BACKGROUND: Diabetic neuroostearthropathy (DNOAP, Charcot’s foot) - is a progressive destructive inflammatory disease of the osteoarticular apparatus of the foot, untimely and inadequate treatment of which can lead to the formation of gross deformities. More often, DNOAP is unilateral, bilateral lesion is relatively rare. It is not always possible to trace the relationship between the debut of DNOAP with trauma and chronic hyperglycemia. There is data demonstrating the role of individual pro-inflammatory factors in the pathogenesis of DNOAP, however, studies combining the evaluation of various metabolic markers of Charcot’s foot formation are currently extremely poor.

AIM: To evaluate the hormonal and metabolic markers of bone formation and resorption in patients with DNOAP and without this diabetic complication.

METHODS: A prospective, controlled trial included 70 patients with type 2 diabetes mellitus (37 men and 43 women) who formed 2 groups: group 1 included patients with DNOAP, group 2 was formed by patients with diabetes without foot skeleton damage. All patients underwent a study of 1,25-OH-vitamin D, sclerostin, pro-MMP-1, C-terminal propeptide type 1 collagen (PICP), type 1 collagen, osteocalcin, PTH, 25-OH-vitamin D, beta-cross-slaps.

RESULTS: The results of the studies confirmed the presence of vitamin D deficiency in all patients with diabetes mellitus included in the study, revealed the absence of statistically significant differences between the groups in the values of sclerostin, pro-MMP-1; 25-OH-vitamin D, type I collagen, and osteocalcin (p > 0.05). However, significant differences were found in the 1.25-OH vitamin D levels: patients with DNOAP presented the lower rates of 1,25-OH-vitamin D in comparison to control group (p <0.05). Beta-cross and PICP levels were significantly higher in DNOAP patients as well (p <0.05). Those findings show the more severe collagen degradation in patients with DNOAP and can be the genetically predisposed cause of DNOAP development. Though further studies are needed.

CONCLUSION: In patients with DNOAP a decrease in 1,25-OH-vitamin D levels was found, as well as the alteration of the synthesis and destruction of collagen (beta-cross-slaps and PICP) compared to patients with diabetes mellitus without ostearticular disorders.

KEYWORDS: diabetes mellitus; Charcot's foot; DNOAP; osteoarthropathy; markers; bone metabolism; remodeling

ОСОБЕННОСТИ КОСТНОГО МЕТАБОЛИЗМА У ПАЦИЕНТОВ С ДИАБЕТИЧЕСКОЙ НЕЙРООСТЕОАРТРОПАТИЕЙ

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ОБОСНОВАНИЕ. Диабетическая нейроостеоартропатия (ДНОАП, стопа Шарко) – прогрессирующее деструктивное воспалительное заболевание костно-суставного аппарата стопы, несвоевременное и неадекватное лечение которого может привести к формированию грубых деформаций, которые нарушают опороспособность. Чаще ДНОАП развивается одностороннее, билатеральное поражение встречается относительно редко. Не всегда удается проследить связь дебюта ДНОАП с травмой и хронической гипергликемией. Имеются данные, демонстрирующие роль отдельных провоспалительных факторов в патогенезе ДНОАП, однако исследований, объединяющих изучение различных метаболических маркеров формирования стопы Шарко, на данный момент крайне мало.

ЦЕЛЬ. Оценить гормональные и метаболические маркеры формирования и резорбции костной ткани у пациентов с ДНОАП и сахарным диабетом (СД) без данного осложнения.

