Entomology and Applied Science Letters
Volume 5, Issue 4, Page No: 1-12

Available Online at: www.easletters.com

ISSN No: 2349-2864

Application of Laboratory and X-Ray Gentral Studies un Early Diagnostics of Metabolic Disturbances of Bone Tissue in Children with Autoimmune Diabetes Mellitus

Dmitry Anatolyevich Domenyuk[1]*, Vladimir Alexandrovich Zelensky[1], Igor Vladimirovich Rzhepakovsky[2], Oksana Ivanovna Anfinogenova[3], Pushkin, Sergey Viktorovich[4]

[1] Department of General Practice Dentistry and Child Dentistry, Stavropol State Medical University of Ministry of Healthcare, Russia
[2] MNOL "Experimental Immunomorphology, Immunopathology and Immunobiotechnology" of the Institute of Living Systems of the North Caucasus Federal University, Russia
[3] Department of Biomedicine and Physiology of the Institute of Living Systems of the North Caucasus Federal University, Russia
[4]Institute of Living Systems of the North Caucasus Federal University, Russia

ABSTRACT
This article focused on the issues related to bone tissue diagnostics in the children with type I diabetes (with different length of disease history). The results of densitometry with automatic calculation of the Z-criterion, allowed evaluating the bone tissues' mineral density in the lumbar spine. The quantitative and qualitative specifics of the jaw bone tissue were based on orthopantomography and cone-beam computed tomography. The mineral and bone metabolism status was studied based on the laboratory-test data (total calcium, ionized calcium, phosphorus, alkaline phosphatase, calcitonin, osteocalcin, parathyroid hormone, 25 OH vitamin D, β-CrossLaps). The earlier stages of the disease were found to feature an increase in the bone tissue remodeling rate along with the escalating bone formation intensity. The children with a long history of type 1 diabetes revealed slower bone remodeling with the bone resorption dominating over the bone formation, as well as the significant decrease in the mineral density with the bone tissue structure demonstrating the dominance of the criteria like "within the expected age norm" and "the low mineral density with respect to the average age norm".

Keywords: type 1 diabetes, osteodensitometry, cone-beam computed tomography, orthopantomography, bone metabolism.

HOW TO CITE THIS ARTICLE: Dmitry Anatolyevich Domenyuk*, Vladimir Alexandrovich Zelensky, Igor Vladimirovich Rzhepakovsky, Oksana Ivanovna Anfinogenova, Application of Laboratory and X-Ray Gentral Studies un Early Diagnostics of Metabolic Disturbances of Bone Tissue in Children with Autoimmune Diabetes Mellitus , Entomol Appl Sci Lett, 2018, 5 (4): 1-12 ,

Corresponding author: Dmitry Anatolyevich Domenyuk
E-mail ✉ domenyukda @ mail.ru
Received: 11/08/2018
Accepted: 06/12/2018

INTRODUCTION

Diabetes is a noteworthy worldwide issue because of drastically expanding around the world [1]. A group of widespread metabolic disorders that possess the phenotype of hyperglycemia has been referred to as Diabetes mellitus [2]. The results of epidemiological studies carried out by the International Diabetes Federation in more than one hundred countries all over the world in the past forty years showed that type 1 diabetes is the leading condition among the endocrine pathologies in children, and the current trend has pointed at the steady increase in the incidence. These data served the basis for adopting the regulatory and legal international

acts aimed at combating type 1 diabetes in children (St. Vincent Declaration of WHO, 1989; Weimar Initiative, 1997; UN Resolution, 2007) [3].

As [4] stated Diabetes is a major contributor to the worldwide burden of ailment. The worst part of type 1 diabetes developing in childhood is that the pathology affects almost all the body organs and systems; the latent nature of the endocrinopathy while the clinical symptoms are manifested at the complete depletion of the pancreatic function; the early development of the severe specific complications; disturbed sexual and physical development followed by the limited capacity and the early disability; the reduced life quality and life expectancy; and the premature mortality [5-7]. The WHO experts claimed (2012) that the case of type 1 diabetes develops in childhood, and such patients' life expectancy is only 50% that of the average value, while the patients do not usually live beyond the age of 40 [8-10]. The challenge of the early identification as well as the high prevalence of type 1 diabetes in children explain the urgency in terms of solving problems associated with the early endocrinopathy detection[11-20].

The available scientific literature has pointed that bone tissue is the key link in the phosphoric-calcium homeostasis system, which can be maintained through the multilevel physiological systems including the operational and regulatory structures that, via neurohumoral mechanisms, interact closely with each other [21-28] carried out, the data on the bone tissue mineral density and phosphorus-calcium metabolism in the children having type 1 diabetes are scarce and still being accumulated. There is a lot of great research and pragmatic value in the fact that, compared with the data from the densitometric studies, the data on calcium-phosphorus bone metabolism obtained through the lab tests are more sensitive, and reveal a faster response to the changes in the intensity of the bone formation (bone resorption). It stands a proven fact that the morphological and functional shifts observed in type 1 diabetes in the children's body correlate with the changes in the calcium-phosphorus metabolism and the bone mineral density. The results of the laboratory and X-ray diagnostics tests for the metabolic disorders of the children's bone tissue with different history of type 1 diabetes will allow establishing the trend and identifying the intensity of the bone formation reactions, the protective-compensatory mechanisms status, the likelihood of the complications development, thus confirming the need to stick to the principles of approaching the body as a holistic system [29-33]

All the above has laid the grounds for the aim of this study.

