JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2014, 65, 3, 359-364
www.jpp.krakow.pl

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EVALUATION OF HYPERPROLACTINAEMIA WITH THE USE OF THE INTERVALS FOR PROLACTIN AFTER MACROFORMS SEPARATION

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Macroprolactin (MaPRL) - a complex of monomeric prolactin (PRL) with immunoglobulin G, may be a cause of laboratory diagnosed hyperprolactinaemia. To quantify MaPRL, a precipitation with polyethylene glycol may be performed. This method involves calculating of recovery ratio but the cut-off value is not precisely determined. Moreover, it is proposed that the assessment of macroprolactinaemia should include also the evaluation of real PRL concentration which means the level of the hormone after macroforms separation. The study included 245 patients with hyperprolactinaemia, in whom precipitation was performed. A recovery ratio ≤40% indicated macroprolactinaemia. The real PRL concentrations of the studied subjects were compared with reference ranges suggested by the assay manufacturer and with new intervals for PRL after macroforms separation. On the base of the recovery ratio after the precipitation, macroprolactinaemia was detected in 21 persons. In these patients true hyperprolactinaemia (elevation of real PRL concentration above manufacturer's reference ranges) was noted in 9 cases. Among 224 patients with a recovery ratio >40%, real PRL concentration turned out to be within the manufacturer's reference range (pseudohyperprolactinaemia) in 36 persons. The new intervals for PRL after macroforms separation were about 20% lower than the manufacturer's reference ranges. After applying new ranges in patients with macroprolactinaemia, true hyperprolactinaemia was observed in 14 persons, while in the group without MaPRL dominance, pseudohyperprolactinaemia was noted in 5 patients. The use of the recovery ratio only to recognize macroprolactinaemia may lead in some subjects to the misclassification of the results. For that reason the assessment of the PRL concentration after macroforms separation that can help to distinguish true hyperprolactinaemia and pseudohyperprolactinaemia, seems to be reasonable. To evaluate the real PRL concentration, the reference intervals suggested by the manufacturer of immunoassay might be used. However, possibly better means to diagnose patients with hyperprolactinaemia accurately is using an appropriate range for the concentration of PRL after macroforms separation.

Key words: *macroprolactin, hyperprolactinaemia, prolactin, precipitation, prolactinoma, separation*

INTRODUCTION

The elevated concentration of prolactin (PRL) in the blood serum, called hyperprolactinaemia, is a commonly encountered clinical condition. It is worth to note that it is in fact not a disease but the effect of some abnormalities occurring in the human body. Hyperprolactinaemia in majority of cases is caused by functional disturbances that affect PRL secretion, for example primary hypothyroidism, liver or kidney failure and some drugs intake. The increase in PRL level may be also due to the tumors of hypothalamic-pituitary region - most often a pituitary adenoma - *prolactinoma* (1-4). On the other hand, the laboratory result indicating hyperprolactinaemia may be the effect of macroprolactin (MaPRL) occurrence in the blood. Macroprolactin is a large PRL isoform (MW about 150 kDa) that consists mainly of the particle of monomeric hormone connected with immunoglobulin G. However, other macroforms of the hormone also may occur in the blood - those are dimers (big-PRL, MW 50–60 kDa) and conglomerates of monomeric or modified (*e.g.* with glycosylation) hormone particles (big-big-PRL, MW>100 kDa, even 600 kDa) (5).

In comparison with monomeric PRL (mPRL), MaPRL and probably other hormone macroforms have limited bioactivity, however, they may react with the components of immunoassays designed for the measurement of PRL concentration. Therefore, the presence of large amounts of MaPRL can lead to the overestimation of PRL level (pseudohyperprolactinaemia) and sometimes to the unsuitable treatment (6-12). To quantify MaPRL in the blood the precipitation with polyethylene glycol (PEG) is the most often used method (8-15). This screening method of MaPRL detection has a simple and cheap procedure but some unclear results may require confirmation by gel filtration chromatography (GFC) which is known as a "gold standard" for the separation of molecules on the basis of their size. However, GFC can not be routinely used because it is a time-consuming as well as expensive method (16).