МЕТОДЫ. В наблюдательное одноцентровое одномоментное контролируемое исследование были включены 70 пациентов с СД 2 типа (СД2) (34 мужчины и 36 женщин), которые сформировали 2 группы: 1-я группа включала пациентов с ДНОАП, 2-й группу составили пациенты с СД2 без поражения скелета стопы. У всех пациентов было проведено исследование паратгормона (ПТГ), 1,25-ОН витамина D и 25-ОН витамина D, маркеров костной резорбции: склеростина, проматриксной металлопротеиназы-1 (pro-MMP-1), остеокальцина и С-концевого телопептида коллагена I типа.
Diabetic foot disease (DFD) is one of the serious late complications of diabetes mellitus (DM). It induces a rapid decrease in the patient’s quality of life and requires significant financial costs of treatment for both the patient and the entire healthcare system [1]. Diabetic neuroosteoarthropathy (DNOAP) is a form of DFD that occurs when a neurological deficiency is caused by diabetic neuropathy and is manifested as the destruction of bones, joints and cartilage tissue lesion of feet as a result of non-infectious inflammation. This may result in osteolysis, decreased bone density, foot deformity and fractures. According to literature, DNOAP incidence varied from 0.08% to 18% among all patients with diabetes [2]. DNOAP is known, like other microvascular and macrovascular complications, to reduce significantly the quality of life of patients and increase mortality [3]. With improper treatment, DNOAP leads to the formation of gross deformities of feet, which results in the loss of the extremity supporting ability. Also, prolapse bones can result in the formation of ulcerative defects, which significantly increases the risk of extremity amputations. Diabetic polyneuropathy (autonomic and sensorimotor), trauma, foot surgery and calcium-phosphorus metabolism disorders are currently recognised as the main reasons for DNOAP formation. Most often, DNOAP develops unilaterally. The relation with a previous injury and severe prolonged hyperglycaemia is not always clearly visible. There are works demonstrating the role of various proinflammatory factors in the formation of DNOAP, but there have not yet been studies published that evaluate comprehensively the role of molecular, hormonal and metabolic disorders in this process. According to the literature, with various types of diabetes, some features in bone remodelling are noted. In patients with type 1 diabetes (DM1), low bone mineral density is registered as well as six to seven times higher risk of fractures than in individuals with normal carbohydrate metabolism. In patients with type 2 diabetes (DM2), normal or high bone mineral density is registered, and the risk of fractures is three times higher than in the control group [4]. Despite the similarity of chronic hyperglycaemia, DM1 and DM2 have different pathophysiological aspects that can affect bone metabolism in various ways. In both cases, the basic mechanisms of reduced bone strength are not entirely understood [5].

According to the literature, in DM2 patients, there is an increase in sclerostin, which is proposed for use as a predictor of femoral neck fractures in post-menopausal women [12, 13]. Another marker of collagen degradation is the matrix metalloproteinase (MMP)-1 enzyme, which destroys this molecule. Some prospective studies have shown that in patients with non-consolidating fractures, significantly higher concentrations of pro-MMP-1 and MMP-8 in the blood serum...
were determined compared with a group of patients with normal consolidation of fractures [14]. Given the increase in the incidence of DM and, as a consequence, DNOAP, the search for new markers of bone metabolism in patients of this category is extremely relevant and will enable to perform a deeper analysis of pathophysiological mechanisms of DNOAP occurrence and subsequently reduce the risk of developing this complication of DM and subsequent amputations.

AIM

The work aimed to evaluate the hormonal and metabolic markers of bone formation and resorption in patients with DNOAP and DM without this complication.

METHODS

Study Design

The work had the design of an observational single-centre cross-sectional controlled study.

Inclusion Criteria
1. DM2 with the use of insulin therapy
2. Age under 65 years
3. DM2 duration of less than 30 years
4. DNOAP (subacute or chronic stage)
5. Lack of osteoporosis (T-test below –2.5)

Exclusion Criteria
1. DM1
2. DM2 without the use of insulin therapy
3. Hyperparathyroidism
4. Osteoporosis (T-test above –2.5)
5. Diabetic nephropathy and chronic kidney disease stage 4–5
6. An acute stage of DNOAP (based on the history, clinical presentation and results of radiography and magnetic resonance imaging (MRI))
7. The presence of DFD, injuries and surgeries on the foot in the anamnesis
8. Hypercalcaemia
9. Hyperparathyroidism (primary and secondary)
10. Therapy with drugs affecting bone mass (anabolic steroids, bisphosphonates, denosumab and teriparatide).

Terms and Conditions

The study was conducted in a multi-field specialised endocrinological hospital (Diabetic Foot Department of the Scientific Research Centre for Endocrinology of the Russian Ministry of Health).

The study of blood samples was performed by a medical laboratory scientist at the clinical diagnostic laboratory of the same institution.

X-ray, MRI research and bone mineral density study were performed at the Department of Radiation Diagnostics, Research Centre for Endocrinology of the Russian Ministry of Health.

Duration of the Study

The study was conducted from January 2018 to June 2019 and included enrolment of patients, the formation of study groups and laboratory testing of blood samples.