Aim of study:

Improving the methodological approaches in the early-stage diagnostics of type 1 diabetes in children based on phosphorus-calcium metabolism and bone mineral density.

MATERIALS AND METHODS

The study implied laboratory-clinical, X-ray examination comprising 114 children (aged 7 to 12) suffering from type 1 diabetes and undergoing treatment in the Endocrinology Department of the Filippsky Child Clinical Hospital (City of Stavropol, Russia). The duration of the endocrinopathy in the children diagnosed with type 1 diabetes varied from eight months to ten years. Given the disease history duration, all the patients were divided into three groups: Group 1 – duration of type 1 diabetes up to one year (n = 33, 28.9%); Group 2 – the disease duration 1-5 years (n = 39; 34.2%); Group 3 – suffering from type 1 diabetes for 5-10 years (n = 42, 36.9%). The comparison group included 35 healthy and basically healthy children (Yu.E. Veltischev, 1994) falling into the same age group.

The densitometric measurement of the bone mineral density relied on the dual-energy X-ray absorptiometry in the lateral and frontal projection of the lumbar spine with the morphometric analysis. The examinations were performed employing the Lunar iDXA densitometer using a cadmium-zinc-telluride detector array and the High-definition direct-digital narrow-angle fan-beam technology. The enCORE™ GE Lunar software was operated on the Windows XP Professional and included a special pediatric program with the age and sex normative indicators installed, which allowed a reliable assessment of the quantitative changes in the children's bone system. In pediatrics, Dual-energy X-ray absorptiometry (DXA) is the gold standard for studying the bones' mineral density. The following bone system parameters were evaluated through the X-ray scanning:

Area – the scanned section projection area (cm^2);

BMC – the Bone Mineral Content (g);

BMD – the Bone Mineral Density, i.e. the volume of the mineralized bone as per unit of the scanned area (BMD = BMC/Area) (g/cm^2).

The bone mineral density estimation (hydroxyapatite quantity per a bone surface unit) using the DXA method is an integral measurement of the cortical and trabecular bone, whereas the densitometric result is expressed as an index of the standard deviation

in relation to the normative value. Following the recommendations of the International Society for Clinical Densitometry, BMD in the pediatric DXA protocol is assessed with the Z-score, which is the standard deviation value of the actual bone mineral density with the respect to the average age norm, while the terms osteopenia, osteoporosis are not employed in the DXA outcomes analysis.

The following evaluation criteria using the Z-score were used: Z-score values below one standard deviation (<-1SD) are described as the normal mineral density; Z-score from one (-1SD) to two (-2SD) standard deviations are defined as within the expected average age norm; Z-score below two standard deviations (<-2 SD) are referred to as low mineral density relative to the average age or below the expected age norm. The spine bone tissues' mineral density data were compared with the reference database of the Lunar iDXA densitometer and the national standards (Scheplyagina L.A. et al, 2004).

Orthopantomography (OPG) of the jaw bones was performed on an ORTHOPHOS XG 3 DS digital orthopantomograph. The OPG analysis focused on the evaluating the height, shape and condition of the cortical plate of the alveolar process and the interalveolar septa, the degree of the expansion of the periodontal gap, the bone tissue resorption in the jaws and in the interalveolar septa. The resorption degree of the jaw body and interalveolar septa was described with the following features: the excessive transparency of the bone substance, the thinned bone trabeculae, the thinned cortical layer, the local bone loss, the restructured fiber arrangement in the bone substance. The Fuchs index (the quantitative index of the decrease in the alveolar bone height) the resorption degree in the interalveolar septa was established relative to the tooth root length (Fig. 1). The Fuchs index evaluation codes included: 0 – missing tooth due to the periodontal pathology, or the tooth outside of the bone tissue; 1 – bone resorption exceeding ⅔ of the root length; 2 – the bone resorption up to ⅔ of the root length; 3 – the bone resorption up to ⅓ root length; 4 – no resorption in the alveolar process. The calculation formula:

$$Index\ Fuchs = \frac{(n \times 0) + (n \times 1) + (n \times 2) + (n \times 3) + (n \times 4)}{\text{number of teeth}}$$

Scoring scale: 0 points – the interalveolar septum bone resorption reaches the tooth root apex; 0.25 points – bone resorption is above ⅔ of the root length; 0.5 points – bone resorption from ⅓ to ⅔ of the root length; 0.7 points – bone resorption up to ⅓ of the root length; 1 point – no loss of the bone tissue in the interalveolar septa.

Fig. 1. The method of quantitative determination of the degree of resorption of interalveolar

partitions relative to the length of the root (Fuchs index)

The X-ray index was used to identify the bone tissue the destruction degree in the alveolar part with respect to the tooth root's total length (Fig. 2). The calculation of the values and their ratios was performed with the AutoCAD Architecture software (2018 Version, 2D format).

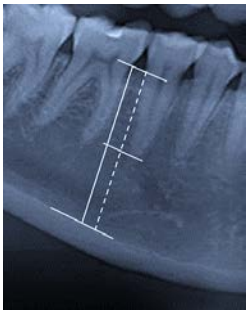

Fig. 2. The method of quantitative determination of the degree of bone tissue destruction of the alveolar part relative to the root length (X-ray index)

The quantitative assessment of the mandible cortical layer was done with the mandibular cortical index (MCI) (by Klemetti E., 1994). The cortical layer was measured on both sides below the foramen mentale using a quadriplying magnifying glass bearing a millimeter grid (step – 0.1 mm) (Fig. 3)

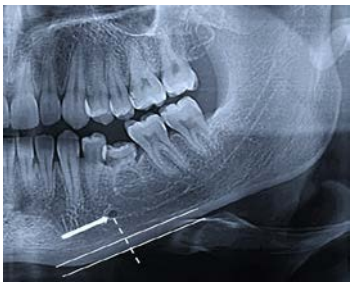

Fig. 3. Method of quantitative determination of the thickness of the cortical layer of the lower

jaw in the region of the mental opening (mandibular-cortical index, MCI)

The qualitative evaluation of the cortical plate below the foramen mentale was performed following the E. Klemetti method (1994) using the following morphological types: C1 – the inner border of the cortical plate is clear and even; C2 – the cortical layer boundary has single semilunar defects with the cortical plate dissection on one or both sides; C3 – the border is unclear, uneven, the cortical plate is multilayered, porous, with the numerous defects (Fig. 4).

