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MOLECULAR BASES OF MUSCULAR DEFINITION: THE ROLE OF MYOSTATIN AND PROTEINKINASE β IN PROGRESSION OF PROTEIN-ENERGY WASTE IN PATIENTS ON HEMODIALYSIS

Abstract

Improving the nephrology service and increasing the availability of hemodialysis create the prerequisites for a deeper analysis of associated complications and diseases. One of the main clinical condition that worsen the patient's prognosis is protein-energy wasting (PEW), which is manifested by loss of muscle mass, strength and performance of skeletal muscles, which also leads to a decrease in quality of life, and often to disability and death. **The objective:** To assess the relationship between myostatin and protein kinase β as markers of the catabolic cascade, and the signs of PEW in dialysis patients. **Materials and methods:** Eighty patients were enrolled in the study (47 men and 33 women); the median age was 51.7 ± 11.6 years. All patients had CKD 5D and were on chronic hemodialysis for an average duration of 33.5 (0.5; 236) months. Clinical examination, anamnestic data collection and muscle strength measurement via hand dynamometry were provided in all patients. The serum levels of MSTN and AKT were determined by enzyme immunoassay (ELISA Kit, USA). Statistical analysis was carried out using Microsoft Office (USA) and Statistica-10.0 (StatSoft Inc., USA) software packages. **Results:** A dependence of the local increase in the skinfold thickness with an increase in the MSTN level, as well as a decrease in the thickness of the subcutaneous fatty tissue with a decrease in AKT ($p = 0.03$) was detected. We proposed a muscle catabolism index for assessing the degree of muscle degradation. It had a statistically proven association with degree of PEW and its clinical signs. The analysis of the effect of systemic inflammation markers on MSTN did not give significant results. However, in the subgroup with elevated AKT on the background of the activation of anabolic processes, we observed a decrease in β2-microglobulin and an increase in serum iron ($p = 0.04$). In the subgroup with a high level of MSTN, higher concentrations of parathyroid hormone (PTH) were determined. We found a direct correlation between the increase in protein kinase β and the annual PTH fluctuation ($r = 0.83$, $p = 0.01$). **Conclusion:** In our study, we found that in patients with CKD 5D on chronic hemodialysis, the activity of myostatin and protein kinase β varies. This leads to an increase in protein degradation over the processes of synthesis, which creates prerequisites for the development of sarcopenia. Taking into account the data obtained, further study of the intermolecular interactions of these markers in the catabolic cascade of muscle proteins is of research interest.

Key words: *chronic kidney disease, protein-energy wasting, sarcopenia, myostatin, protein kinase β*

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AKT — protein kinase β; ISRNM — International Society of Renal Nutrition and Metabolism; MSTN — myostatin; PEW — protein-energy wasting; MTCI — muscle tissue catabolism index; PTH — parathyroid hormone; CKD — chronic kidney disease; CRF — chronic renal failure

Introduction

Improving nephrology service and increasing the accessibility of hemodialysis as a method of renal replacement therapy create the conditions for a more detailed analysis of complications and diseases associated with it. One of the main clinical conditions that significantly worsen the patient's prognosis is protein-energy wasting (PEW) and the development of muscle mass loss, decrease of strength and performance of skeletal muscles, referred to as sarcopenia, on the background of uremic intoxication. These changes lead to the deterioration of quality of life and often to disability and death. [3].

The International Society of Renal Nutrition and Metabolism (ISRNM) identifies sarcopenia as a clinical manifestation of PEW [7]. According to the latest data, 20–70 % of patients with CKD on hemodialysis suffer from complications associated with PEW [1]. According to ISRNM guidelines, the main criteria in the diagnosis of PEW are: low body weight, including a reduction in fat mass and BMI; decrease in muscle mass (based on mid-arm circumference, double X-ray absorptiometry, bioelectrical impedance analysis) or changes in blood creatinine; reduced protein/energy intake; detection of biochemical markers of protein catabolism [10].

A slight but persistent imbalance between protein synthesis and its degradation in patients with kidney diseases causes a significant loss of protein. At present, there are no perfect methods to prevent muscle atrophy caused by chronic renal failure (CRF). However, mechanisms have been identified that regulate the metabolism of muscle protein. Their study will create conditions for the development of therapeutic approaches aimed at the pharmacological correction of intermolecular relationships in the muscle metabolism pathway and will allow controlling this process.

