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Original article

The level of microelements and heterogeneity of joint hypermobility as an endophenotype of undifferentiated connective tissue dysplasia

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Abstract: *Objective* — The aim of the work was to study serum concentrations of magnesium, copper, zinc, phosphorus and calcium in individuals with undifferentiated connective tissue dysplasia (uCTD) and joint hypermobility (JH) in an isolated and combined state.
Material and methods — the concentrations of magnesium, copper, zinc, phosphorus and calcium were measured by the direct colorimetric method in 55 people with joint hypermobility and in 34 – without hypermobility.
Results — There were no significant differences between serum concentrations of microelementsin groups with and without JH. In patients with mild and severe uCTD significant decrease in serum magnesium concentrations was noted (U=2.12, p=0.034 and U=3.7, p=0.012). In patients with isolated JH significant serum zinc concentrationdecrease was revealed compared with the control group (U=3.12, p=0.022). Serum magnesium concentrations were reduced in all patients with uCTD and JH; in the groups with isolated dysplasia and combined pathology, the differences reached the level of statistical significance (U=2.78, p=0.024 and U=3.2, p=0.018).
Conclusion — The study revealed significant associations of a decrease in serum magnesium concentrations with the development of uCTD in an isolated and combined with JH state and decrease in serum zinc concentrations with the development of isolated JH.

Keywords: trace elements, zinc, magnesium, undifferentiated connective tissue dysplasia, joint hypermobility.

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Introduction

Joint hypermobility (JH) is a condition in which the amplitude of active and / or passive movements in a joint exceeds the average norm. The first literature references date back to the 80s of the 20th century. Since then, the views on the etiology and pathogenesis of JH have undergone a number of changes, but to date, a number of questions about classification, pathogenesis and diagnosis of JH are still unclear. The importance of this problem is due to the development of pain [1, 2] andearly degenerative pathology of the musculoskeletal system [3-5]. In the English-language literature JH is mainly considered like a part of monogenic forms of connective tissue dysplasia (CTD) – Ehlers-Danlo syndrome, Marfan syndrome, osteogenesisimperfectaand several others [6]. Russian scientists, along with these nosologies, distinguish an undifferentiated variant of connective tissue dysplasia (uCTD), which can also be associated with JH [7]. However, the presence of joint hypermobility in a number of individuals without signs of CTD leaves the questions of their connection, development andetiopathogenesis open. It is also worth remembering that an increased range of motion in the joints as a variant of the norm is possible in children and adolescents or acquired doing special exercises, during sports or some professional activities [8]. In addition to genetic markers, whose contribution to the development of CTD and JH is beyond doubt, there is an assumption about the role ofof microelements-magnesium, zinc, copper, calcium, phosphorus – in the development of these conditions. A number of studies have been carried out, mainly related to uCTD and its individual phenotypes [9-11]. However, studies of the role of microelementsin patients with isolated joint hypermobility orhypermobility combined with uCTDhave not been conducted.

The aim of the work is to estimate serum concentrations of magnesium, copper, zinc, phosphorus and calcium in individuals with uCTD and JH in an isolated and combined state.

Material and Methods

Patients`s characteristics

250 healthy individuals of young (18-25 years old) age were examined for the presence of joint hypermobility and/or uCTD, and 89 of them were included in the study. all patients signed the voluntaryinformed consent. The exclusion criteria were: systemic connective tissue diseases, secondary JH on the background of sports exercises or professional activities, the monogenic form of CTD, active bacterial or viral infection, traumatic injuries of joints in the anamnesis, pregnant or lactating women, refusal to participate in the study.

Table 1. Serum concentrations of microelementsin patients with and without JH

Parameters	*Presence of JH, n=55*	*Absence of JH, n=34 (control group)*
Copper, mmol/l	804.3 (750.2, 837.7), p=0.792	803.5 (758.2, 828.2)
Zinc, mkmol/l	13.2 (11.38, 15.0), p=0.875	13.2 (11.38, 15.5)
Phosphorus, mmol/l	1.19 (1.06, 1.28), p=0.257	1.21 (1.06, 1.31)
Magnesium, mmol/l	0.8 (0.7, 0.9), p=0.890	0.8 (0.7, 0.9)
Calcium, mmol/l	2.2 (2.13, 2.3), p=0.171	2.24 (2.2, 2.3)

p-level is for comparison with control group. Data presented as median with low and upper quartiles – Me (LQ, UQ).

Table 2. Serum concentrations of microelementsin patients with varying severity of uCTD.

Parameters	*Severe uCTD, n=6*	*Mild uCTD, n=48*	*Absence of uCTD, n=35 (control group)*
Copper, mmol/l	762.2 (620.5, 802.7) p=0.206	804.2 (754.9, 840.8) p=0.830	812.2 (750.2, 839.2)
Zinc, mkmol/l	13.2 (13.2, 16.39) p=0.190	12.75 (10.9, 15.0) p=0.495	13.6 (11.38, 15.48)
Phosphorus, mmol/l	1.06 (0.9, 1.14) p=0.083	1.2 (1.07, 1.31) p=0.219	1.18 (1.06, 1.25)
Magnesium, mmol/l	0.7 (0.6, 0.8) p=0.024	0.75 (0.7, 0.9) p=0.018	0.9 (0.7, 0.9)
Calcium, mmol/l	2.2 (2.2, 2.3) p=0.940	2.2 (2.1, 2.3) p=0.420	2.2 (2.2, 2.3)

p-level is for comparison with control group. Data presented as median with low and upper quartiles – Me (LQ, UQ).