Fig. 4. Method of qualitative assessment of the type of cortical plate of the lower jaw

The cone-beam computed tomography (CBCT) was performed on a 21-slice digital panoramic PaX-i3D SC device featuring the function of a computer tomograph and FOV cephalostat with the accessories subject to the scanning protocol for Sim Plant. The processing, storage and export of the X-ray images were done with the Ez Dent-i^{Tm} software; the multiplanar reconstruction and 3D reconstruction – with 3D tomography Ez Dent-i^{Tm} software for 3D diagnostics; viewing of the saved data with an import option – using the Viewer software [13-25] The tomographic section thickness was 1 mm, the reconstruction step – 1 mm, the rotation step – 1 mm, the reconstruction mode was set as BONE and STDN (standard) [14,17,33]. The radiological density of the mandibular bone was examined based on the mathematical reconstruction of the attenuation of the coefficients expressed in Hounsfield units (HU) [9,10,26,28,30]. The areas for the investigation which was selected included the mandibular body at the second molar and the mandibular angle (C. Ulm, 2009). The cortical bone thickness was measured at the foramen mentale level (Fig. 5).

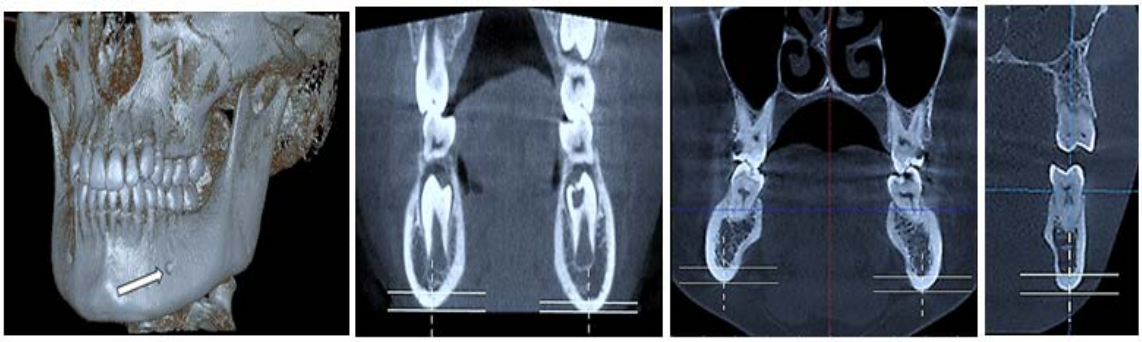

Fig. 5. Cone-beam computed tomography in the region of premolars, angle of mandible (a). Determination of the thickness of the cortical layer on the frontal sections (b, c, d)

The COBAS 6000 Hoffmann-LaRoche Diagnostics analyzer for the biochemical and immunochemical analysis (with commercial test kits) was employed to detect the level of the inorganic phosphorus, calcium (total, ionized), activity of the bone isoenzyme of alkaline phosphatase (AP) in all the patients' blood serum. The contents of serum immunoreactive parathyroid hormone (PTH), osteocalcin, calcitonin, 25-hydroxyvitamin D were determined via solid-phase enzyme immunoassay (EIA) using Vector-Best commercial test systems. The optical density of the samples was recorded on an enzyme-linked plate analyzer Statfax 4200. The bone resorption was assessed based on the serum levels of the degradation product of the C-terminal telopeptide of type I collagen spiral proteins (β-CrossLaps) using the «Serum CrossLapsTMElisa» diagnostic test systems (96 catalog number AC-02F1). The statistical data processing was performed employing the StatPlusV25 software application package using the parametric and nonparametric methods.

Table 1 shows the radiographic features of the jaw bone tissue status in the groups involved.

RESULTS AND DISCUSSION

Table 1. The radiographic features of the jaw bone tissue status in the groups involved, (M±m)

Object of study	Research groups			
	Comparison group	First group	The second group	Third group
Fuchs index, (points)				
Upper jaw	1,0	0,83±0,03*	0,72±0,02*	0,64±0,05*
Lower jaw	1,0	0,92±0,04*	0,79±0,05*	0,71±0,04*
The averaged index	1,0	0,87±0,06*	0,75±0,07*	0,68±0,08*
X-ray index, (points)				
Upper jaw	0,0	0,02±0,01*	0,06±0,01*	1,13±0,02*
Lower jaw	0,0	0,05±0,01*	0,11±0,02*	1,16±0,01*
The averaged index	0,0	0,03±0,01*	0,08±0,01*	1,14±0,02*
Mandibular-cortical index, MCI, (mm)				
Lower jaw	3,8±0,3	3,7±0,1*	3,5±0,2*	3,4±0,1*
Frequency of occurrence of cortical plate types, (%)				
Lower jaw	C1 – 74,3 C2 – 25,7 C3 – 0	C1 – 57,6 C2 – 42,4 C3 – 0	C1 – 30,8 C2 – 53,8 C3 – 15,4	C1 – 23,8 C2 – 40,5 C3 – 35,7

Note:* - p≤0.05 is statistically significant in comparison with the parameters of patients in the comparison group.

