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Original article

Connective tissue dysplasia: a risk factor for osteopenia in children and adolescents

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Abstract
Introduction: in addition to genetic predisposition, a significant exogenous factor in the formation of Undifferentiated connective tissue dysplasia (UDCTD) is the deficiency of osteotropic micronutrients such as vitamins (D, A, C, E, K); macroelements (calcium, phosphorus, magnesium), trace elements (copper, manganese, zinc, boron, selenium, silicon), so essential for the connective tissue matrix and, above all, for bone tissue. A small number of studies of osteotropic micronutrients and the state of bone tissue in adolescents with UDCTD served as the basis for this comprehensive study.
Materials: a randomized study of 130 adolescents aged 10–16 years in the 1–2 health groups. The first group (primary) was 90 subjects with detected UDCTD. The second group (comparative) was 40 people with no signs of dysplasia.
Methods: included the definition of: vitamin D – 25(OH)D: micronutrients; calcium ductation: spinal column densitometry at L_{II}–L_{IV} level; physical development and psycho-emotional stress levels.
Availability disorders of 25(OH)D, low calcium consumption, magnesium deficiency, and shifts in micronutrient content correlated with densitometric data showing a 75% decrease BMD in Group 1 adolescents, while in Group 2 only 27.5%.
The results of a comprehensive study showed that osteotropic micronutrient deficiency is a serious exogenous trigger for the development and progression of UDCTD with osteopenia/osteoporosis formation. Osteopenia/osteoporosis, low physical development, disorders in the psycho-emotional sphere indicate the seriousness of the prognosis of UDCTD in adolescents.

Keywords: connective tissue dysplasia, osteotropic micronutrients, osteopenia, osteoporosis, macro- and microelements, vitamin D

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Оригинальная статья

Дисплазия соединительной ткани: фактор риска остеопении у детей и подростков

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Резюме

Введение: кроме генетической предрасположенности, значимым экзогенным фактором формирования недифференцированной дисплазии соединительной ткани (НДСТ) является дефицит остеотропных микронутриентов, таких как витамины (D, A, C, E, K), макроэлементы (кальций, фосфор, магний), микроэлементы (медь, марганец, цинк, бор, селен, кремний), столь необходимых для соединительнотканного матрикса, и прежде всего для костной ткани. Немногочисленные исследования остеотропных микронутриентов и состояния костной ткани у подростков с недифференцированной дисплазией соединительной ткани послужили основанием для настоящего комплексного исследования.

Материалы: рандомизированное исследование 130 подростков в возрасте 10–16 лет 1–2-й группы здоровья. Первая группа (основная) – 90 человек с выявленной недифференцированной дисплазией соединительной ткани. Вторая группа (сравнительная) – 40 человек с отсутствием признаков дисплазии.

Методы включали определение: витамина D – 25(OH)D: микроэлементов; суточного потребление кальция: денситометрию позвоночного столба на уровне L_{II}–L_{IV}; уровня физического развития и психоэмоционального напряжения.

Нарушения обеспеченности 25(OH)D, низкое потребление кальцийсодержащих продуктов, дефицит магния, сдвиги в содержании микроэлементов коррелировали с денситометрическими данными, выявившими снижение МПКТ у 75% подростков 1-й группы, тогда как во 2-й группе только у 27,5%.

Результаты комплексного исследования показали, что дефицит остеотропных микронутриентов является серьезным экзогенным триггером развития и прогрессирования недифференцированной дисплазии соединительной ткани с формированием остеопении/остеопороза. Остеопения/остеопороз, низкое физическое развитие, нарушения в психоэмоциональной сфере свидетельствуют о серьезности прогноза недифференцированной дисплазии соединительной ткани у подростков.

Ключевые слова: дисплазия соединительной ткани, остеотропные микронутриенты, остеопения, остеопороз, макро- и микроэлементы, витамин D

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INTRODUCTION

Connective tissue dysplasia (CTD) is a group of polymorphic pathological conditions caused by hereditary or congenital defects in collagen synthesis and accompanied by disorders of the functions of the musculoskeletal system and internal organs. CTD includes genetically determined differentiated syndromes (Marfan syndrome, Ehlers-Danlos syndrome, brittle bone disease, Beals syndrome, Stickler syndrome, etc.) and undifferentiated connective tissue dysplasia (UCTD), which also has a genetic basis but is manifested by multifactorial effects in embryonic and postnatal periods.

The prevalence of syndrome forms is low. Thus, the frequency of Marfan syndrome in the population is 1:10000–1:15000 [1]. The population frequency of UCTD in Russia is 8.5%, but its individual features are determined in 60–70% of the population. In children within the population UCTD is observed in 20–70% [2–4]. There is no data on UCTD prevalence in the sources of foreign authors.

