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ASSESSMENT OF VARIOUS BIOLOGICAL FORMS OF PROLACTIN IN THE BLOOD OF PATIENTS WITH RESISTANT PROLACTINOMAS

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ОЦЕНКА УРОВНЯ РАЗЛИЧНЫХ БИОЛОГИЧЕСКИХ ФОРМ ПРОЛАКТИНА В КРОВИ У ПАЦИЕНТОВ С РЕЗИСТЕНТНЫМИ ПРОЛАКТИНОМАМИ

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REZISTENT PROLAKTINOMALI BEMORLAR QONIDA PROLAKTINNING TURLI BIOLOGIK SHAKLLARI DARAJASINI BAHOLASH

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Цель: *оценка содержания различных форм пролактина (с молекулярной массой 23 и 150-170 кДа) при пролактиномах с различной восприимчивостью к терапии агонистами дофамина и их корреляции с другими клиническими параметрами.* ***Материал и методы:*** *общая выборка случаев пролактиномы включала 128 пациентов. Резистентность к терапии определялась как отсутствие нормализации уровня пролактина крови и/или отсутствии уменьшения объема аденомы на 50% и более от исходного на фоне приема максимально переносимых доз агонистов дофамина. Для определения уровня макропролактина использовался метод ПЭГ-преципитации.* ***Результаты:*** *у обследованных не выявлено макропролактинемии, во всех случаях имела место истинная гиперпролактинемия. Установлено, что для пациентов с резистентной пролактиномами, помимо более высокого среднего значения уровня общего пролактина (387±213,1 нг/мл), была характерна более низкая доля высокомолекулярного макропролактина (150-170 кДа) (37,8±6%) и более высокая доля низкомолекулярного биологически активного пролактина (23 кДа) (62,2±6%). При корреляционном анализе зарегистрирована заметная прямая связь между уровнем биопролактина и степенью инвазии и заметная обратная связь между содержанием макропролактина и степенью инвазии.* ***Выводы:*** *отсутствие феномена макропролактинемии у пациентов, резистентных к агонистам дофамина, позволяет предположить, что резистентность к терапии агонистами дофамина не связана с феноменом макропролактинемии.*

Ключевые слова: *пролактиномы, пролактин, высокомолекулярный пролактин, низкомолекулярный пролактин.*

Maqsad: *dopamin agonist terapiyasiga turlicha moyillikka ega bo'lgan prolaktinomalarda turli xil prolaktin shakllarining (molekulyar og'irligi 23 va 150-170 kDa) darajasini va ularning boshqa klinik parametrlar bilan o'zaro bog'liqligini baholash.* ***Material va usullar:*** *prolaktinoma holatlarining umumiy namunasi 128 bemorni o'z ichiga olgan. Terapiyaga chidamlilik qon prolaktin darajasining normallashmasligi va/yoki dopamin agonistlarining maksimal toqat qilinadigan dozalarini qabul qilishda adenoma hajmining boshlang'ich ko'rsatkichdan 50% yoki undan ko'proqqa kamaymasligi sifatida aniqlangan. Makroprolaktin darajasi PEG cho'ktirish usuli yordamida aniqlandi.* ***Natijalar****: tekshirilgan bemorlarda makroprolaktinemiya aniqlanmadi; barcha hollarda haqiqiy giperprolaktinemiya mavjud edi. Rezistent prolaktinomali bemorlar, o'rtacha umumiy prolaktin darajasining yuqoriligidan (387±213,1 ng/ml) tashqari, yuqori molekulyar og'irlikdagi makroprolaktinning pastroq ulushi (150-170 kDa) (37,8±6%) va past molekulyar og'irlikdagi biologik faol prolaktinning yuqori ulushi (23 kDa) (62,2±6%) bilan tavsiflanganligi aniqlandi. Korrelyatsiya tahlili bioprolaktin darajasi va invaziya darajasi o'rtasida sezilarli to'g'ridan-to'g'ri bog'liqlikni, shuningdek, makroprolaktin darajasi va invaziya darajasi o'rtasida sezilarli teskari bog'liqlikni aniqladi.* ***Xulosa:*** *dopamin agonistlariga chidamli bemorlarda makroprolaktinemiyaning yo'qligi dopamin agonist terapiyasiga qarshilik makroprolaktinemiya bilan bog'liq emasligini ko'rsatadi.*

Kalit so'zlar: *prolaktinomalar, prolaktin, yuqori molekulyar og'irlikdagi prolaktin, past molekulyar og'irlikdagi prolaktin.*

The distinctive feature of prolactinomas (PRL), which sets them apart from other hormonally active pituitary adenomas, is their high susceptibility to drug therapy with dopamine receptor agonists (DA). In most cases, this therapy allows for the stabilization of prolactin levels and reduction of tumor size [1,4,5]. However, there is a proportion of PRL patients who respond unsatisfactorily even to high doses of DA, meaning they are resistant to this group of drugs. Their share reaches approximately 10-15% [2,3]. It should be noted that there is still no unified interpretation of the concept of "DA therapy resistance." In light of the above, the issues of factors, causes, and pathogenesis of DA treatment resistance development, methods for timely diagnosis of this condition, and approaches to overcoming resistance remain extremely relevant.