Table 2. shows the radiological density of the mandibular body bone tissue in the groups under examination.

Object of study	Research groups			
	Comparison group	First group	The second group	Third group
The mandibular body in the region of the 35 tooth, (Hounsfield units, HU)				
Average value, (M)	348,1	263,5*	39,2*	-126,7*
Standard deviation, (SD)	108,7	102,8*	127,4*	133,8*
Width of the window	71,0-669,0	37,0-422,0*	-83,0-198,0*	-271,0-23,0*
The angle of the lower jaw, (Hounsfield units, HU)				
Average value, (M)	736,4	539,1*	156,3*	5,6*
Standard deviation, (SD)	149,1	126,3*	71,8*	37,4*
Width of the window	372,0-1097,0	238,0-794,0*	106,0-429,0*	-46,0-73,0*
The thickness of the cortical layer of the lower jaw, (mm)				
	2,8±0,4	2,5±0,1**	2,1±0,2**	1,7±0,3**

Note. * - p≤0,001 is statistically significant in comparison with the patients of the comparison group;
** - p≤0.005 is statistically significant in comparison with the patients of the comparison group.

The outcome analysis revealed that the increase in the duration of type 1 diabetes in the children comes along with a significant decrease in the radiographic density of the bone tissue in the mandibular angle (body), a decrease in the interalveolar septa height, and the width (thickness) of the mandible cortical layer, associated with an increase in the share of the patients featuring a slightly (type C2) or severely damaged (type C3) cortical layer (Fig. 6).

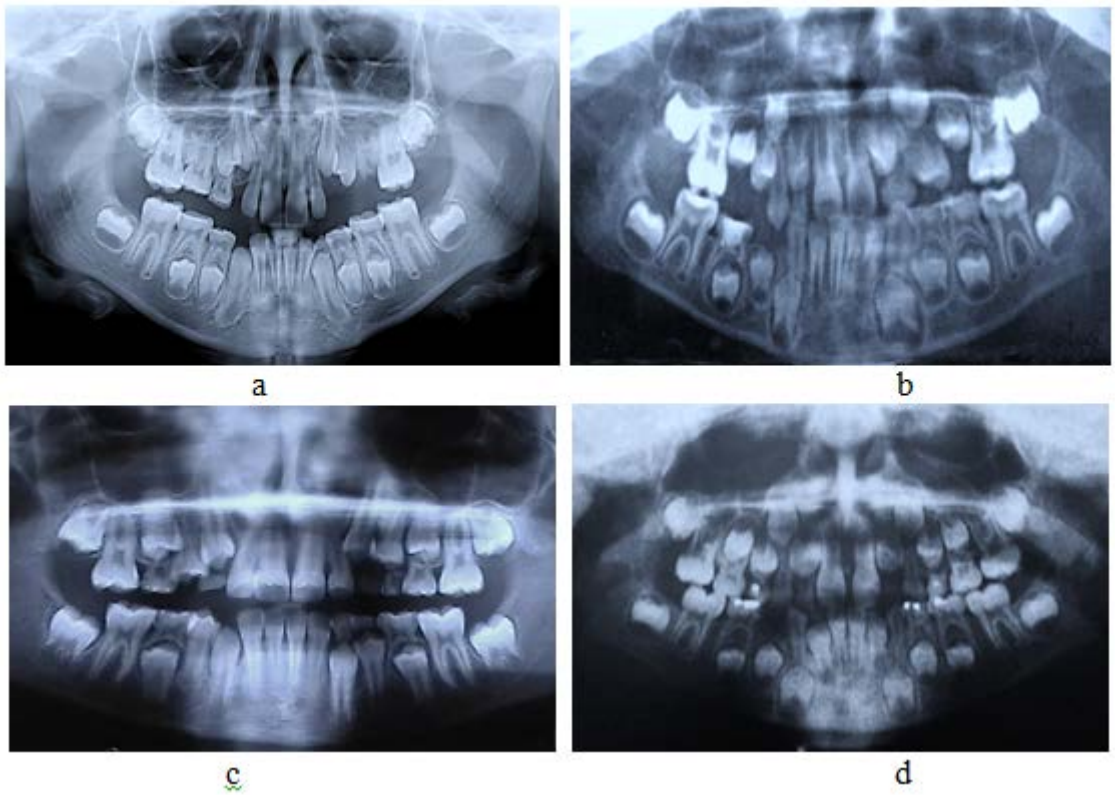

Fig. 6. X-ray characterization of the state of the bone tissue of the jaws in children of the studied groups: a - healthy children; b - children with experience of type 1 diabetes less than a year; в - children with the experience of type 1 diabetes from 1 year to 5 years; g - children with experience of type 1 diabetes from 5 to 10 years

Thus, the children with an endocrinopathy history of up to one year (the Fuchs index for the group 0.87 ± 0.06; X-ray index 0.03 ± 0.01; MCI 3.7 ± 0.1; X-ray density, body of lower jaw 263.5 ± 102.8 HU; X-ray density, angle of lower jaw 539.1 ± 126.3 HU) and from 1 to 5 years (the Fuchs index for the group 0.75 ± 0.07; X-ray index 0.08±0.01; MCI 3.5±0.2; X-ray density, body of lower jaw 39.2±127.4 HU; X-ray density, angle of lower jaw 156.3±71.8 HU) revealed a uniform, insignificant, generalized decrease in the interalveolar septa height (less than ⅓ of the root length), combined with an early degree of resorption (3-8%) of the bone tissue. The children with a 5-10 year-long history of type 1 diabetes (the Fuchs index for the group 0.68 ± 0.08; X-ray index 1.14±0.02; MCI 3.4±0.1; X-ray density, body of lower jaw 126.7±133.8 HU; X-ray density, angle of lower jaw 5,6±37,4 HU) featured a uniform generalized decrease in the interalveolar septa height (within ⅓ of the root length) at the initial resorption degree (14%) of the bone tissue.