Due to the polymorphism of clinical signs, UCTD has become an interdisciplinary problem that concerns not only pediatricians, but also doctors of other specialties (rheumatologists, cardiologists, orthopedists, gastroenterologists, etc.). Unfortunately, practitioners are not sufficiently informed about the nature of UCTD, the recognition of its manifestations even at the first examination, resulting in a lack of accurate specific recommendations for the patient on physical activity, treatment, prevention of progression of connective tissue dysplasia. The clinical manifestations of UCTD are extremely diverse (*Table 1*).

The dynamics of UCTD signs is progressive, mainly up to the age of 35 years. At early age the symptoms of UCTD are minimal: at the age of 4–5 years – heart valve prolapse begins to form; at 5–7 years – deformations of chest and spine, flat feet, myopia; at adolescent and young age – joint hypermobility, autonomic dysfunction, vascular syndrome [1, 5, 6]. The critical period is adolescence, when the increase in the number of signs of connective tissue dysplasia is maximal, which is due to the growth spurt with an increase in total mass of connective tissue. At the age of over 35 years, the risk of a new sign of UCTD is unlikely and the main problem is complications of dysplasia (varicose veins, thrombophlebitis, recurrent dislocations, valve prolapse of the 2–3 stage, diverticular bowel disease and etc)[1] [1, 3, 5].

[1] Pathology of connective tissue – the cause of most diseases. Access mode: http:// nsp.ayurve-

Table 1. Manifestations of UDCTD in children and adolescents

Appearance
Tallness Long upper and lower extremities Arachnodactyly, thumb syndrome Epicanthic fold, big eyes, blue sclerae, protruding ears Malocclusion and tooth growth disorders, high palate
Musculoskeletal system
Posture disorders Chest deformities (pectus excavatum, pectus carinatum, flat chest) Spinal deformities (scoliosis, kyphoscoliosis) Hypermobile joints Joint clicks Sprains and subluxations Flat feet Osteopenia/osteoporosis
Skin, muscles, aponeurosis
Thin, easily stretchable skin Multiple growth of lanugo hairs Kelloid scars Muscular hypotension Different localized hernias
Visceral organs and nervous system
Cardiovascular system: prolapses, heart septum aneurysms, phlebeurysm in adolescents (incl. varicocele) GIT: ptosis, refluxes, hiatal hernia, biliary anomalies and dyskinesia, dolichosigmoid, diverticules and cysts Urinary system: nephroptosis Nervous system: vegetative dysfunction

It is known that connective tissue comprises more than 50% of body weight and its structure is well studied. The main function of connective tissue is structural support, a kind of «exoskeleton» for all other tissues of the body [7]. Unlike other tissues, connective tissue has an excess of extracellular matrix with a fairly small number of cells (osteoblasts, chondroblasts and fibroblasts).

The extracellular matrix consists of macromolecules of collagen, elastin, proteoglycans. The matrix is dominated by collagen content. The synthesis of collagens in the body is encoded by more than 50 genes, for which more than 1300 mutation types have been described[2]. This leads to a variety of clinical manifestations of UCTD.

From biochemical point of view collagen fibers are formed from proteins – amino acids (lysine, glycine, proline), which are synthesized by matrix cells and intestinal microbiota. Collagen fibres, interacting with each other and with matrix cells, provide structural integrity and strength of connective tissue. To date, 20 types of collagen fibers with different localization have been identified, of which the main ones are:

- type I collagen, localization: bone, cartilage, tendons;
- type II collagen – cartilage;
- type III collagen – extracellular matrix of connective tissue;
- type IV collagen – basal layer of the epithelium.

Thus, the maturation of native collagen is ensured by corresponding genes and proteins. Connective tissue dysplasia may occur at various stages of collagen formation, in particular, in case of synthesis disorders, its excessive degradation, insufficient cross-linking of fibers [6].

Immediate causes of UCTD are various types of effects on the fetus, resulting in a genetically determined change of extracellular matrix. These include burdened course of pregnancy, unfavourable environmental conditions, malnutrition, bad habits, stress, diseases of the expectant mother. In children and adolescents, the genetic defect may manifest itself when triggered by such factors as unbalanced physical activity, psycho-emotional overstrain, unfavourable ecology, protein-energy malnutrition due to irrational nutrition.

However, one of the most powerful factors is micronutrient deficiency, which is so necessary for the connective tissue matrix, and above all for bone tissue. Its matrix (osteoid) consists of approximately 90% collagen. The bone is not just a supporting organ. From modern points of view, the bone is a dynamic living tissue with high sensitivity to various regulatory mechanisms as well as to endo- and exoeffects [8]. The process of precipitation and accumulation of minerals, i.e., mineralization of bone tissue, largely depends on the structure and functional state of the bone matrix.