It is known that prolactin can exist in three different forms: biologically active or low molecular weight prolactin (molecular mass 23 kDa), dimeric prolactin (molecular mass 50-60 kDa), and high molecular weight immunoreactive prolactin, or macroprolactin (molecular mass 150-170 kDa) - which consists of low molecular weight prolactin bound to immunoglobulin G. These forms of prolactin differ in their level of biological activity. The low molecular weight form has the highest activity, while dimeric prolactin and macroprolactin have low biological activity. When dimeric and macroprolactin predominate in blood serum, hyperprolactinemia may be asymptomatic. If the percentage of bioactive prolactin is less than 40% of the total prolactin, the condition is classified as macroprolactinemia [6-8,10,12].

Purpose of the study

To assess the content of various forms of prolactin (with a molecular weight of 23 and 150-170 kDa) in prolactinomas with different sensitivities to dopamine agonist therapy and determine their correlation with other clinical parameters.

Material and method

The scientific work was conducted from 2021 to 2024. As part of the study, clinical, imaging, and laboratory stages of research were carried out at the Republican Specialized Scientific and Practical Medical Center of Endocrinology named after Academician Yo.Kh.

Turakulov of the Ministry of Health of the Republic of Uzbekistan.

The patient selection criteria for the study included the following inclusion and exclusion criteria.

Inclusion criteria:

Cases of hormonally active prolactinomas (PRL), confirmed by clinical, laboratory, and imaging studies:

- microprolactinomas (less than 10 mm);
- macroprolactinomas (more than 10 mm);
- giant PRLs (more than 4 cm);

These included PRLs with an aggressive course (APRL) (recurrent, resistant, rapidly growing, and with imaging characteristics of aggressive growth) and PRLs resistant to therapy (RPRL) (therapeutic resistance was defined as the absence of blood prolactin level normalization and/or the absence of a decrease in adenoma volume by 50% or more from baseline while taking maximum tolerable doses of dopamine agonists, but not less than 15 mg/day of bromocriptine or 3 mg/week of cabergoline, for at least 6 months).

Exclusion criteria:

- History of cancer;
- Concomitant genetic diseases;
- Hypothyroidism and other endocrine diseases;
- Functional hyperprolactinemia;
- NFPA, corticotropinoma, somatotropinoma, gonadotropinoma, thyrotropinoma;
- Liver cirrhosis, chronic kidney disease, chronic heart failure.

Based on the selection results, a general sample of 128 PRL cases was formed, with a mean age of 44.2±8.7 years. In the total sample of patients with PRL, women predominated (64.06%).

The PEG precipitation method was used to determine the level of macroprolactin. For quantitative assessment of biologically active prolactin in blood serum, PEG 6000 was used (0.25 ml of serum was mixed with an equal volume of 25% (wt/vol) PEG in buffer (pH 7.4) and left for 30 minutes at room temperature). After centrifugation (1500 rpm/30 min), the quantitative level of bioactive prolactin was determined. The amount of macroprolactin was calculated by subtracting bioactive prolactin levels from total prolactin.

Results and discussion

The most significant factor in diagnosing PRL was determining prolactin levels in blood serum. Table 1 presents the results of total prolactin level assessment in patients across the studied groups.

Table 1

Results of total prolactin level assessment in patients across the studied groups

Indicator	Patients with PRL sensitive to AD therapy			Patients with PRL resistant to AD therapy		
	Total group, n=78	Men, n=22	Women, n=56	Total group, n=50	Men, n=24	Women, n=26
Median	164	294	142	350	531	192
Maximum	692	692	297	893	893	729
Minimum	76	116	76	162	167	162
Mean value (M)	198.1	319	149.9	387	515.1	266.1
Standard deviation (SD)	120.9	161.7	45.2	213.1	191.8	150.5

Note. *No statistically significant differences were found between the M±SD values in the studied groups at p<0.05.