One of the main catabolic pathways that cause the degradation of muscle proteins is the activation of the ubiquitin-proteasome system, which operates through myostatin and protein kinase β [13].

Myostatin (MSTN) is a member of the transforming growth factor-beta family, whose catabolic effects are due to the activation of Smad2/Smad3 signaling molecules and the blocking of protein kinase β (AKT) phosphorylation on the one hand. As a result, signal transmission through the IGF-1/PI3K/AKT pathway is disrupted, the level of the active factor FoxO1 increases and the genes associated with atrophy are activated [11]. On the other hand, the myostatin signal in the AKT-mTOR pathway blocks endogenous protein synthesis, also leading to the development of muscle atrophy. Another effect of MSTN overexpression is the development of fibrosis [9] and the suppression of the activity of satellite cells involved in muscle fiber repair processes [5].

The mechanisms of muscle tissue degradation described can be initiated by complications of chronic renal failure: metabolic acidosis, defective insulin signaling, inflammation, uremic intoxication, hormonal imbalance, hypodynamia, and abnormal appetite regulation.

The objective of our study was to assess the relationship between myostatin and protein kinase β, as markers of the catabolic pathway, and PEW manifestations in patients on hemodialysis.

Materials and Methods

The study involved 80 subjects, 47 men and 33 women; the median age was 51.7 ± 11.6 years. All patients had CKD 5D and were treated with chronic hemodialysis for a mean duration of 33.5 (0.5; 236) months. Anamnestic data was collected, an anthropometric evaluation of the upper and lower extremities was carried out (the diameter and the circumference of the mid-arm, wrist, neck, thigh; the subcutaneous skinfold thickness in the biceps, triceps, above and below the scapula, in the iliac region were measured). Muscle strength was measured by hand dynamometry (DMER-120-0.5, Russia). The PEW stage was evaluated using a complex method in G. L. Bilbrey

and T. L. Cohen modification [1]. MSTN and AKT serum concentrations were determined by enzyme-linked immunoassay using the Myostatin ELISA Kit (USA), Protein Kinase B Beta ELISA Kit (USA). Statistical data analysis was performed using Microsoft Office (USA) and Statistica-10.0 (StatSoft Inc., USA) software packages.

The statistical significance of mean difference was determined using Student's t-test when the sample distribution was normal, and using Mann-Whitney test when the sample distribution deviated from a normal distribution. Correlation analysis was carried out using the Pearson coefficient when the trait distribution was normal and using Spearman coefficient when the trait distribution deviated from a normal distribution. A single- and multifactor linear regression analysis was performed. Parametric analysis of variance was performed using ANOVA analysis, Levene's test, and Brown-Forsythe test. Differences were considered statistically significant with $p<0.05$.

Results

We noted a high prevalence of PEW in the study group: manifestations of muscle atrophy varying in degree were discovered in 90 % of the subjects. At the same time, most of the disorders were related to mild and moderate degree of PEW (61.25 % and 27.5 %); a significant protein imbalance was detected in 1 patient. Correlation dependence was found between the combined attribute of the presence of PEW and the decrease in muscle strength. We divided the data obtained into three subgroups, where 0 is the absence of a decrease in muscle strength, 1 is a decrease in muscle strength of the hand or leg, 2 is a decrease in the muscle strength of both limbs (Table 1).

The mean MSTN value in the study group was 8.47 ± 1.27 ng/ml, the mean AKT value was 3.15 ± 2.15 ng/ml, ranging from 0.08 to 11.6 ng/ml, and the distribution in both cases did not differ from normal. The ANOVA analysis and the Brown-Forsythe test made it possible to

Table 1. *Correlation of the combined attribute of the presence of PEW and the decrease in muscle strength with clinical parameters*

Clinical parameters	r, Spearman
Gender (male — 0, female — 1)	0.30*
Dynamometry on a fistula-free hand, N	–0.50*
Dynamometry on the left hand, N	–0.50*
Dynamometry on the right hand, N	–0.50*
Decreased hand muscle strength	0.37*
Test with raising the leg, sec	0.37*
Reduced leg muscle strength	–0.62*
The degree of decrease of the muscle strength of the limbs	0.75*