In puberty the maximum bone mass increase is observed, which increases more than 2 times at the age of 10–17 years [9, 10]. For the accumulation of bone mass, first of all, the body needs nutritional support with osteotropic micronutrients (vitamins D, A, C, E, K; macroelements – calcium, phosphorus, magnesium; micronutrients – copper, manganese, zinc, boron, selenium, silicon) [11].

The synergy between calcium, phosphorus and vitamin D on bone mineralization is widely covered in the literature. However, the effect of vitamin D on connective tissue is significantly less well known. The results of studies of genetic effects of vitamin D receptors have shown that active forms of vitamin can lead to changes and expression of more than 400 genes, including those whose function is directly or indirectly related to the structure of different types of connective tissue [12].

Vitamin C is essential for collagen formation and stimulation of osteoblasts differentiation.

The biological effects of B vitamins (B1, B2, B3, B6) are due to the fact that their derivatives are cofactors of more than 120 proteins, including those needed for collagen synthesis. Nowadays B6 (pyridoxine) is assigned to osteotropic micronutrients directly involved in osteogenesis, or more precisely in synthesis of bone matrix collagen. Experimental studies have confirmed

da-land.ru/p0233.htm.

[2] Pathology of connective tissue – the cause of most diseases. Access mode: http:// nsp.ayurve-da-land.ru/p0233.htm.

the fact that pyridoxine deficiency impairs the mechanical properties of connective tissue and leads to dysplasia. Pyridoxine deficiency has been found to disturb collagen stability by weakening the bonds between collagen fibers [13]. It was noted that pyridoxine is a magnesium synergist, contributing to the element's inflow into cellular structures [14]. Despite the fact that pyridoxine is found in a large number of products, vitamin deficiency is widespread in Russia, especially among schoolchildren. Normal vitamin B6 supply is found in only 36% of children (15).

Vitamin A is essential for the proper development and growth of bone tissue right from the fetal life. Due to its anabolic action, vitamin A contributes to the formation of the fetal skeleton [16]. Active vitamin metabolites regulate complex interactions of genes involved in the growth and differentiation of cells, including the cells of the connective tissue matrix. Vitamin A slows down the decomposition of collagen and elastin [17].

Vitamin E, being a biological antioxidant, blocks peroxidation of membrane lipids and prevents damage of cells and subcellular structures. It contributes to intracellular accumulation and retention of calcium inside cells, which is especially important for muscle tissue. It cannot be excluded that vitamin E deficiency is one of the causes of muscle hypotension, so typical for UCTD. Previously, experimental studies have shown that vitamin E is involved in the maturation and preservation of collagen stability through the stabilization of intra- and intermolecular bonds.

Osteogenic vitamins include fat-soluble vitamin K. The role of vitamin K in metabolic processes is so important that Danish biochemist Henrik Dam was awarded the Nobel Prize for the discovery and establishment of its chemical structure. Numerous studies in recent years have shown that vitamin is essential for bone tissue. Vitamin exists in two vital forms: K1 (phylloquinone) and K2 (menaquinone). The functions of the two forms are different: K1 is mainly responsible for blood coagulation and also participates in supporting cognitive functions, K2 is responsible for metabolism and bone formation[3].

K2 activates the main proteins of the bone matrix (osteocalcin, protein S, Gla-protein), stimulates the differentiation of osteoblasts and causes apoptosis of bone resorption cells, activates mineralization by directing calcium «as intended» to the bone tissue and preventing its deposition in soft tissues and vessels [8]. Moreover, K2 provides interaction of calcium with vitamin D, without which absorption of calcium is impossible[4].

Vitamin K2 practically does not enter the body with food, but is actively produced by intestinal microbiota. The only known K2 food source today is natto, traditional Japanese food made from fermented soybeans. They're also used to make K2 medications.

[3] Wonder Vitamin. The Russian Association on Osteoporosis. Access mode: http://www.osteoporoz.ru/index2.php?option=com_content&task=emailform&id=2373&itemid=70.
[4] Ibid.

Macro- and micronutrients are no less important for collagen synthesis processes and, consequently, for bone mineralization [11, 18, 19]. An important bone nutritive support is the provision of the organism with magnesium, an element regulating the mineralization, isogonic growth and strength of bone tissue. Magnesium deficiency causes a decrease in bone mass and, as a consequence, osteoporosis development [14, 20]. In addition, the importance of magnesium in the formation and maintenance of bone structure is associated with the fact that in case of its chronic deficiency the most important factor of bone mineralization – the ratio of Mg:Ca (normal ratio is 1:3, ie, per 1000 mg of Ca – 350–400 mg of Mg) is violated. When the ratio decreases towards magnesium deficiency, the mineralization of bone tissue slows down sharply.