The table data show that prolactin levels in patients resistant to antidepressant (AD) therapy are significantly higher compared to patients sensitive to therapy. In the group with resistance to AD therapy, a wider range of values is observed, which may indicate higher variability in prolactin levels. In the group resistant to AD therapy, both the mean value and standard deviation are higher, which may suggest more diverse and variable results.

It was noted that no statistically significant differences were found between the groups at p<0.05. Despite differences in indicators, these differences do not reach the level of statistical significance at the chosen significance level. This means that the differences do not necessarily represent real differences between groups. Data were also analyzed with patients divided by sex. A trend towards higher prolactin levels was observed in men compared to women, both in the group of patients with sensitive prolactinomas and in the group with resistant prolactinomas.

Thus, the level of total prolactin differs in PRL patients depending on their sensitivity or resistance to AD therapy. The AD therapy-resistant group has higher mean values, a higher median, and greater data dispersion compared to the therapy-sensitive group. Statistical significance has not been confirmed, which necessitates additional research and consideration of other factors that may influence the level.

A state of macroprolactinemia can be discussed if the content of big-big-prolactin, or high-molecular-weight macroprolactin, in blood serum exceeds 60%. If its content is less than 60%, the condition is considered “true hyperprolactinemia.” The study showed that among 128 patients with PRL, no cases of macroprolactinemia were detected, and in all cases, true hyperprolactinemia was present. Of particular interest was the quantitative determination of the content (%) and level of macroprolactin by methods of subtracting low-molecular-weight bioprolactin from the total prolactin content, the content of which was determined using PEG.

Table 2 presents the results of assessing the content and level of high-molecular-weight prolactin (150-170 kDa) in patients in the studied groups.

Table 2

Results of assessing the content and level of high-molecular-weight prolactin (150-170 kDa) in patients in the studied groups

Indicator	Patients with PRL sensitive to AD therapy						Patients with PRL resistant to AD therapy					
	Total group, n=78		Men, n=22		Women, n=56		Total group, n=50		Men, n=24		Women, n=26	
	%	ng/ml	%	ng/ml	%	ng/ml	%	ng/ml	%	ng/ml	%	ng/ml
Median	44	69	41	115	46	67	36	126	36	190	36	70
Maximum	55	277	54	277	55	107	48	250	48	250	40	204
Minimum	34	40	34	56	36	40	28	63	28	78	28	63
Mean value (M)	44.1	83.8	41.7	129.5	45.1	65.7	36.3	135.3	37.8	184.9	34.9	88.7
Standard deviation (SD)	6.0	44.9	6	61.1	5.7	13.7	5.1	65.5	6	48	3.4	38.9

Note. *No statistically significant differences between M±SD indicators in the studied groups were detected at p<0.05.

Table 3

Results of assessing the level of low molecular weight prolactin (23 kDa) in patients in the studied groups

Indicator	Patients with PRL sensitive to AD therapy						Patients with PRL resistant to AD therapy					
	Total group, n=78		Men, n=22		Women, n=56		Total group, n=50		Men, n=24		Women, n=26	
	%	ng/ml	%	ng/ml	%	ng/ml	%	ng/ml	%	ng/ml	%	ng/ml
Median	56	96	59	163	54.5	76	64	217	64	340	65	124
Maximum	66	415	66	415	64	190	72	643	72	643	72	525
Minimum	45	37	46	56	45	37	52	88	52	88	60	97
Mean value (M)	55.9	114.3	58.3	189.6	54.9	84.2	63.7	251.7	62.2	330.1	65.1	177.4
Standard deviation (SD)	6.0	78	6	104	5.7	33.1	5.1	151.2	6	146.8	3.4	111.8

Note. *No statistically significant differences between M±SD indicators in the studied groups were detected at p<0.05.

The results showed that the level of high-molecular-weight prolactin is higher in patients with prolactinoma sensitive to antidepressant therapy compared to those resistant to treatment. Both indicators (percentage and concentration) are quite high, but the group with resistance to antidepressant therapy shows a slightly lower maximum level. The mean values indicate a significant difference in high-molecular-weight prolactin levels between the two groups. The group resistant to antidepressant therapy has a lower average percentage but a higher average concentration. This group also has a higher standard deviation, indicating greater data dispersion and more significant variability in high-molecular-weight prolactin levels. The level of high-molecular-weight prolactin differs among patients depending on their sensitivity or resistance to antidepressant therapy. The group resistant to antidepressant therapy has a lower average percentage but a higher average concentration and greater data dispersion.