Note: *— $p<0.05$

Table 2. *The values of clinical parameters (M ± SD) in subgroups depending on the level of MSTN*

Parameter	MSTN		SS	SS error	MS error	F	p
	≤8.49 ng/ml	>8.49 ng/ml					
Lymphocytes, 10^3 cells/μl	1.58 ± 0.5	1.54 ± 0.5	0.001	7.94	0.10	0.01	0.91
Transferrin, g/l	1.9 ± 0.45	1.8 ± 0.36	0.05	5.9	0.08	0.71	0.40
Ferritin, μg/l	386 ± 367	366 ± 263	79,042	4,241,027	55,803	1.42	0.24
Serum iron, g/l	10.5 ± 2.9	10.5 ± 3.6	2.7	396	5.1	0.53	0.47
Albumin, g/l	41.8 ± 2.3	41.7 ± 2.3	0.01	173	2.22	0.005	0.94
HbA1c, %	5.9 ± 1.2	7.5 ± 1.1	0.03	4.44	0.64	0.04	0.84
β2-MCG, ng/ml	119 ± 32	125 ± 29	0.30	39,883	511	0.0006	0.98
CRP, mg/l	124 ± 26	118 ± 42	2,438	60,401	774	3.15	0.07

distribute quantitative traits into two subgroups depending on the myostatin value. The concentrations of parathyroid hormone (PTH) were higher in the subgroup with a high MSTN value, which indicates a possible activation of the catabolic pattern of muscle metabolism in patients with CKD 5D on the background of secondary hyperparathyroidism. Regarding the increase in protein kinase β, a correlation was found with the annual PTH fluctuation (r = 0.83, p = 0.01). In the subgroup with high PTH fluctuation, a more pronounced decrease in PTH value was observed during the observation period (–123 ± 12 versus 11 ± 18 ng/ml, p = 0.021), as well as more frequent prescription of hormone replacement therapy (38.5 % versus 11.1 %, p = 0.04).

When assessing the effect of non-specific markers of systemic inflammation on blood myostatin concentration, no statistically significant results were obtained. (Table 2).

However, an increase in serum iron and a decrease in β2-microglobulin were observed in the subgroup with an elevated AKT value on the background of the activation of anabolic processes (Table 3).

When assessing the effect of anthropometric measurements on the MSTN value, it was found that an increase in its concentration (above the median ≥8.49 ng/ml) was associated with an increase in the skinfold thickness above the biceps and in the iliac region (Fig. 1, Fig. 2). At the same time, in patients with a low AKT value, there was an increase in the skinfold thickness above the biceps more than the mean value (Fig. 3). These data indicate the activation of lipid metabolism in the regions discussed with an increase in catabolic processes in muscle tissue.

Table 3. *The values of clinical parameters (M ± SD) in subgroups depending on the level of AKT*

Parameter	AKT		SS	SS error	MS error	F	p
	≤2.55 ng/ml	>2.55 ng/ml					
Lymphocytes, 10^3 cells/μl	1.6 ± 0.5	1.5 ± 0.5	0.18	7.8	0.09	1.88	0.17
Transferrin, g/l	1.86 ± 0.4	1.81 ± 0.4	0.007	6.4	0.08	0.09	0.77
Ferritin, μg/l	327 ± 255	427 ± 366	67,467	4,125,783	54,286	1.25	0.27
Serum iron, g/l	10.3 ± 2.5	14.7 ± 3.8	20.3	380	4.9	4.2	0.04
Albumin, g/l	41.9 ± 2.2	41.6 ± 2.4	0.19	174	2.2	0.08	0.77
HbA1c, %	5.9 ± 1.2	7.5 ± 1.1	0.03	4.44	0.64	0.04	0.84
β2-MCG, ng/ml	119 ± 32	125 ± 29	0.30	39,883	511	0.0006	0.98
CRP, mg/l	124 ± 26	118 ± 42	2,438	60,401	774	3.15	0.07