Studies have shown that the «health» of connective tissue is directly related to the magnesium content in the body. The mechanism of magnesium ion deficiency effect on connective tissue is an increase in the degradation of collagen and possibly elastin fibers. The influence of element deficiency on collagen cross-links leads to their splitting into «slabs», which is expressed in the reduction of mechanical strength of connective tissue, while a sufficient level of magnesium prevents degradation and contributes to the accelerated synthesis of new collagen molecules [21–23].

An important mineral component of bone tissue is phosphorus, whose content in the skeleton as hydroxyapatite is 85% of the total amount in the body. Phosphorus is necessary both for the mineralization of the skeleton and for increasing the functional activity of osteoblasts. The optimal ratio of calcium to phosphorus is 1.5:1.

Although the role of minerals and vitamin D in osteogenesis is undeniable, complete metabolism and mineralization is impossible in case of osteotropic micronutrients (MN) deficiency. This applies primarily to such essential MNs as copper, zinc, manganese, selenium, silicon, and conditionally essential MN – boron. Among the variety of metabolic functions of the above-mentioned MN in the body, their direct involvement in the synthesis of basic structural proteins of connective tissue and bone matrix should be emphasized [19].

Copper determines the activity of the lysil oxidase enzyme involved in the formation of cross-links of collagen and/or elastin chains, which gives the connective tissue matrix maturity, resilience and elasticity.

Zinc is necessary for the functioning of many metalloenzymes that directly regulate the synthesis of collagen and the bone formation process. There is evidence that zinc affects the absorption activity of calcium through the intestinal wall [24]. Changes in the structure of vitamin D receptors caused by zinc deficiency reduce their quantity and activity, which violates the regulatory effect of vitamin D on various biological processes in the body, including osteogenesis [25]. However, the effect of vitamin D on zinc assimilation and the rate of its meta-

bolism in the body has been described (26). This means that vitamin D deficiency leads, among other things, to zinc deficiency in the body.

Manganese activates a number of enzymes necessary for the synthesis of major proteins of connective tissue (proteoglycans and collagen), which determine growth and structure of bone, cartilaginous and connective tissues [27].

Boron improves the calcium assimilation by bone tissue and also directly participates in the synthesis of collagen and major osteogenesis proteins [2, 18, 19].

Selenium is a micronutrient that every cell of various organs and tissues needs. It is a component of more than 30 biological substances that are essential for the organism. Selenium is part of the active centers of natural enzymes of antioxidant protection, so their activity directly depends on the content of selenium in the body. Selenium-dependent enzymes are essential for the synthesis of thyroid hormones, in particular calcitonin. Hormone, by stimulating osteoblasts, activates bone metabolism, as well as mineralization by increasing the efficiency of calcium ion transport into bone tissue [28].

Recently there has been increased attention to silicon, the essential MN required for the synthesis and proper development of connective tissue, primarily of bone, cartilage, tendons [29]. It was found that silicon promotes the formation of connective tissue matrix components, providing physical and chemical properties of bone by activation of some enzymes (lysil oxidases) and inhibition of others (hyaluronidases) [30]. There is evidence that silicon can regulate the activity of the gene encoding the type I collagen molecule and simultaneously act as an enzyme cofactor (prolylhydroxylase) required for its synthesis [31]. However, it should be noted that the biological role of silicon and the potential for its involvement in osteogenesis have been studied so far insufficiently.

There are many works in the literature devoted to the study of UCTD. The studies show peculiarities of pathology of heart, respiratory organs, gastrointestinal tract, bone fractures course in patients with UCTD as well as changes in level of certain osteogenic and collagen-specific micronutrients in biological substrates [4, 6, 11, 32]. It has been noted that the most frequent shortages are silicon (100%), selenium (95.6%), calcium (64.1%), copper (58.7%), manganese (53.8%) and magnesium (47.8%) [33].

A few studies of osteotropic micronutrients and the state of bone tissue in adolescents with UCTD were the basis for this comprehensive study.

MATERIALS AND METHODS

A randomized study of 130 adolescents aged 10–16 years of the 1st-2nd health group hospitalized at the Pediatric Department of the Children's City Clinical Hospital named after Z.A. Bashlyaeva due to vegetative dysfunction and at the Central Clinical Sanatorium «Malakhovka» in Moscow region was conducted. The study did not include adolescents who had taken vitamin and mineral complexes, vitamin D and calcium within three months before the present study.

The first group (main group) consisted of 90 people with identified UCTD. The 2nd group (comparative) included 40 people with no signs of dysplasia. The surveyed groups were comparable by sex and age.