Table 3 presents the results of assessing the level of low-molecular-weight prolactin (23 kDa) in patients from the studied groups. The results showed that low-molecular-weight prolactin levels were higher in patients with prolactinoma resistant to antidepressant therapy compared to those sensitive to the therapy. Both indicators (percentage and concentration) are relatively high, but a higher maximum level is observed in the group resistant to antidepressant therapy. The mean values indicate a significant difference in low-molecular-weight prolactin levels between the two groups. The group resistant to antidepressant therapy has a higher average percentage and average concentration. This group also has a higher standard deviation, indicating greater data dispersion and more significant variability in low-molecular-weight prolactin levels. Low-molecular-weight prolactin levels vary among patients depending on their sensitivity or resistance to antidepressant therapy. The group resistant to antidepressant therapy demonstrates a higher average percentage, average concentration, and greater data dispersion.

Figure 1 shows the correlation between the level of total prolactin in the blood and the content of high-molecular-weight macroprolactin in the blood. The Pearson correlation coefficient was found to be -0.615. The negative value of the coefficient indicates an inverse relationship between the level of total prolactin and the content of high-molecular-weight macroprolactin. As one parameter increases, the other decreases, and vice versa. In this case, the higher the level of total prolactin, the lower the content of high-molecular-weight mac-

roprolactin, and vice versa. The Pearson correlation coefficient demonstrates a moderate inverse relationship between the level of total prolactin and the content of high-molecular-weight macroprolactin.

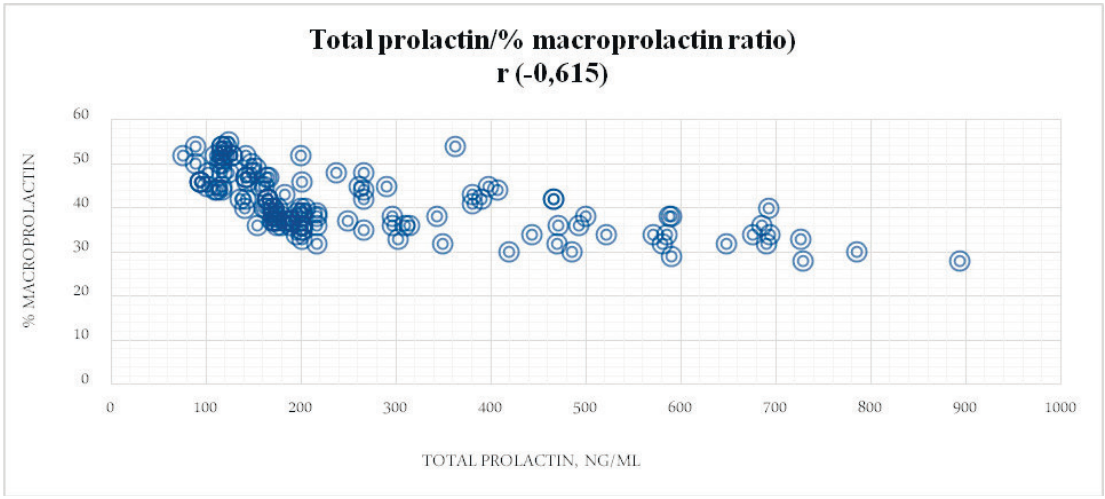


Fig 1. Correlation between the level of total prolactin in the blood and the content of high-molecular-weight macroprolactin in the blood.

Figure 2 shows the correlation between the content of high-molecular-weight macroprolactin in the blood and the degree of prolactinoma invasion. The Pearson correlation coefficient was -0.570. The negative value of the coefficient indicates an inverse relationship between the content of high-molecular-weight macroprolactin and the degree of prolactinoma invasion. That is, the higher the content of high-molecular-weight macroprolactin, the lower the degree of prolactinoma invasion, and vice versa. The Pearson correlation coefficient indicates a moderate inverse relationship between the content of high-molecular-weight macroprolactin and the degree of prolactinoma invasion. The z-statistic and confidence intervals confirm the statistical significance and direction of the correlation.