Description of Medical Intervention

Patients underwent fasting blood sampling in Vacutainer SST II Advance and K2E (ethylenediaminetetraacetic acid) tubes, followed by centrifugation to obtain serum and plasma.

All patients underwent peripheral sensitivity testing. Vibration sensitivity was measured using a graduated tuning fork (Kircher & Wilhelm, Germany) at standard points (medial ankle and the pollex base). Tactile sensitivity was determined using a 10-g monofilament (North Coast Medical, Inc., USA) at standard points. Temperature sensitivity was evaluated using a standard type-therm cylinder (Neue Medizintechnic GmbH, Germany).

The patients underwent standard X-ray osteodensitometry (Lunar GE iDEXA, Prodigy, USA) with measurement of bone mineral density, T-criteria and Z-criteria in the lumbar vertebrae and proximal femur.

If it was difficult to determine the DNOAP stage based on the clinical and radiological findings, MR imaging was performed. MRI was performed on a GE Optima MR450w 1.5T tomograph (USA) using a specialised 8-channel GEM coil for studies of the shin and foot joints. The DNOAP stage was determined according to the existing classification of Chantelau and Grutzner, as well as the diagnostic criteria presented in the current algorithms of specialised medical care for diabetic patients [15].

The blood flow through the dorsalis pedis, anterior tibial and posterior tibial arteries, as well as the state of the venous outflow, was assessed by duplex scanning on a Voluson 730 apparatus (General Electric, USA) in the Diabetic Foot Department of the Scientific Research Centre for Endocrinology.

Primary Study Outcome

The aspects of bone metabolism in DNOAP patients were studied based on laboratory determination of indicators such as 1.25-OH vitamin D, sclerostin, pro-MMP-1, PICP and type 1 collagen.

Additional Study Outcomes

Bone resorption markers studied in routine clinical practice were additionally evaluated, namely, osteocalcin, parathyroid hormone, total calcium and ionised calcium, C-terminal telopeptide, 25-OH vitamin D, bone mineral density determination by X-ray densitometry and MRI of the foot. To assess the condition of arterial blood flow, all patients underwent duplex ultrasound (DU) of lower extremity arteries.

Subgroup Analysis

According to the study design, two groups were formed. Group 1 consisted of patients with DM2 and DNOAP (subacute or chronic stage).

Group 2 (comparison group) included patients with DM2 without DNOAP or DFD in the history.

Patients with acute stage of Charcot foot were not included in the group since the study aimed to identify possible predictors of DNOAP development. According to previous studies, the most pronounced increase in bone resorption markers is found in the acute stage of complications, which can lead to false or ambiguous data.
The groups were formed by DM2 patients (due to the aspects of bone metabolism and increased osteoclast activity in DM1 patients [7]).

Methods of Outcome Registration
All laboratory studies of blood serum samples were conducted in the clinical and diagnostic laboratory of the Scientific Research Centre for Endocrinology of the Russian Ministry of Health. Routine determination of bone resorption markers was performed according to standard methods.

Radioimmunoassay was used to study 1,25-dihydroxyvitamin D and pro-MMP-1, and sclerostin levels were determined using the Sandwich enzyme-linked immunosorbent assay (ELISA) method. PICP and type I collagen were investigated using ELISA.

Ethical Examination
The local ethics committee under the Scientific Research Centre for Endocrinology of the Ministry of Health of the Russian Federation decided to approve the possibility of conducting this research work, extract from protocol no. 2 dated 31 January 2018. All the patients signed informed consent to participate in the study.

STATISTICAL ANALYSIS

Principles for Calculating Sample Size
The sample size was not preliminary calculated.

Methods of Statistical Data Analysis
For the study result analysis, the Statistica 7.0 program (TIBCO Software Inc., USA) was used. The description of quantitative data is presented in the form of a median and quartiles (percentiles 25 and 75, Q25–Q75). To describe the qualitative data, absolute (n) and relative values (%) were calculated. The type of distributions was analysed using the Shapiro–Wilks test. The independent groups were compared by quantitative characteristics using the non-parametric method using the Mann–Whitney U-test. The differences were considered statistically significant at \( p < 0.05 \).

RESULTS

Objects (Participants) of the Study
The study included 70 patients. Group 1 consisted of patients with DM and DNOAP (27 patients), and Group 2 (control) included patients with DM2 without DNOAP (43 patients).