Precipitation method involves calculating the recovery of mPRL - that means the percentage ratio of the hormone concentration measured after and before the separation of PRL

macroforms. The cut-off value of recovery differs broadly from paper to paper which creates some difficulties in the proper evaluating of macroprolactinaemia (5, 11, 17-20). Therefore, assessing not only the recovery value but also the concentration of PRL after removing MaPRL (real PRL concentration reflecting the concentration of mPRL) may be a better way to distinguish true and pseudohyperprolactinaemia as some authors have proposed (9, 21-23). Moreover, it has been suggested that each laboratory undertaking MaPRL screening, has to establish a method - specific reference intervals derived by the use of PEG-treated sera from healthy individuals (21-23).

For that reason the aim of our study was to check if the intervals for PRL after macroforms separation differ from manufacturer's reference ranges and to compare the results of PEG precipitation with the use of the criterion of recovery value and the criterion of real PRL concentration.

MATERIAL AND METHODS

The study protocol was approved by Bioethical Committee of the Medical University of Lodz (RNN/378/08/KB).

Hyperprolactinaemic sera

The study included 245 patients (224 women and 21 men) hospitalized in Department of Clinical Endocrinology, Medical University of Lodz. The inclusion criterion was the concentration of PRL exceeding 30 ng/mL measured in the fasting state. The study group consisted of 45 patients with a pituitary tumor (14 *macroprolactinoma*, 20 *microprolactinoma* and 11 non-functioning pituitary adenoma) and 200 persons with functional hyperprolactinaemia (the idiopathic origin, group of hyperandrogenic women with polycystic ovary morphology, primary hypothyroidism and drugs-induced hyperprolactinaemia).

Normoprolactinaemic sera

In order to check the difference between concentrations of PRL before and after the separation of macroforms in normoprolactinaemic sera, the samples were collected in the fasting state from apparently healthy people (with no evidence of endocrine disorders and without intake of drugs that may change PRL level). Finally, 120 sera were included into the study (89 from women aged 21–61 years and 31 from men aged 21–62 years).

Prolactin immunoassay

The prolactin concentration was measured by enzyme-amplified chemiluminescent immunoassay (Immulite 1000, Siemens). Analytical sensitivity of the assay is 0.5 ng/mL. Intra-assay and inter-assay coefficients of variation (CV) are respectively 6.1% (PRL concentration 6.3 ng/mL) and 9.6% (PRL concentration 14.1 ng/mL). Reference ranges are 1.9–25.0 ng/mL for women and 2.5–17.0 ng/mL for men. Prolactin Immulite kit was standardized to the World Health Organization's third international standard for prolactin, 84/500.

Precipitation with polyethylene glycol

The precipitation with PEG was performed similarly to a method proposed by Olukoga and Kane (11) and also followed a protocol recommended by Diagnostic Products Corporation (nowadays owned to Siemens). Equal volumes of PEG (Sigma) and serum were mixed, incubated at room temperature for 10 minutes and next centrifuged at 3000 rpm for 30 minutes. The supernatant obtained after this procedure was diluted 10-fold and checked for PRL concentration (PRL_{PEG}). The result was compared with PRL concentration in 10-fold diluted, untreated serum (PRL_{total}). In accordance with the majority of literature data, we assumed that the recovery of monomeric prolactin (the percentage ratio PRL_{PEG}/PRL_{total}) equal or below 40% means that MaPRL dominates in the serum sample (macroprolactinaemic subjects) (5, 11, 19, 24-26).

Statistical analysis

The data obtained from the experiment was recorded on Excel (MS Office 2007) worksheets. The McNemara test was used to analyze the diagnostic concordance between the recovery ratio values compared with the real PRL concentration evaluated both in the context of manufacturer and new ranges for PRL.