METHODS

- The measurement of vitamin D – 25(OH)D in serum is performed using the chemiluminescent immunoassay (Roche/Hitachi Cobas analyzer, Switzerland). The normal level of 25(OH)D was considered to be within 30–50 ng/ml, insufficiency – 21–29 ng/ml, deficiency – 10–20 ng/ml, marked deficiency – <10 ng/ml [34, 35].
- Hair levels of boron, copper, manganese, zinc, magnesium were studied by inductively coupled plasma mass spectrometry (ICP-MS) using Nexion300D+NWR213 («Perkin Elmer», USA). The research was conducted in the laboratory of the Autonomous Nonprofit Organization «Center for Biotic Medicine» (Moscow).
- Evaluation of daily calcium intake was made according to tables on the basis of analysis of home nutrition using the formula: daily calcium intake (mg) = calcium in dairy products (mg) + 350 mg [36].
- Spinal column densitometry at LII-LIV level was performed by dual-energy X-ray absorptiometry (HOLOGIC densitometer, QDR 4500C, USA). Presence of osteopenia was established at values of Z-score from -1 to -2 SD and on the basis of quantitative definition of projective bone mineral density BMD (in g/cm2) [37]. Researches were carried out on the basis of Clinical hospital № 86 FMBA.
- Physical development was assessed by the results of measurement of hand-held dynamometry, vital capacity, Ruffier index (HR before and after 15 squats), height and body weight with the subsequent data entry into the hardware software complex «Origins of Health» (HSC, Rospatent Certificate №2004610012, series № 7000202) [38].
- The level of psycho-emotional stress was determined by the results of the modified Lüscher color test, included in the program of HSC [19].
- Statistical analysis: the obtained data was processed using the software package STATISTICA v. 10.0 (StatSoft Inc., USA). Parametric (Student's t-test, Pearsen and Fisher rank correlation coefficient) and non-parametric methods of statistical processing (Wilcoxon signed-rank test, Spearmen's rank correlation coefficient) were used.

RESULTS OF THE STUDY

UCTD in adolescents was identified based on external and somatic features (*Table 2*).

Table 2. External and somatic signs of UDCTD in the surveyed adolescents (in abs./%)

	External signs	n	%
1	Hypermobile joints	90	100
2	Asthetic physique	33	36,6
3	Scars on the skin	15	16,6
4	Thin fragile skin	12	13,3
5	Flat feet	69	76,6
6	Posture disorders	75	83
7	Gothic palate	9	10
8	Malocclusion	48	53,3
9	Protruding ears	6	6,6
10	Hernias in anamnesis	15	16,6
	Somatic signs		
1	Gallbladder anomalies	72	80
2	MVP and others IADH	84	93,3
3	Nephtoptosis	9	10
4	Myopia, astigmatism	48	53,3

From the data presented in *Table 2*, it follows that among the examined adolescents, the most frequent external features of UCTD were joint hypermobility, posture disorders and flat feet. It should be emphasized that these signs are very rarely noticed by a pediatrician when examining a patient.

The evaluation of physical development on the basis of physiometric data (VC, hand-held dynamometry, Ruffier index) and also on the basis of body mass index showed that 42 persons of the 1st group (46%) had very low functional indices and were below average level (*Fig. 1*). Adolescents in the comparative group had mostly average physical development, while 8 had above average physical development (20%). Decrease in body weight was observed in 36% of Group 1 adolescents, while in Group 2, 73% of the surveyed had normal or even excessive body weight.

Figure 1. Physical health level of adolescents with UDCTD

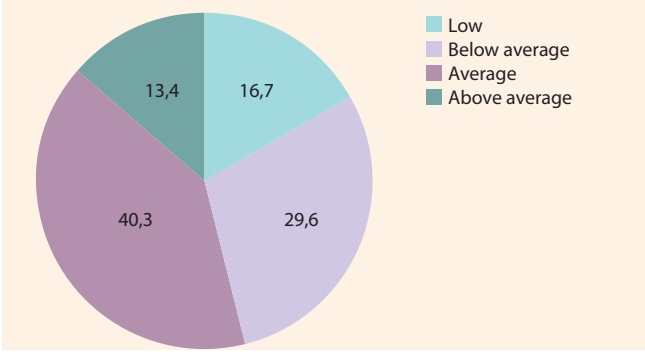


Figure 2. Assessment of psycho-emotional tension in adolescents with UDCTD according to the results of a modified color test on the Hardware and Software Complex