The groups under study were comparable in terms of age, gender characteristics and diabetes duration and its control. Table 1 presents the clinical and laboratory characteristics of the patients examined.

Primary Study Outcomes
The absence of changes in the values of parathyroid hormone and calcium confirmed the absence of hyperparathyroidism and other disorders of calcium-phosphorus metabolism in the patients studied.

Table 1. Clinical and laboratory characteristics of the patients examined (n = 70).

<table>
<thead>
<tr>
<th>Index</th>
<th>Group 1 (n = 27)</th>
<th>Group 2 (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>54 [47; 61]</td>
<td>61 [54; 64]</td>
</tr>
<tr>
<td>Gender, m/f</td>
<td>16/11</td>
<td>18/25</td>
</tr>
<tr>
<td>Duration of DM, years</td>
<td>18 [11; 27]</td>
<td>15 [7; 27]</td>
</tr>
<tr>
<td>Distal diabetic polyneuropathy (n):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Yes</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>Diabetic retinopathy (n):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Non-proliferative</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Proliferative</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Proliferative</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Diabetic nephropathy, CKD, stage (n):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>C2</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>C3a</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>C3b</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>9.1 [7.4; 10.1]</td>
<td>8.8 [7.4; 10.7]</td>
</tr>
<tr>
<td>LDL-C, mol/L</td>
<td>2.7 [2.15; 3.56]</td>
<td>3.2 [1.1; 6]</td>
</tr>
<tr>
<td>Haemoglobin, g/L</td>
<td>131 [121; 142]</td>
<td>137 [84; 186]</td>
</tr>
<tr>
<td>White blood cells, 10⁶/L</td>
<td>7.2 [5.9; 8.3]</td>
<td>7.4 [4.7; 11.7]</td>
</tr>
<tr>
<td>Total serum protein, g/L</td>
<td>73 [71; 77]</td>
<td>73 [54; 84]</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>33.5 [14; 46]</td>
<td>22.7 [2; 88]</td>
</tr>
<tr>
<td>GFR (MDRD), mL/min·1.73 m²</td>
<td>67.5 [41; 91]</td>
<td>75.0 [62.0; 86.0]</td>
</tr>
<tr>
<td>Total calcium, mmol/L</td>
<td>2.31 [2.24; 2.38]</td>
<td>2.39 [2.3; 2.45]</td>
</tr>
<tr>
<td>Ionised calcium, mmol/L</td>
<td>1.10 [1.07; 1.14]</td>
<td>1.09 [0.92; 1.17]</td>
</tr>
</tbody>
</table>

Notes: The groups were comparable in the characteristics listed (\( p > 0.05 \)).
DM, diabetes mellitus; CKD, chronic kidney disease; HbA1c, glycated haemoglobin; LDL-C, low-density lipoprotein cholesterol; GFR, glomerular filtration rate; ESR, erythrocyte sedimentation rate; MDRD, Modification of Diet in Renal Disease.
During the study, no significant differences in the values of sclerostin, pro-MMP-1, 25-OH vitamin D, type I collagen and osteocalcin were obtained. However, a significant difference in the indices of 1.25-OH vitamin D is noteworthy, as in DNOAP patients. The level of this vitamin D metabolite was lower than in patients of the comparison group (p < 0.05). Beta-CrossLaps and PICP markers were statistically significantly higher than in the comparison group.

The main results are presented in Table 2 and Figures 1–3.

### Additional Study Results

During the study, most patients of both groups had a pronounced deficiency of 25-OH vitamin D, which required the administration of vitamin D preparations at the recommended doses.

According to X-ray densitometry, there was no decrease in bone density in 49% of the examined patients of both groups; osteopenia was recorded in 35%, and pronounced osteoporosis of different localisation was noted in 9.6% of cases. In 5.4% of patients, this study was not conducted for technical reasons.