The results of the study of the computer tomograms of the cross-sections of the alveolar part of the mandible of the healthy children allowed to visualize the integrity of the trabecular packet, the wide cortical plates (vestibular, lingual, palatine), the presence of the interconnected wide trabeculae (Fig. 7a,b). In the studied images of the bone sections in the children with the experience of type 1 diabetes from 1 to 5 years, the damage to the trabecular packet (free-standing, destroyed trabeculae), thinning of the cortical plates from the vestibular surface was observed (Fig. 7c,d). The evaluation of the computer tomograms of the mandibular cross-sections of children with experience of type 1 diabetes from 5 to 10 years revealed the following violations of the microarchitecture of the bone tissues: the destruction of the trabecular packet, the multi-layer (stratification) of the cortical plate, the presence of the thinned bone trabeculae not related to each other (Fig. 7e,f).

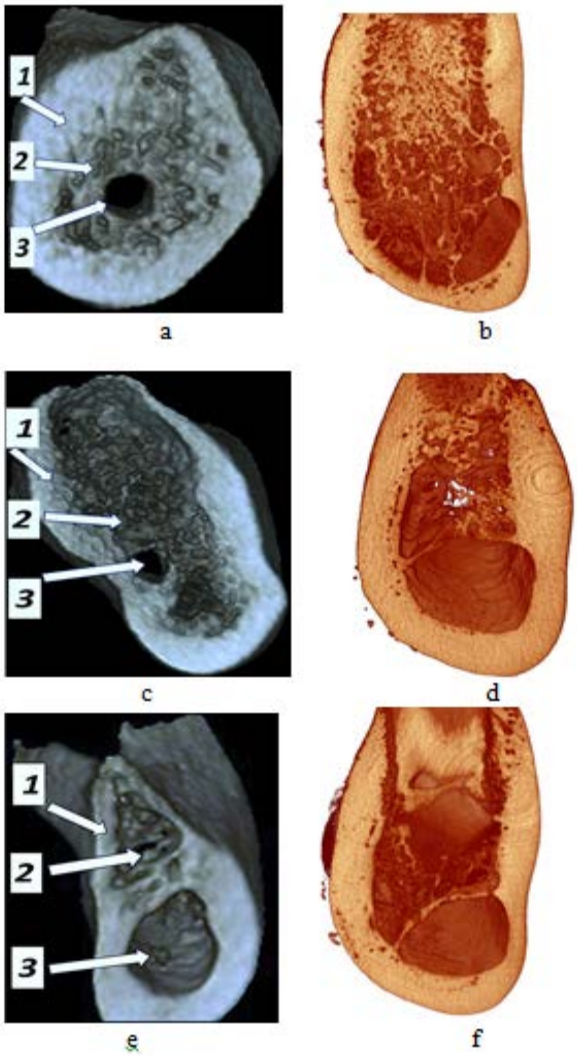

Fig. 7. Cone-beam computed tomography cross-section of the alveolar part of the mandible of a healthy child (a,b) and a child with the experience of type 1 diabetes from 1 year to 5 years (c,d) and a child with the experience of type 1 diabetes more than 5 years (e,f): 1-cortical lamina, 2-bone trabeculae, 3-mandibular canal

Systematizing the jaw bones X-ray data, it could be stated that the increased bone resorption is the most prominent in the children with the history of diabetes of type 1 exceeding 5 years, which has its course along with the insufficient metabolic control, whereas the alveolar bone loss degree exceeded the similar values for the mandible in all the examined groups. An analysis of the qualitative indicators of the bone tissue (based on OPG and CBCT) in children of Group 3 pointed at the generalized nature of the inflammatory-destructive changes that have been manifested as a uniform decrease in the interalveolar septa height (within ⅓ of the root length); a widening periodontal gap; a density decrease and disturbed microarchitectonics of the bone tissue in the jaws body (the increased transparency of the bone substance, the thinned bone trabeculae, the restructured fiber arrengement, the indistinctness, the porosity, the multilayered cortical plate all over, the large-mesh pattern of the spongy bone).

Deteriorated periodontological status in the children diagnosed with type 1 diabetes along with the long-term chronic hyperglycemia resulted in the disorganized morphology of the periodontium. The reduced compensation of the endocrine pathology along with an increasing history of type 1 diabetes in children was combined with the intensified bone resorption and remodeling, and progressing the proteolytic degradation that correlated with the uncontrolled destruction of the intercellular matrix, which was manifested through the disturbed barrier, protective, cushioning, trophic and plastic functions of the periodontal tissues. The experts have found that one of the most common bone tissue complications in case of diabetes mellitus is a decrease in the mineral density. Since there are no complaints reported

regarding the decreasing bone density in children with type 1 diabetes (or as such issues may be difficult to define), the identification of this parameter is an important criterion when diagnosing the endocrinopathy and assessing the rate of bone of the complications' development.