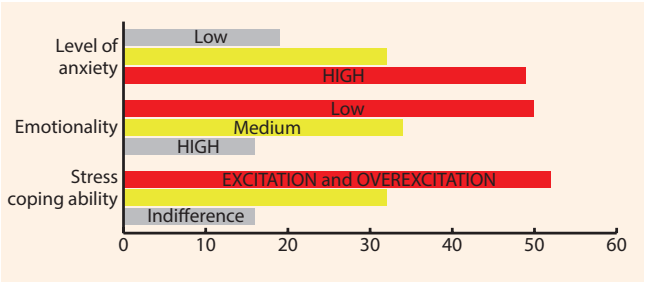
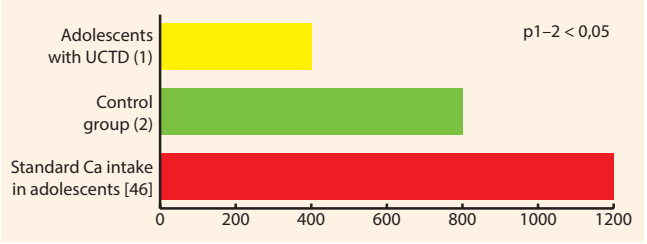


Figure 3. Daily calcium intake by adolescents in both groups (mg)



The state of psycho-emotional sphere as one of the manifestations of autonomic dysfunction, which is one of the markers of dysplasia, showed a high level of anxiety combined with emotional lability in half of the 1st group patients (*Fig. 2*).

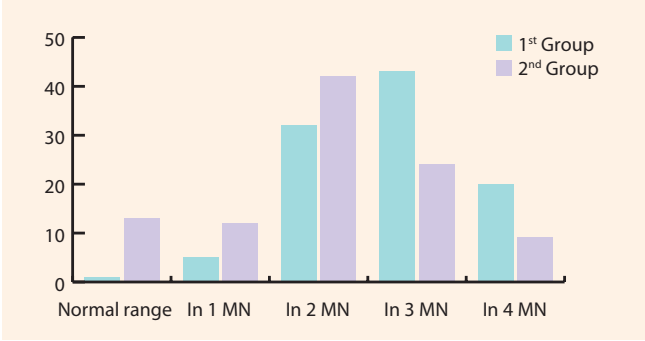
Low stress tolerance, manifested by excitement or overexcitement, was observed in 47 adolescents (52%). Attention was drawn to a decrease in emotional dynamics expressed by low levels of anxiety, indifference and absence of reaction to a stress factor in 15 people (17%).

The analysis of food diaries of adolescents revealed insufficient consumption of meat – in 40%, fish – in 80%, dairy products – in 62%, eggs – in 56%, vegetables and fruits – in 45% of adolescents. The evaluation of daily calcium intake showed that both groups of adolescents had insufficient consumption of calcium-containing products. However, significantly lower intake was found in Group 1 teenagers of 422 ± 18 mg, which was significantly lower than in Group 2 – 855 mg ± 25 mg ($p < 0.05$) (*Fig. 3*).

Analysis of the diet in general showed that the adolescents have not only a calcium deficiency, but also osteogenic vitamins and MN deficiency.

25(OH)D deficiency was found in all adolescents in both groups, while vitamin deficiency (22.1 ± 1.4 ng/ml) was found in 24 (18.5%) adolescents, deficiency (13.8 ± 2.8 ng/ml) in 76 (58.4%), and marked deficiency (7.8 ± 1.2 ng/ml) in 30 (23%).

Figure 4. Micronutrient frequency in adolescents in two study groups



Results of magnesium examination, an element so necessary for the connective matrix and BMD, are given in *Table 3*.

The results showed that magnesium deficiency in Group 1 adolescents was twice as high as in Group 2 (58.8 and 30%, respectively).

The MN study revealed changes in both Group 1 and 2, but shifts in boron, copper, manganese and zinc content were observed in all Group 1 patients, while in Group 2 13% of patients had normal levels. The number of shifts in the studied micronutrient complex in patients of two groups is illustrated in *Fig. 4*.

From the given data it follows that in the 1st group microelementosis of the 3rd and 4th micronutrients was more often encountered, in the 2nd group – of the 1st-2nd micronutrients. The quantitative average content of the investigated micronutrients in hair (μg/g) is illustrated in the *Fig. 5*.

Presented data indicates that the content of boron and manganese in the hair of teenagers is reduced, but in the 1st group the deficit is more significant and reliable in comparison with the 2nd group. The marked manganese deficiency in Group 1 is probably due not only to an unbalanced diet, but also to magnesium deficiency. There is evidence that under magnesium deficiency conditions, manganese is able to replace it in the active centers of certain enzymes involved in collagen synthesis and osteogenesis, and perform the same functions [16]. It follows from the aforesaid that magnesium deficiency can be one of the factors of manganese deficiency in conditions of connective tissue dysplasia.

