PRL was lower than in the work of the Warsaw group [37]. Conversely, Jeske et al. [37] obtained a two thirds lower frequency of macroprolactinaemia (4% vs. 12%) in patients with prolactinoma. But when we presume that dominancy of MaPRL confirmed by PEG precipitation concerns only one patient, we have exactly the same frequency — 4%. The gold standard for estimating various forms of PRL is gel chromatography, but in routine laboratory work, precipitation with PEG is applied more often. However, PEG treatment is not a perfect technique for detecting MaPRL because PEG may precipitate not only macroforms of PRL but also some monomeric PRL. Furthermore, PEG may cause immunoassay interference on the analysers, including Immulite which was used to measure PRL concentration in our study [21, 22, 36]. Therefore, screening for macroprolactinaemia may sometimes require other techniques. One of the proposed methods is centrifugal ultrafiltration. There are only a few papers concerning ultrafiltration in world literature [21, 23–25, 27]. In our study, we noted a positive correlation and a general high diagnostic concordance between the investigated methods. We observed that in patients with functional hyperprolactinaemia, the results of both methods based

on a 40% cut-off point were all concordant, except for one case. However, in patients with pituitary adenoma, the results of precipitation and ultrafiltration vary considerably. Previous studies have shown a disagreement in recovery after ultrafiltration compared to PEG precipitation for samples without macroprolactin; in such cases, the results of ultrafiltration may exhibit apparent (false-positive) macroprolactinaemia [21, 23, 25].

In our study, such a situation might concern two patients with pituitary adenoma (No. 2 and 3 — Table III) and a woman with idiopathic hyperprolactinaemia (No.7 — Table III) — where the well-established precipitation method indicated that there was no MaPRL domination in their sera. The lower hormone recovery seen after ultrafiltration than after precipitation can be explained by the fact that in some cases of hyperprolactinaemia with high concentrations of PRL (higher than 100 ng/mL) the passage of different forms of PRL through the separating membrane during ultrafiltration depends not only on their molecular size, but also on net charge and three-dimensional structure of particles [25]. On the other hand, the lower hormone recovery after precipitation than after ultrafiltration (in our study — patient No. 1 — Table III) may be due to the co-precipitation of monomeric PRL and "big PRL" by PEG. This may lead to an underestimation of monomeric PRL concentrations by 25% or even more [21, 39]. Moreover, according to the manufacturer of the Microcon ultrafilter, during the ultrafiltration almost all molecules up to 45 kDa freely cross the membrane of the filtration unit, but only 50% of particles with molecular weight, like "big PRL" (~60kDa), do so (Microcon leaflet).

Hyperprolactinaemia in women with PCOS has been widely described for decades but the mechanism of this coexistence remains largely unknown [40–44]. Recent studies have even suggested that the elevation of PRL concentration and PCOS are independent disorders and they recommend that patients should be investigated for other causes of hyperprolactinaemia [45, 46]. Our results showed that PRL concentration in PCOS was very similar to idiopathic hyperprolactinaemia. Moreover, the recoveries of PRL after PEG precipitation and after ultrafiltration were comparable (Table I). However, the occurrence of macroprolactinaemia in PCOS women was the lowest among three examined groups (5.8% *vs.* 12% for prolactinoma and 9.3% for HPRL).

From the clinical point of view, it is important to realise that the statement of macroprolactinaemia does not exclude hyperprolactinaemia. Especially in patients with high basal PRL concentration, recovery of PRL of lower than 40% may occur, but real hormone concentration, after macroforms separation, may be still above the reference range. Here, treatment with appropriate drugs, such as dopamine agonists, should be applied. On the other hand, in patients who have even a small number of macroforms (recovery higher than 40%) it may turn out that real PRL concentrations remain in the reference range. In our study, this situation has concerned 12 subjects (10%) — Table IV. Some of them (~ 40%) have no clinical symptoms of hyperprolactinaemia and probably those patients, similarly to subjects with macroprolactinaemia, have not required drugs which inhibit the secretion of PRL.

In conclusion, we can state that screening for MaPRL should be performed in hyperprolactinaemic sera, particularly in non-organic hyperprolactinaemia. Although the Immulite test for the measurement of PRL is classified as a "middle-reacting" assay with MaPRL [36], our results show that macroprolactinaemia in hyperprolactinaemic patients seems not to be a major clinical problem because the frequency of macroprolactinaemia in our study was rather low.

Moreover, on the basis of the findings, we can conclude that the effectiveness of the precipitation and the ultrafiltration methods for detecting MaPRL is comparable in functional hyperprolactinaemia, but the usefulness of ultrafiltration in patients with pituitary adenoma requires further examination, including the verification of obtained results by using gel chromatography.

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