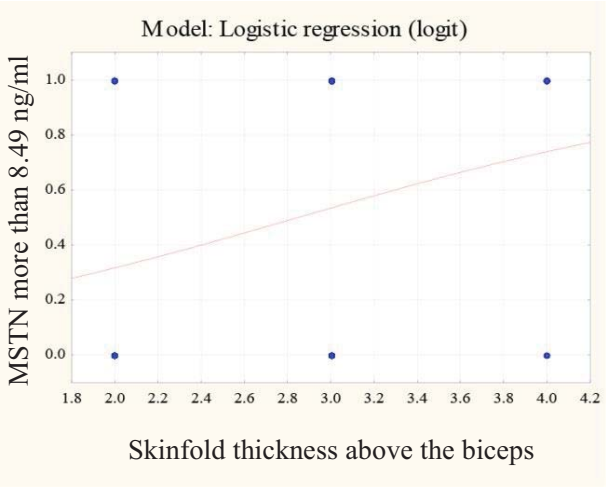

Figure 1. *The probability of the myostatin level increase, depending on the skinfold thickness above the biceps*

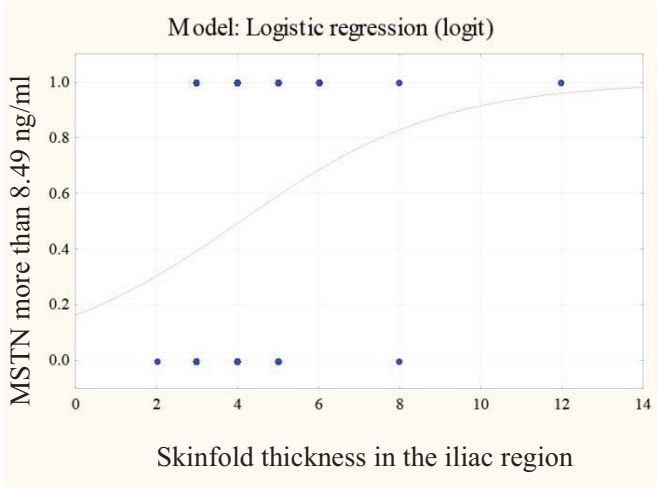

Figure 2. *The probability of the myostatin level increase, depending on the skinfold thickness in the iliac region*

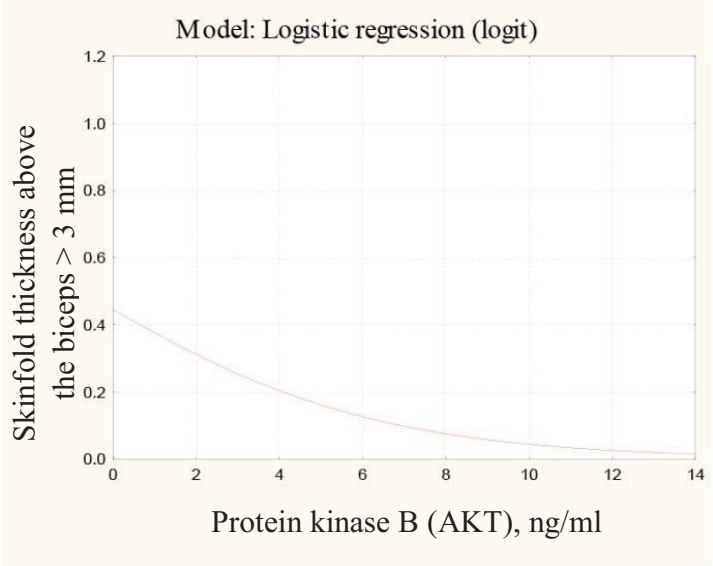

Figure 3. *The skinfold thickness above the biceps depending on the level of AKT*

When studying the association between MSTN and protein kinase β, a relationship was established between these biomarkers. We have proposed a muscle tissue catabolism index (MTCI), which is calculated by the ratio of MSTN to AKT. It can be a basis for indirect determination of the direction of skeletal muscle metabolism in patients with CKD 5D. We separated the patients into three subgroups: 0 — low MTCI, which is determined by the prevalence of anabolic processes (low myostatin, high AKT), 1 — moderate MTCI, protein synthesis and degradation processes are balanced (low myostatin, low AKT or high myostatin, high AKT), 2 — high MTCI, in which degradation of muscle proteins significantly prevails over their synthesis (high MSTN, low AKT).

According to the regression analysis, there was an increase in the proposed coefficient with an increase in the stage of PEW (Fig. 4).