### Table 2. Results of laboratory studies of bone metabolism markers in the studied groups.

<table>
<thead>
<tr>
<th>Index, Me [25; 75]</th>
<th>Group 1 (n = 27)</th>
<th>Group 2 (n = 43)</th>
<th>P-value</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25-OH dihydroxyvitamin D, ng/mL</td>
<td>0.015 [0.010; 0.020]</td>
<td>0.018 [0.014; 0.024]</td>
<td>0.049</td>
<td>0.016-0.081</td>
</tr>
<tr>
<td>Sclerostin, pmol/L</td>
<td>53.0 [37.2; 64.7]</td>
<td>46.2 [34.5; 61.9]</td>
<td>0.42</td>
<td>0-240</td>
</tr>
<tr>
<td>Pro-MMP-1, ng/mL</td>
<td>6.0 [4.0; 9.75]</td>
<td>5.57 [3.66; 8.27]</td>
<td>0.50</td>
<td>0.912-9.34</td>
</tr>
<tr>
<td>Beta-CrossLaps, ng/mL</td>
<td>0.37 [0.27; 0.54]</td>
<td>0.26 [0.21; 0.32]</td>
<td>0.001</td>
<td>0.01–0.69</td>
</tr>
<tr>
<td>25-OH vitamin D, ng/mL</td>
<td>12.4 [8.2; 23.7]</td>
<td>12.1 [7.7; 18.0]</td>
<td>0.50</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Parathyroid hormone, pg/mL</td>
<td>36.8 [26.4; 42.7]</td>
<td>32.2 [26.2; 45.3]</td>
<td>0.87</td>
<td>15–65</td>
</tr>
<tr>
<td>Osteocalcin, ng/mL</td>
<td>17.1 [12.9; 20.0]</td>
<td>14.4 [10.5; 21.4]</td>
<td>0.15</td>
<td>11–43</td>
</tr>
<tr>
<td>PICP, ng/mL</td>
<td>83.8 [65.6; 101.7]</td>
<td>54.4 [48.6; 79.8]</td>
<td>0.007</td>
<td>-</td>
</tr>
<tr>
<td>Type I collagen, pg/mL</td>
<td>57.8 [47.4; 75.5]</td>
<td>58.9 [51.2; 74.5]</td>
<td>0.7</td>
<td>-</td>
</tr>
</tbody>
</table>

Me, median; pro-MMP-1, promatrix metalloproteinase-1; PICP, procollagen type I carboxyterminal propeptide.
No data were obtained for the presence of haemodynamically significant stenosis of the arteries of the lower extremities, according to DU data of the arteries of the lower extremities.

Adverse Events
During the study, no adverse events were recorded.

DISCUSSION

Summary of the Main Outcome of the Study
The examination revealed that the groups of patients did not differ significantly in age, duration of diabetes and the degree of its control, and there were no significant differences in lipid values. In addition, the presence of anaemia, renal failure signs (glomerular filtration rate corresponded to chronic kidney disease stage 1–3B), impaired calcium and phosphorus metabolism, and an acute inflammatory reaction and hypoproteinemia were ruled out in all patients included in the study. The increase in ESR was non-specific.

In the current study, pro-MMP-1 values were within reference values and did not differ between the groups.

According to the results obtained in this study, there were no differences between groups in sclerostin values; also, this indicator in both groups was within the reference values. This is not consistent with the literature that describes an increase in the level of sclerostin in DM2 patients [12].

Another marker of collagen degradation is the MMP-1 enzyme, which destroys this molecule [14].

In the current study, pro-MMP-1 values were within reference values and did not differ between the groups.

Study Limitations
Because of the relatively low incidence of DNOAP, the comparison group was slightly larger than the main group. Patients receiving only insulin therapy in various modes were selected because the difference in hypoglycemic therapy could affect the results obtained. It is planned to continue this study in the future with a larger sample and subject to more stringent inclusion criteria.

CONCLUSION
During the study, a pronounced deficiency of vitamin D was recorded in all the patients examined. Given the increased risk of osteoporosis and fractures, it is extremely important to control this indicator in patients with DM. A disorder of the synthesis and degradation of collagen in patients with Charcot foot (increased beta-CrossLaps and PICP) was revealed, which, perhaps, enables to clarify the mechanisms of DNOAP development; however, studies with a larger sample are required to understand more fully the pathogenesis of DNOAP, as well as population studies with the identification of genetic markers for the development of this complication in DM2 patients.

ADDITIONAL INFORMATION.

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Conflict of Interest. The authors declare no obvious or potential conflicts of interest related to the publication of this article.

Contribution of Authors. E.L. Zaitseva collected the clinical material, analysed and wrote the article. A.Yu. Tokmakova developed the study design and wrote the article. V.M. Zhilyaev conducted laboratory research. N.I. Sazonova performed X-ray densitometry. G.R. Galstyan developed the study design and wrote the article. A.V. Vorontsov performed the MRI scanning.

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