When assessing the results of the zinc and copper study, there were significant shifts in the MN content in the adolescents of Group 1, while in Group 2 there was only a tendency for shifts (p in comparison with the norm > 0.05) (*Fig. 6*). The increased level of copper may

Figure 5. Quantitative average content of manganese and boron in hair (μg/g)

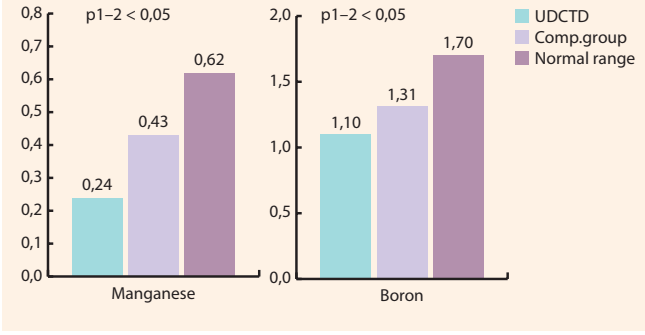


Figure 6. Quantitative average copper and zinc content in hair (μg/g).

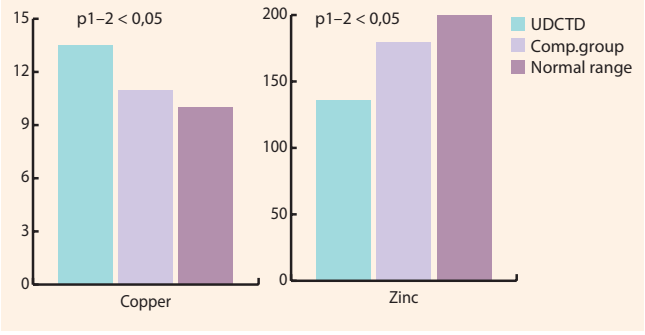


Table 3. Magnesium content in the hair of adolescents in both groups

Main group	Magnesium content in hair in UCTD (μg/g)							
	Level in patients	n	Mean	Median	Min	Max	Std.	p
Girls 13–16 y.o. Normal range: 40–105	decrease	12	19,4	22,1	11,1	24,9	6,3	0,005
	normal range	10	44,1	44,9	44,6	51,6	6,46	
Boys 13–16 y.o. Normal range: 25–50	decrease	15	12,2	4,4	4,4	20,0	3,2	0,001
	normal range	9	32,2	21,6	26,2	46,2	10,2	
Comparison group	**Magnesium content in hair in comparison group (μg/g)**							
Girls, boys 12–16 y.o.	normal range	28	43,3	48,2	43,9	54,8	5,6	0,001
	decrease	12	24,8	27,6	11,7	29,5	3,8	

be due not only to the mutual influence of copper and zinc, but also to the calcium deficiency. There is evidence that calcium deficiency leads to inhibition of copper exchange with acceleration of micronutrient accumulation rate in hair [39]. Besides, the increased level of copper can be caused by formation of micronutrient complexes with protein (metalloprotein) and their retention in various tissues, including hair. Complexes significantly reduce the turnover rate of copper and make it inaccessible for rapid integration into micronutrient-dependent functions of the body [40].

The results of the study of osteogenic MN complex showed that in the body of adolescents with UCTD their distinct imbalance has a negative impact on the metabolism and mineralization of bone tissue.

A densitometric study of adolescents in both groups revealed a decrease in BMD, but its incidence and severity varied. In Group 1 osteopenia was diagnosed in 53 persons (Z-score: -1 to -2), BMD = 0.84 ± 0.13 g/cm^2, and in 15 persons osteoporosis was diagnosed as BMD values were significantly reduced, averaging 0.74 ± 0.01, below BMD-1SD). In general, a decrease of BMD in Group 1 was observed in 68 people (75%). In the 2nd group osteopenia was found in 11 people (27,5%). Normal BMD values (1,09 ± 0,15 g/cm2) corresponding to height, sex and age were found in 51 (37%) adolescents in both groups.

The study of correlation between the results of densitometric studies and the level of MNs revealed the presence of reliable correlations in Group 1 adolescents. There was a moderate positive correlation between BMD and boron level ($r = 0.45$; $p < 0.001$); between manganese, zinc and BMD – strong positive correlation ($r = 0.57$; 0.52; $p < 0.001$); between copper and BMD – negative correlation ($r = -0.35$; $p < 0.001$). The presence of a reliable correlation between BMD and MNs confirms the influence of the investigated micronutrient complex on osteogenesis.