In our study, the link between parameters of muscle strength decrease and the catabolic markers being studied is statistically significant only for an increase in myostatin ($\chi^2 = 4.15$, $p = 0.041$). However, the use of MTCI allows defining pathogenetic interactions more clearly. Thus, a decrease in muscle strength along with an increase in MTCI was noted in 91 %

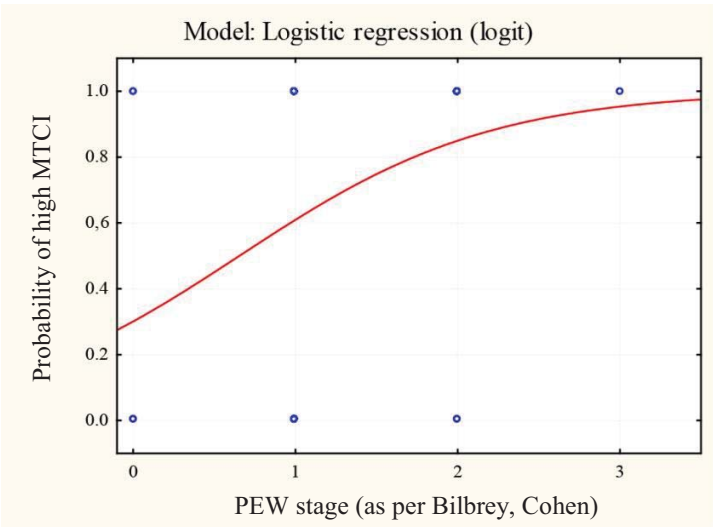

Figure 4. *The regression equation and the graphic representation of the probability of the MTCI increase depending on the stage of PEW*

Table 4. *Values of parameters (M ± SD) depending on the presence of PEW and MTCI*

Parameter	no PEW			PEW		
	MTCI					
	0	1	2	0	1	2
Dynamometry of muscle strength on a fistula-free hand, N	41.8 ± 30	39.0 ± 19	23.2 ± 7.4	37.7 ± 13	36.2 ± 13	32.7 ± 11
	SS = 595, MS = 119, F = 2.81, ρ = 0.022					

of cases, versus 63 % in its absence (χ^2 = 3.67, ρ = 0.048). ANOVA analysis and statistical significance assessment using the Levene's test allows to perform a quantitative analysis of traits in subgroups depending on the presence or absence of PEW and the degree of MTCI (Table 4).

Discussion

Dissociation in the processes of protein kinetics in end-stage CRF is associated not only with nutritional deficiency, as previously thought, but also with impaired synthesis and degradation of proteins [6]. In the case of CKD-induced muscle atrophy, the prevalence of protein degradation processes is more important than a decrease in the synthesis [12]. Such changes in muscle tissue are characterized by the development of PEW, accompanied by a decrease in the mass and function of muscle fiber [8].

One of the leading roles in maintaining skeletal muscle homeostasis is played by myostatin and protein kinase β [2], the activity of which is determined by a number of exogenous and endogenous factors, in particular, vitamin D imbalance and the development of secondary hyperparathyroidism [4]. At the same time, metabolic acidosis and systemic inflammatory response on the background of uremia can also have a trigger effect on the system of muscle proteolysis [5].

As shown by our study, the activity of the discussed biomarkers changed in patients with CKD who received therapy with chronic hemodialysis, resulting to the prevalence of protein degradation over synthesis processes, which creates the conditions for the development of sarcopenia.

Detection of the serum MSTN and AKT concentrations, as well as the coefficient of their intermolecular interaction, can serve as an additional minimally invasive method for diagnosis of PEW and indirect assessment of the severity of sarcopenia in patients with CKD 5D.

Conclusion

The effect of elevated myostatin and low protein kinase β levels on the parameters of muscle strength in patients with CKD 5D has been determined. The dependence of changes in these factors in the anthropometric assessment of the skinfold thickness in various body segments was determined, which may be related to the processes of fibro-adipogenesis in muscle fiber.

The original muscle tissue catabolism index developed can be used in a comprehensive assessment of the nutritional status in CKD patients, who receive long-term hemodialysis.

Further study of the intermolecular interactions of MSTN and AKT in the catabolic pathway of muscle proteins is of research interest. In particular, the question of the intracellular determination of the biomarkers discussed, as well as the integrative role of the mTOR serine-threonine protein kinase in muscle tissue metabolism in patients with CKD and sarcopenia, requires in-depth study.

Conflict of interests

The authors declare no conflict of interests.

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