The results of studies focusing on the bone mineral density in the lumbar spine using the DXA method allowed the identification of the following structure in the bone tissue: the patients of Group 1 – within the age norm were 29 (87.9%) children; within the expected age norm were 4 (12.1%) children; Group 2 – within the age norm were 13 (33.3%) children; within the expected age norm were 17 (43.6%) children; the low mineral density compared to the average age norm were 9 (23.1%); Group 3 – within the expected age norm were 24 (57.1%) children; and the low mineral density compared to the average age norm were 18 (42.9%) children. The recommendations adopted by the International Society for Clinical Densitometry (ISCD, 2005) suggested that children's (adolescents') increase in the bone mass is mainly due to the prominent bone mineralization against an increase in the growing body's skeleton size. Given this, the obtained BMD values were compared with the bone age, the body length or put against the standards that allow calculating the Z-score mathematically in view of the age and body length. The interpretation of the skeleton bones' densitometric parameters in children (adolescents) took applying the regional (populational) databases (standards) calculated with a large sample pool and the specific for a particular geographic area (population). The age, sex, ethnicity, race, genetics, hormonal background, health status and physical activity, as well as the nutrition, growth, etc. have been proven to have a significant influence on the bone mass increase. This study used the pediatric reference database included in the Lunar iDXA densitometer software. Systemathisization of the obtained results allowed stating that an increase in the history of type 1 diabetes in the children came along with a significant decrease in the bone mineral density (Z-score <-1SD), while the bone tissue structure revealed the prevalence of the criteria like within the expected age norm and low mineral density compared to the average age norm. A statistically significant decrease in the bone mineral density in children with a history of type 1 diabetes exceeding five years points, as it was seen, at absolute insulin deficiency of pancreatic β-cells and an early debut of endocrine pathology during the bone tissue growth and development, served as an impetus for osteopenic syndrome development.

Table 3 shows the calcium-phosphorus metabolism and the parameters of calcium-regulating hormones in the blood serum of the children within the studied groups.

Table 3. The state of calcium-phosphorus metabolism and parameters of calcium-regulating hormones in blood serum in children of the study groups, (M±m)

Indicators, units of measurements	Reference intervals	Research groups			
		Comparison group	First group	The second group	Third group
Ca total, mmol/l	2,12-2,55	2,39±0,03	2,30±0,04*	2,21±0,02*	2,01±0,03*
Ca $^{++}$, mmol/l	1,12-1,32	1,23±0,02	1,17±0,02*	1,06±0,01*	0,98±0,02*
P, mmol/l	1,12-2,05	1,76±0,05	1,82±0,02*	1,68±0,04*	1,88±0,03*
Ca total / P	1/0,5-1/1,2	1/0,7	1/0,8	1/0,8	1/0,9
Ca $^{++}$ / P	1/1,10-1/1,50	1/1,43	1/1,56	1/1,58	1/1,92
The alkaline phosphatase, U/L	145,0-560,0	391,64±13,41	556,13±17,43*	302,75±9,81*	188,42±15,67*
Calcitonin, pg/ml	0,0-10,0	5,37±0,29	6,98±0,34*	3,63±0,47*	22,18±1,66*
Osteocalcin, ng/ml	2,80-41,00	30,38±2,96	104,51±7,26*	136,26±11,84*	24,27±1,68*
Parathyroid hormone, pg / ml	11,00-65,00	28,23±4,06	37,84±1,43*	69,07±3,51*	18,14±0,16*
25 OH vitamin D, nmol / l	27,70-107,00	47,63±1,84	35,06±2,38*	38,19±1,27*	29,34±1,91*

Note:* - p≤0.05 is statistically significant in comparison with the parameters of patients in the comparison group.

The results of studying the serum phosphorus-calcium metabolism in children with type 1 diabetes revealed a bidirectional dynamic in view of the endocrinopathy history. In case of an increasing history of type 1 diabetes, the serum levels of total (Ca_{total}) and ionized calcium (Ca^{2+}) were below the reference values at the normal levels of inorganic phosphorus (P) (i.e. within the physiological limits). An increasing history of type 1 diabetes came along with an increase in the ratios of Ca_{total}/P and Ca^{2+}/P, while the dependence of Ca_{total}/P varied within the

reference intervals, and Ca^{2+}/P went beyond the normative values, correlating with the severity of the metabolic disturbances. The obtained outcomes were consistent with the data claiming that an decrease in the bone mineral density in children with type 1 diabetes features a compensatory increase in the intensity of the bone development, was confirmed by a progressive decrease in the blood levels of Ca^{2+}.

Wave-like fluctuations in the alkaline phosphatase levels (AP) within the normative values showed that the earlier stages of endocrinopathy were associated with a growing rate of the bone tissue remodeling. The later stages of type 1 diabetes showed a decrease in the activity of AP, a marker of the bone tissue development and a parameter for the bone metabolism evaluation, which pointed at a decrease in the bone development intensity and the gradual prevalence of the bone resorption in the body.