Table 3. Serum concentrations of microelementsin patients with JH and uCTD in an isolated and comorbid state

Parameters	*uCTD+JH+, n=30*	*uCTD+JH-, n=25*	*uCTD-JH+, n=8*	*uCTD-JH-, n=26 (контроль)*
Copper, mmol/l	803.9 (743.0, 837.7) p=0.870	815.4 (735.8, 853.6) p=0.329	825.75 (768.85, 837.25) p=0.340	787.4 (758.2, 820.2)
Zinc, mkmol/l	13.4 (11.38, 15.6) p=0.890	12.3 (10.0, 14.11) p=0.770	11.0 (10.94, 14.94) p=0.022	12.97 (11.38, 15.5)
Phosphorus, mmol/l	1.16 (1.01, 1.25) p=0.160	1.18 (1.12, 1.27) p=0.640	1.19 (1.1, 1.27) p=0.730	1.21 (1.05, 1.33)
Magnesium, mmol/l	0.80 (0.7, 0.8) p=0.031	0.75 (0.7, 0.8) p=0.033	0.85 (0.8, 0.95) p=0.144	0.9 (0.7, 0.9)
Calcium, mmol/l	2.2 (2.1, 2.3) p=0.220	2.2 (2.1, 2.3) p=0.910	2.25 (2.2, 2.3) p=0.170	2.24 (2.2, 2.3)

"+" presence of a sign, "-" absence of a sign. p-level is indicated for comparison with the control group. Data presented as median with low and upper quartiles – Me (LQ, UQ).

Study design

A case-control study was conducted in three stages.

At the first stage, the presence of JHwas evaluated without presence of uCTD using Beighton criteria [12]. The following tests were carried out:

i) Passive extension of the little finger of the brush more than 90° (right hand and left hand)

ii) Passive pressing of the thumb of the hand to the inner side of the forearm (right hand and left hand).

iii) Re-extension in the elbow joint of more than 10° (right hand and left hand).

iv) Re-extension in the knee joint of more than 10° (right leg and left leg).

v) Front torso with palms touching the floor with straight legs.

For each positive test, 1 point was assigned, the maximum possible number of points – 9, a positive result – the sum of points from 4 inclusive and higher. Among the examined individuals, 55 people were identified; the comparison group consisted of 34 people without JH.

At the second stage, the presence of uCTD was evaluated without presence of JH using the diagnostic algorithm according to T.I. Kadurina in the authorsmodification [13, 14]. This algorithm is a series of phenotypic characters with a diagnostic value assigned to each one in points. After examining and filling out the appropriate questionnaire, a summation is made and the conclusion on the presence of uCTD is formed by the total of points: from 8 to 16 points – mild uCTD, more than 17 points – severe uCTD. Among the examined individuals, 54 people with uCTDwere identified, among them mild – in 48 people, severe – in 6 people. The comparison group consisted of 35 people without uCTD.

At the third stage, the combined and isolated availability of JH and uCTDwas assessed. A combination of pathologies was detected in 30 people, isolated JH – in 25 people, isolated uCTD – in 8 people. The comparison group consisted of 26 people without JH and uCTD.

Microelement levels measurement

The serum concentrations of copper (mmol/L), zinc (μmol/L), phosphorus (mmol/L), magnesium (mmol/L) and calcium (mmol/L) were determined In all the formed groupsby direct colorimetric method on a BioChem360 (USA) using diagnostic kits from Hospitex (Russia), Cobas (Japan).

Statistic alanalysis

For statistical data processing, the software packages MSOfficeExcel 2007 (Microsoft, USA), Statistica v.6.2 (StatSoft, USA) were used. The calculation of the median (Me) and the interquartile range (LQ, UQ) was used. The normality of the distribution of quantitative indicators was checked using the Shapiro-Wilk criterion, an intergroup comparison of the obtained data was carried out taking into account the volume and normality of the data distribution using the nonparametric Mann-Whitney criterion (U). In all cases the result considered statistically significant at $p<0.05$.

Results

We estimate serum concentrations of copper, zinc, phosphorus, magnesium and calcium in individuals with JH and without it, regardless of the presence of uCTD. The results are presented in *Table* 1. There were no statistically significant differences between serum concentrations of microelementsin the studied groups. This fact can be explained by the heterogeneity of JH and various etiological factors underlying it.