RESULTS

Comparison of recovery values after precipitation with real prolactin concentrations (manufacturer's reference ranges)

The calculation of recovery of monomeric PRL after macroforms separation with the use of the precipitation method showed macroprolactinaemia in 21/245 samples (8.6%) - in four persons with *prolactinoma* and in 17 subjects with functional hyperprolactinaemia. We noted that in the patients with recovery ≤40% real PRL concentration was elevated above manufacturer's reference ranges (it means that those persons have indeed true hyperprolactinaemia) in 9 cases - in almost all (3/4) persons with organic and in 6/17 patients with functional hyperprolactinaemia (*Table 1*).

Among 224 patients without macroprolactinaemia based on recovery values (ratio >40%), real PRL concentrations were within the manufacturer's reference range (it means that these patients have pseudohyperprolactinaemia - elevation of "native" PRL level was due to macroforms) in 36 cases. Those were six women with hypothalamic-pituitary area disease other than *prolactinoma* and 30 with functional hyperprolactinaemia. The remaining 188/224 persons had PRL concentration above the manufacturer's reference range - that is true hyperprolactinaemia.

New intervals for prolactin after macroforms separation

In order to check the difference between concentrations of PRL before and after the separation of macroforms in normoprolactinaemic subjects, we performed the precipitation in 120 normoprolactinaemic (according to manufacturer's reference ranges for PRL) sera. The mean recovery after the precipitation was 79.3±0.62%. It means that PRL concentration in the sera of potentially healthy people decreases by about 20%. Therefore, we modified the manufacturer's reference range by decreasing limit values by 20% and we obtained new ranges for PRL concentration after macroforms separation (*Table 2*).

Comparison of recovery values after precipitation with real prolactin concentrations (new ranges for prolactin after macroforms separation)

Applying new ranges for PRL in the group of patients with macroprolactinaemia (recovery value ≤40%), we observed elevated PRL level (true hyperprolactinaemia) in 14 persons - 3/4 with organic and 11/17 subjects with functional hyperprolactinaemia (*Table 1*). In 224 patients without

Table 1. Patients with macroprolactinaemia - characteristics, recovery ratio and values of real PRL concentration.

No/sex	Clinical diagnosis	PRL [ng/mL]	recovery-PEG [%]	PRL-PEG (real PRL conc.) [ng/mL]
1/M	*prolactinoma*	6766	29	1962
2/W	*prolactinoma*	157	40	64
3/W	*prolactinoma*	190	17	32
4/W	*prolactinoma*	87	18	16
5/W	functional hyperprolactinaemia	494	15	74
6/W	functional hyperprolactinaemia	313	15	47
7/W	functional hyperprolactinaemia	129	18	23
8/W	functional hyperprolactinaemia	111	17	19
9/W	functional hyperprolactinaemia	103	27	28
10/W	functional hyperprolactinaemia	88	6	4
11/W	functional hyperprolactinaemia	87	20	17
12/W	functional hyperprolactinaemia	80	31	25
13/M	functional hyperprolactinaemia	66	25	16
14/W	functional hyperprolactinaemia	60	27	16
15/M	functional hyperprolactinaemia	59	33	20
16/W	functional hyperprolactinaemia	45	22	10
17/M	functional hyperprolactinaemia	41	27	11
18/W	functional hyperprolactinaemia	144	36	52
19/W	functional hyperprolactinaemia	92	31	28
20/W	functional hyperprolactinaemia	80	28	22
21/W	functional hyperprolactinaemia	57	39	22

PRL - prolactin level; recovery-PEG – recovery ratio after precipitation with polyethylene glycol; PRL-PEG - prolactin level after precipitation with polyethylene glycol.

macroprolactinaemia (recovery value >40%), real PRL concentration was within new ranges for PRL (pseudohyperprolactinaemia) only in five persons. It means that the usage of new values for PRL showed true hyperprolactinaemia in 219/224 persons, including 31 among 36 patients with the normal hormone level according to the manufacturer's reference range. The summary of the obtained results has been comprised in *Table 3*.