The regulation of the bone tissue remodeling is an extremely complex and multilevel process, while the most significant regulatory factors included parathyroid hormone, osteocalcin and calcitonin. The children with a type 1 diabetes history of up to one year had parathyroid hormone and calcitonin levels that are virtually no different from those of the healthy children, which proved the intactness of the hormonal regulation mechanisms at the earlier stages of the endocrinopathy. A sharp rise in the parathyroid hormone level in children with a history of type 1 diabetes from 1 to 5 years should be viewed as the compensatory hyperparathyroidism, which helps in maintaining proper blood calcium levels through inhibiting calcium excretion in the urine, and stimulating the osteoclasts activity. Besides, along with the insulin deficiency and hypocalcemia, the increased production of parathyroid hormone is one of the key factors in diabetic osteopenia pathogenesis. In children with a history of type 1 diabetes exceeding 5 years, a significant reduction in the parathyroid hormone content down to the reference values indicated an improper response of the calcium-regulating hormone to hypocalcemia. The calcitonin level, which is a functional parathyroid hormone antagonist, is within the physiological values in children with the history of type 1 diabetes ranging from 1 to 5 years. A sharp increase in the calcitonin level in children with a history of type 1 diabetes beyond 5 years should be seen, on the one hand, as a compensatory response aimed at reducing the bone resorption, while on the other – as a total result of the discoordination in the mechanisms ensuring the bone remodeling.

A prominent increase in the osteocalcin content (biochemical marker of bone development) in the children whose history of type 1 diabetes fell in the groups of up to 1 year, and between 1 to 5 years, as it was seen, pointed at an increased osteoblasts and odontoblasts metabolic activity, the stimulation of the bone mineralization, the potentiation of histomorphometric rearrangement and the rate of the young bone development. A significant decrease in the osteocalcin level as a prognostic indicator of the increased osteoporosis and demineralization in the children with the endocrinopathy history beyond 5 years, revealed the decreased bone development, the predominance of the bone resorption under immature bone development deficiency, and the disturbed ossification (bone tissue development). A decrease in the content of 25 hydroxyvitamin D in the children with type 1 diabetes along with an increasing history of the endocrinopathy within the reference intervals, stood as a proof to some tensions in the mechanisms ensuring the calcium phosphoric and bone metabolism maintenance. A decrease in the 25 hydroxyvitamin D level down to the minimum threshold values potentiated the disturbance in the calcium absorption into the intestine, thereby increasing the parathyroid hormone level (secondary hyperparathyroidism) and the osteoclasts activity.

In this study, it was believed that the insulin deficiency is the main pathogenetic mechanism in the diabetic osteopathy development and the disturbed bone tissue metabolism in type 1 diabetes. Insulin has been proven to stimulate the cell growth in different tissues, amino acid transport, protein biosynthesis, to have a direct stimulatory effect on collagen and hyaluronate synthesis, as well as an anabolic effect on the bone tissue metabolism. Besides, insulin is involved in osteoblasts differentiation, prolonged the absorption of calcium and amino acids in the intestine, and enhanced their incorporation into the bone tissue. The absolute insulin deficiency in case of type 1 diabetes would inhibit the osteoblasts activity, reduce the collagen production by osteoblasts, which is necessary to develop the bone matrix and its mineralization, and potentiate the metabolic acidosis, which increase the osteoclasts activity.

The results of biochemical studies focusing on the bone metabolism markers in the children with a history of type 1 diabetes exceeding 5 years have revealed, on the one hand, the dissociation of the bone remodeling towards the slower bone metabolism and, on the other, the predominance of the resorption along with a decreasing bone development intensity. The

range of the identified metabolic disorders were observed at the longer treatment course of the endocrinopathy in the children could be attributed to early manifestations of bone tissue damage.

Table 4 shows the level of collagen type I C-terminal telopeptide, as a marker of the collagen type I degradation and the disorganization of the extracellular matrix, in the blood serum of the children belonging to the groups within this study.

Table 4. The level of the C-terminal telopeptide of type I collagen in the serum of children in the study groups, (M ± m), (ng / ml))

Reference intervals	Research groups			
	Comparison group	First group	The second group	Third group
0,101 - 0,580	0,106± 0,03	0,187± 0,026*	0,266± 0,049*	0,127± 0,014*

Note:* - p≤0.05 is statistically significant in comparison with the parameters of patients in the comparison group.

The progressive increase in the β-CrossLaps level, which correlated with an increase in the osteocalcin content in children with a history of type 1 diabetes of up to 1 year, and ranging within 1 to 5 years, indicated the activation in the bone remodeling mechanisms with the resorption predominance, increased degradation of the interstitial type I collagen along with increasing the clinical manifestations of the endocrinopathy as well as structural and functional destructive changes in insulin-producing β cells of the islets of Langerhans. A significant decrease in the β-CrossLaps level, accompanied with a decrease in the osteocalcin content in children with a history of the endocrine pathology above than 5 years, revealed the development of the irreversible degenerative-dystrophic changes in the pancreas β-islet cells, a decrease in the extracellular matrix of the active destruction, the bone metabolism reduction, the predominance of the resorption over the bone development, as well as the young bone tissue development deficit combined with the disturbed ossification.

CONCLUSIONS.

1. The blood serum in children at their early stage of developing type 1 diabetes, featured an increase in the ratio gradients of Ca_{total}/P and Ca^{2+}/P, the alkaline phosphatase activity, the levels of calcitonin, osteocalcin, parathormone, β-CrossLaps along with a decrease in the levels of calcium (total, ionized) and 25 hydroxyvitamin D, which pointed at an increase in the bone remodeling rate that correlated with an increase in the bone development intensity. The later stages of the endocrinopathy showed a further increase in Ca_{total}/P and Ca^{2+}/P, the calcitonin level with decreasing calcium (total, ionized), the alkaline phosphatase activity, the osteocalcin, the parathyroid hormone and 25 hydroxyvitamin D, and β-CrossLaps, which were indicative of slowing bone remodeling with the bone resorption predominance over the bone development.