Similar examination was conducted in individuals with the presence and absence of uCTD of varying severity. The results are

presented in *Table* 2. In patients with mild and severe uCTD, a statistically significant decrease in serum magnesium concentrations was observed (U=2.12, p=0.034 and U=3.7, p=0.012, respectively) compared with a group of individuals without uCTD. The serum concentration of copper decreased according to severity of dysplasia, but the differences did not reach the level of statistical significance. Serum concentrations of zinc, phosphorus and calcium in the studied groups did not differ significantly.

To differentially assess the involvement of microelementsin the development of JH and uCTD, their concentrations were measured in groups with isolated and combined variants of connective tissue dysplasia and joint hypermobility. The results are presented in *Table* 3. Serum zinc concentration in patients with isolated JH was significantly revealed compared with the control group (U=3.12, p=0.022). Serum zinc concentrations were comparable in patients with isolated uCTD and combined pathology to those in the control group. Serum magnesium concentrations were significaly reduced in groups with isolated dysplasia and combined pathology relative to the control group (U=2.78, p=0.024 and U=3.2, p=0.018, respectively). Copper concentrations were generally lower in patients with pathology of the connective tissue, compared with the control group, the differences were trend-like. There were no differences in serum concentrations of phosphorus and calcium in the studied groups.

Discussion

According to current data, magnesium is the second most important trace element after sodium. It takes part in more than 600 enzymatic processes of the human body [14]. The value of magnesium in the functioning of the connective tissue system and its derivatives was also studied. Bones loses its quality characteristics according to reduced concentrations of this trace element, which leads to the development of osteoporosis [15]. In a long-term prospective study, which included 2,245 men aged 42 to 61 years, an association of decreased serum magnesium concentration with fractures of the peripheral skeleton was revealed [16]. In a study of chondrocalcinosis in the Chinese population, Zeng et al. found that patients with lower serum magnesium levels, even within the normal range, had a higher prevalence of chondrocalcinosis of knee [17]. This can by explained by magnesium effect on the calcium and vitamin D metabolism, which play a significant role in the connective tissue metabolism in general [18]. A histological examination of biopsy specimens of the enlarged thoracic aorta in patients with uCTD revealed a decrease in magnesium level, while zinc and copper levels were close to normal [19]. The role of magnesium in the activation of anabolic processes of connective tissue was proved by a group of Swedish scientists who found that titanium coated bone prostheses containing this microelement enhanced bone formation and increased levels of anabolic process markers in the perioperative zone in experimental animals [20]. According to the results of our research, a decrease in the serum magnesium concentration is associated with the presence of JH and uCTD.

The influence of other microelementson the structure and function of connective tissue is less studied. Copper is a cofactor in the collagen and elastin synthesis, which form the structural basis of connective tissue [21, 22] and, along with nickel, is more concentrated in cartilage and tendons than in bone tissue [23]. Increased daily intake of phosphorus can lead to a disturbance of calcium balance and, as a consequence, metabolic processes in the bone with the development of osteopenia [24]. Zinc deficiency and molecular disorders of its transporter genes are associated with the development of a number of skin diseases, such as alopecia, prolonged wounds healing, pellagra and several others. In addition, a mutation in the ZIP13 transporter gene causes the phenomenon of hyperelasticity of the skin and hypermobility of small joints, which is characteristic of Ehlers-Danlo syndrome [25]. Studying experimental osteoarthritis on mutant cell lines of chondrosarcoma SW1353 and model animals, it was found that increasing of zinc concentration in cell culture or adding it to the diet of rats reduces general oxidative stress, secretion of interleukin 1b, interleukin 13 and matrix metalloproteinase 13, thereby reducing the risk of osteoarthritis [26]. Studying the levels of microelementsin patients with rheumatic diseases, a decrease in zinc concentration was revealed only in osteoarthritis, magnesium concentration was reduced in osteoarthritis, rheumatoid arthritis, copper concentration was elevated in psoriatic arthritis [27]. According to the results of our research, a reduced concentration of zinc is associated with isolated JH.

Studies of magnesium, phosphorus and calcium concentrations in children 6-17 years old with signs of connective tissue dysplasia showed a significant decrease in serum calcium concentration, magnesium concentration was tended to decrease, phosphorus concentrations did not differ from those in the control group [28]. A tendency to the copper concentration decrease in the with uCTD was observed, not reaching the level of statistical significance. Concentrations of phosphorus and calcium in the studied groups did not differ from control group.

Thus, the results revealed associations between the serum concentrations of a number of microelementsand the presence of joint hypermobility and uCTD, which makes medical and nutritional correction possible.

Conclusion

A decrease in serum magnesium concentrations with the development of uCTD in an isolated and combined with JH state and a decrease in circulating zinc concentrations is significantly associated with the development of isolated JH.

Study Limitations

The main limitations of the study were the sample size, which had the ability to conduct biochemical diagnostics, which limited the possibility of using multivariate analysis methods, as well as the inability to compare the concentrations of microelementsin various body fluids and derivatives (skin, nails, hair), for a more comprehensive analysis of their content.

Ethical approval

All procedures involved in human studies comply with the ethical standards of the institutional and national research committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Conflict of interest

The authors declare no conflict of interest.

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