With the use of the McNemara test, we showed that the diagnostic concordance of recovery ratio values compared with real PRL concentration assessed in the context of new ranges for PRL after macroforms separation (89.4%) is statistically significantly higher ($p<0.05$) than the diagnostic concordance of recovery ratio values compared with the real PRL concentration evaluated in the context of the manufacturer reference range (76.7%).

DISCUSSION

Macroprolactinaemia is a common problem in a medical practice because, according to our previous study, it occurs in at least every tenth patient with hyperprolactinaemia (17). Some studies indicate that this frequency may be much higher and may achieve over 40% (27, 28). For that reason the proper estimation of MaPRL amounts in serum is very important. The precipitation with PEG - method that may be used to quantify MaPRL in blood, involve the separation of macroforms and the calculation

of recovery of mPRL. The value of recovery indicates whether MaPRL predominates in serum or occurs in an insignificant amount. However, the most often proposed cut-off value for precipitation is 40%, but the values equal to 30 or even 50% were used (11, 13, 18-20). Therefore, the diagnosis if serum sample is macroprolactinaemic or not differs depending on the accepted cut-off value. Moreover, the most often used 40% cut-off value has unsatisfactory diagnostic specificity and can lead to misinterpretation when both an excess amount of macroprolactin and a very high concentration of monomeric prolactin are present simultaneously (9, 23).

Hence, in cases of hyperprolactinaemic sera it seems to be reasonable to assess not only the recovery value but also the concentration of PRL after the separation of macroforms. Real PRL concentration will indicate if the amount of the monomeric - biologically active (responsible for symptoms) form of the hormone in serum is elevated (9, 23). Thus, while evaluating the studied group of subjects we observed that in above half of persons (57%) with recovery less than 40% (significant macroprolactinaemia), real PRL concentration was within the reference ranges proposed by the manufacturer. It means that these patients indeed had pseudohyperprolactinaemia - the presence of MaPRL was responsible for elevated PRL level obtained in a laboratory. On the other hand, 43% of patients with macroprolactinaemia - mainly people with *prolactinoma* and very high PRL levels, had the raised concentration of mPRL (according to manufacturer's reference ranges) and had, in fact, true hyperprolactinaemia. These are specific cases in which using only the criterion of recovery and finding macroprolactinaemia, which is thought to be responsible for elevated PRL level, could lead to a misdiagnosis and the withdrawal from treatment. Our results showed that it may be a significant problem.

Conversely, in 224 patients without macroprolactinaemia based on cut-off value, 16% persons had normal PRL concentration according to manufacturer's reference ranges. They were persons with a slightly increased PRL level in whom the separation procedure led to the obtainment of correct real hormone concentration. Bearing in mind that precipitation has disadvantage of depleting serum not only from macroforms but also from smaller than MaPRL forms of PRL (including mPRL), it seems possible that even in serum with no MaPRL, the recovery value will be a little below 100% and real PRL concentration will be lower than "native". Moreover, some studies have shown that small amounts (1–3%) of MaPRL are present in the sera of healthy people with normal PRL level (29, 30). Therefore, it would be more appropriate to assess a real PRL concentration not in the context of reference ranges designed by manufacturer for "native" hormone level but referring it to a new range of values established for PRL concentration after macroforms separation. Our results are rather similar to those reflecting nonspecific precipitation of mPRL and to the new intervals for PRL after macroforms separation presented by some other authors (decrease in serum PRL about 20–30%) (21, 29, 31, 32). We would like to accentuate that our study was performed with the use of Immulite 1000 that is thought to be medium-reactive towards MaPRL (33). Possibly when using immunoassay with higher or lower effectiveness, the percentage decrease of PRL level after macroforms separation may be a little different. Therefore, we also would like to underline that each laboratory or at least each region should elaborate their own reference values for tested parameters.