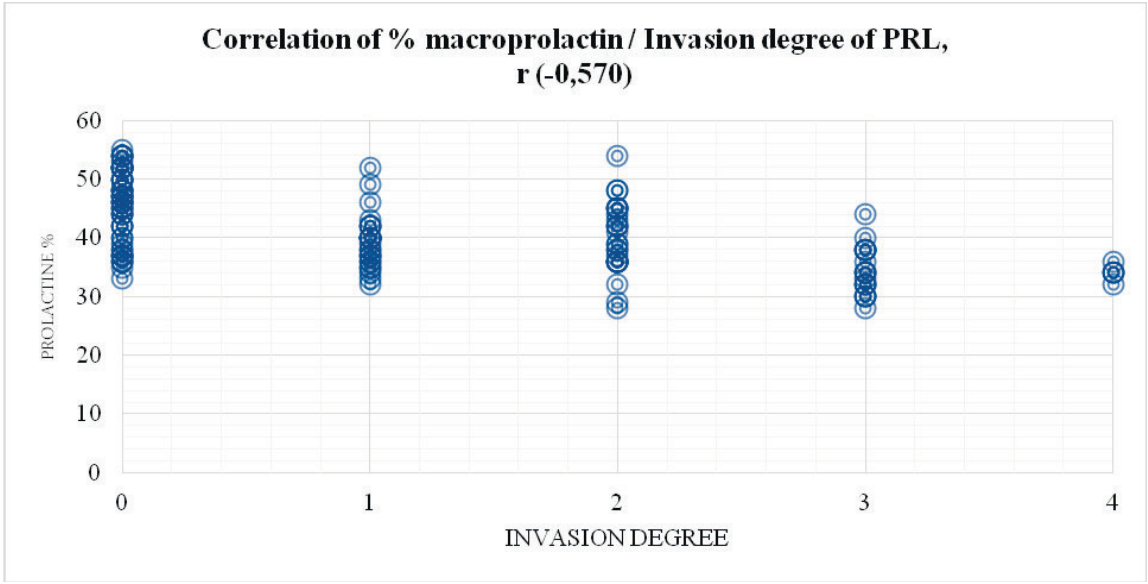


Fig. 2. Correlation between high-molecular-weight macroprolactin content in the blood and the degree of prolactinoma invasion.

Our research established that blood prolactin levels were significantly higher in patients resistant to dopamine agonists compared to those sensitive to these drugs. In line with previous studies that examined prolactinomas, regardless of their response to dopamine agonists, we found that prolactin levels in men are higher than in women, which is confirmed by our results. Most studies [1,9,11] do not differentiate between dopamine-sensitive and dopamine-resistant patients when examining gender differences. However, in our study, we observed differences in prolactin levels between men and women in both patient groups. Women resistant to dopamine agonists were found to be younger and had higher prolactin levels, which explains the high percentage of menstrual cycle disorders, particularly primary amenorrhea.

In this study, unlike other research, we did not observe the phenomenon of macroprolactinemia in patients resistant to dopamine agonists. This suggests that resistance to dopamine agonist therapy is not associated with the phenomenon of macroprolactinemia.

Conclusion

1. It was established that patients with resistant prolactinoma, in addition to having a higher average level of total prolactin (387±213.1 ng/ml), were characterized by a lower proportion of high-molecular-weight macroprolactin (150-170 kDa) (37.8±6%) and a higher proportion of low-molecular-weight biologically active prolactin (23 kDa) (62.2±6%).

2. Additionally, correlation analysis revealed a notable direct relationship between bioprolactin levels and the degree of invasion in patients, as well as a notable inverse relationship between macroprolactin levels and the degree of invasion in patients (r (-570).

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Objective: *To assess the levels of different forms of prolactin (with molecular weights of 23 and 150-170 kDa) in prolactinomas with varying susceptibility to dopamine agonist therapy and their correlation with other clinical parameters.* ***Material and methods:*** *Based on selection criteria, a total sample of 128 patients with prolactinoma was formed. Resistance to therapy was defined as the failure to normalize blood prolactin levels and/or the absence of a 50% or greater reduction in adenoma volume from baseline while taking the maximum tolerated doses of dopamine agonists. The PEG precipitation method was used to determine macroprolactin levels.* ***Results:*** *Among 128 patients with prolactinomas, no cases of macroprolactinemia were detected, and true hyperprolactinemia was present in all cases. It was established that patients with resistant prolactinomas, in addition to having a higher average level of total prolactin (387±213.1 ng/ml), were characterized by a lower proportion of high-molecular-weight macroprolactin (150-170 kDa) (37.8±6%) and a higher proportion of low-molecular-weight biologically active prolactin (23 kDa) (62.2±6%). Correlation analysis revealed a notable direct relationship between bioprolactin levels and the degree of tumor invasion in patients, as well as a notable inverse relationship between macroprolactin levels and the degree of invasion in patients.* ***Conclusions:*** *The absence of macroprolactinemia in patients resistant to dopamine agonists suggests that resistance to dopamine agonist therapy is not associated with macroprolactinemia.*

Key words: *prolactinomas, prolactin, high-molecular-weight prolactin, low-molecular-weight prolactin.*

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