Thus, the combined results of the study clearly showed that the marked deficiency of osteotropic micronutrients is a serious exogenous trigger of UCTD development and progression with the development of osteopenia/osteoporosis in most adolescents. It should always be remembered that bone is also connective tissue and their structure and metabolism are identical. This is why connective tissue dysplasia is one of the powerful risk factors for osteopenia/osteoporosis. The formation of osteopenia/osteoporosis, pronounced micronutrient deficiency in combination with low physical development, with shifts in the psycho-emotional sphere indicate the seriousness of UCTD prognosis in adolescents.

Important conditions for the effective correction and prevention of the progression of UCTD in children and adolescents are the following:

- High-protein nutritious diet – a source of amino acids needed for the synthesis of collagen and products containing osteogenic macro- and micronutrients (*Table 4*).
- Dosed physical activity, isometric exercises, aerobic contactless sports (swimming, running, walking). Not indicated – group sports, gymnastics, power and static exercises.
- Prolonged, almost constant intake of multivitamin and mineral complexes. Among the diversity of MMC reception of Multi-tabs complexes is preferable. Комплексы Multi-tabs complexes are characterized by balanced multi-component composition, as well as innovative production technologies that allow to preserve the efficiency of all components and avoid their inactivation in the process of interaction. The most important In UCTD are: Multi-Tabs Kid Calcium Plus, containing calcium, 12 vitamins, 6 MNs, including osteogenic ones. The complex is recommended for children from the age of three, adolescents and adults (in the appropriate dose); Multi-Tabs Junior – a drug containing a full range of osteotropic vitamins and MNs. The complex is prescribed to children and adolescents aged from 4 to 11 years; Multi-Tabs Immuno Plus – a complex which, in addition to osteogenic vitamins, including magnesium 90 mg and MNs, includes *Lactobacillus rhamnosus* (LGG) for normalization of microflora and synthesis of vitamin K in the gut. This is particularly important as probiotics currently hold a specific place in UCTD correction. The conducted studies have established that microorganisms that make up the intestinal microbiota play an important role in the development of dyspla-

Table 4. Products containing osteogenic macro- and micronutrients

Element	Macro- and micronutrient rich foods
Calcium	Cheese, curdled dairy products, milk, cereals (buckwheat, oatmeal), vegetables (peas, carrots, green onions, beetroot), fruits (apricots, grapes, citrus, cherry, plum), fish
Phosphorus	Condensed milk, cream, cheese, fish, meat, cereals (pearl, oatmeal), dried fruits (hook, curaga, raisins)
Magnesium	Cereals (buckwheat, wheat, oats), watermelon, hazelnut, peas, seafood
Boron	Root vegetables, grapes, pears, apples, nuts.
Manganese	Rye bread, bran, soybeans, peas, nuts, vegetables (potatoes, beetroots, tomatoes), berries (blueberries, currants, raspberries, gooseberries), green tea.
Copper	Meat, fish, seafood, cereals (buckwheat, oatmeal, pearl), potatoes, fruits (apricots, pears), berries (gooseberries)
Zinc	Meat products (beef, liver), fish, eggs, legumes, bran, pumpkin seeds
Silicon	Grains, cereals (buckwheat, rice), corn and corn oil, beans, bananas, vegetables (red sweet pepper, pumpkin), nuts, mineral water [18]
Selenium	Nuts, barley groats, lentils, dairy products, eggs, various types of meat, seafood, mushrooms, wholemeal bread

sia[5]. Cascade of microbiota enzymes promotes the synthesis of vitamins (K2, etc.), amino acids (lysine, proline, glycine) required for the synthesis of collagen and, consequently, of the bone matrix. Disturbances of intestinal microbiota revealed in children reduce the synthesis of amino acids, which leads to the synthsis of less strong, unstable collagen fibers [41]. That is why probiotics are included in the complex of corrective measures in UCTD[6]. In this respect, Multi-Tabs Immuno Plus can be a drug of choice in UCTD in adolescents.

- Prolonged intake (regardless of MMC intake) of calcium and vitamin D drugs to stabilize mineral metabolism.
- Course intake of magnesium drugs to stimulate collagen synthesis (Magne B6 forte, mineral water «Donat Mg», etc.).
- Course intake of Coenzyme Q10, L-Carnitine for correction of bioenergy potential of the body.
- Literary sources have recommendations for the prescription of combined chondroprotectors in the presence of complaints and serious changes in the musculoskeletal system, with increased daily urinary excretion of hydroxyproline and decreased concentration of free amino acids in blood serum[7] [33].

CONCLUSION

Therefore, the results of the studies show that the diagnostics and correction of UCTD should be carried out at an earlier age than in adolescence. This can counteract the symptoms of UCTD and completely prevent the progression and development of diseases originating in connective tissue dysplasia.

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