By the comparison of the real PRL concentrations with a new reference ranges for PRL after macroforms separation, we showed that in group of 21 subjects with macroprolactinaemia, 14 persons had true hyperprolactinaemia. This result is higher than the one obtained with the use of manufacturer's reference ranges (9/21 patients) and indicates that in as many as 66% of cases there is a risk of omission of hyperprolactinaemia due to the result of MaPRL dominance obtained with the use of recovery ratio which falsely suggests that there is no hyperprolactinaemia in the serum sample. Conversely, in patients without macroprolactinaemia the application of new ranges for PRL showed that only five persons had a normal hormone level. This result is much lower than that obtained with the use of the manufacturer's reference range (36 persons, only women). However, in the cases of slightly elevated "native" hormone levels, real PRL concentration may be differently classified taking into account that the manufacturer's and our new reference limit are close (25 and 20 ng/mL respectively for women). Therefore, in 5/224 persons results negative for MaPRL dominance and hence suggesting hyperprolactinaemia, turn out to be false because their real concentration of PRL was actually normal (pseudohyperprolactinaemia). The above data seem to indicate that with the use of the new ranges for PRL after macroforms separation the number of misleading results for MaPRL might be much lower than with the use of the reference ranges proposed by manufacturer.

It is rather obvious that the self-established new intervals would be more appropriate than reference ranges developed by the manufacturer in a distant country on a probably different

Table 2. Reference ranges for PRL according to manufacturer of Prolactin Immulite Kit and ranges of hormone after macroforms separation.

	Women	**Men**
Manufacturer's reference ranges for PRL [ng/mL]	1.9 – 25	2.5 – 17
Ranges for PRL concentration after macroforms separation [ng/mL]	1.5 – 20	2 – 13.6

Table 3. Comparison of statements of macroprolactinaemia and true hyperprolactinaemia with the use of the criterion of recovery value and the criterion of PRL concentration after macroforms separation (manufacturers' reference ranges and new ranges for PRL after macroforms separation).

	manufacturer's reference ranges		new ranges for PRL after macroforms separation	
	HPRL(+)	**HPRL(-)**	**HPRL(+)**	**HPRL(-)**
PRL recovery ≤40% (macroprolactinaemia)	9	12	14	7
PRL recovery >40%	188	36	219	5

HPRL(+) – true hyperprolactinaemia (PRL level after macroforms separation above upper limit); HPRL(-) – pseudohyperprolactinaemia (PRL level after macroforms separation within reference range).

population. Particularly as regards the values for PRL after macroforms separation procedure, the above statement seems very logical. Our results confirmed that the interpretation of real PRL concentration with the use of manufacturer's and new intervals for PRL may differ significantly, mainly in subjects with functional hyperprolactinaemia. Obviously we are aware that, despite of suggestions, the elaboration of new intervals for PRL after macroforms separation by each laboratory or even each region is very difficult due to technical and financial aspects. Therefore, we would like to underline that while assessing the real PRL concentration, it should be kept in mind that sometimes using manufacturer's reference ranges may be a little incompatible with the values of real PRL concentration.

In summary, we conclude that evaluating of macroprolactinaemia only with the use of the recovery ratio may be not sufficient for a proper diagnosis in hyperprolactinaemic patients. The laboratory result preferably should contain the recovery value given together with a real PRL concentration. To assess PRL level after macroforms separation, reference intervals suggested by the manufacturer of immunoassay might be used but better means to identify patients with true hyperprolactinaemia accurately is using the appropriate ranges for concentration of PRL after macroforms separation.

Acknowledgements: This study was financially supported by the Medical University of Lodz (Grant No 503/5-020- 02/503-01).

Conflict of interests: None declared.

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Received: October 24, 2013
Accepted: February 13, 2014

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