2. In children with type 1 diabetes, an increase in the endocrinopathy history has been registered to come along with a significant decrease in the bone mineral density (Z-score <-1SD), with bone tissue structure showing the prevalence of the criteria such as within the expected age norm and low mineral density in relation to the average age norm. A statistically significant decrease in the bone mineral density in the children with a history of type 1 diabetes exceeding 5 years indicated the absolute insulin deficiency of the pancreatic β-cells, an early debut of the endocrinopathy during the bone tissue growth and development, which were the impetus towards developing the osteopenic syndrome.

3. An increase in the history of type 1 diabetes in children involved a significant deterioration in the periodontological status, which was due to the hormonal changes, the disturbed salt-water exchange, and the metabolic disorders. It was proven that as type 1 diabetes in children progresses, the bone tissue radiological density in the mandible angle decreases; the interalveolar septa height goes down and so does the mandible cortical layer width (thickness); there was also disturbed microarchitectonics of the bone tissue in the jaws body (bone substance increased transparency, the thinned bone trabeculae, the restructured fiber arrengement, the indistinctness, the porosity, multilayered cortical plate all over, the large-mesh pattern of the spongy bone), while there was an increase in the share of the children with insignificantly (type C2) or severely damaged (type C3) mandibular cortical layer.

4. The children diagnosed with type 1 diabetes, revealed the disturbed bone metabolism at the later stages of the endocrinopathy, while such disturbance featured a low level of the bone metabolism, a decrease in the bone resorption activity and bone development, the destruction of the trabecular structure, a change in the extracellular matrix structure which were due to a disturbed balance between the bone proteins

tissues' synthesis and degradation, which involved the development of irreversible dystrophic changes. An increase in the history of type 1 diabetes, combined with the poor metabolic control, increased the risk of developing not diabetic osteopenia alone yet also other specific diabetic complications.

REFERENCES

1. Munir, A. Malik, S. I. Aslam, S. Mehmood, A. Amjad, S. Malik, K. A. Younis, M. Shah, A. H. & Shah, G.M. (2018), "Medicinal plants are effective inhibitors of type I and II diabetes", Pharmacophore, 9(5), 1-7.
2. Najafipour, M. Bani Mohammad, M. Zareizadeh, M. & Najafipour, F. (2018)."Step by step in management of type 2 diabetes", International Journal of Pharmaceutical and Phytopharmacological Research, 8(5), pp.68-71.
3. King H., Aubert R.E., Herman W.H. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care, 1998; 21(9): 1414-1431.
4. AL-Asiri, I. Sulaiman, H. Alotaibi, F. Predictors of Depression among Diabetes Mellitus Outpatient Attending King Abdul-Aziz Specialist Hospital in Taif, Saudi Arabia (2018). International Journal of Pharmaceutical Research & Allied Sciences, 2018, 7(4):97-103.
5. Dedov I.I., Mel'nichenko G.A., Fadeyev V.V. Endokrinologiya: Uchebnik. M.: Meditsina. 2000; 632 p. (In Russ.).
6. Balabolkin M.I., Klebanova Ye.M., Kreminskaya V.M. Differentsial'naya diagnostika i lecheniye endokrinnykh zabolevaniy: Rukovodstvo. M.: Meditsina. 2002; 752 p. (In Russ.).
7. Kostolanska J., Jakus V., Barak L. Glycation and lipid peroxidation in children and adolescents with type 1 diabetes mellitus with and without diabetic complications. J. Pediatr. Endocrinol. Metab. 2009; Vol. 22; 7: 635-643.
8. Kemmis K. Diabetes and osteporotic fractures. Diabetes Educator, 2005; Vol. 31; 2: 187-196.
9. Dedov I.I., Kurayev T.K., Peterkova V.A. Sakharnyy diabet u detey i podrostkov: Rukovodstvo. M.: GEOTAR-Media. 2013; 272 p. (In Russ.).
10. Roe T.F., Mora S., Costin G. Vertebral bone density in insulin-dependent diabetic children. Metabolism, 1991; 40: 967-971.
11. Bykov I.M., Ivchenko L.G., Domenyuk D.A., Kostyukova N.Y., Storozhuk A.P., Ilijev D.M. Salivary the level of proinflammatory cytokines in children with autoimmune diabetes mellitus in different phases of compensation endocrinopathy. Kuban Scientific Medical Bulletin. 2017; 24(4): 39-48. (In Russ., English abstract). DOI: 10.25207/1608-6228-2017-24-4-39-48.
12. Davydov B.N., Gilmiyarova F.N., Domenyuk D.A., Ivchenko L.G. Clinical and diagnostic significance of the activity of matrix metalloproteinase and their tissue inhibitors in assessing the condition of periodontal tissues in children with type 1 diabetes mellitus. Part I. Children's dentistry and prevention. 2017; Vol. XVI; 4 (63): 14-19. (In Russ.).
13. Vedeshina E.G., Domenyuk D.A. Buccal epithelium reactivity indicators as markers of morphological and functional impairment in patients with dentoalveolar pathology. Kuban Scientific Medical Bulletin. 2016; (4): 23-32. (In Russ., English abstract). DOI:10.25207/1608-6228-2016-4-23-32.
14. Domenyuk D.A., Davydov B.N., Vedeshina E.G., Dmitrienko S.V. Perfection of diagnostic methods of dentoalveolar anomalies by results of studying functional shifts in the system of oral homeostasis (Part I). The Dental Institute. 2016; 71(2): 74-77. (In Russ.).
15. Ivchenko L.G., Domenyuk D.A. Diagnosis of immunometabolic disorders in children with type I diabetes mellitus. Kuban Scientific Medical Bulletin. 2017; 1(2): 73-82. (In Russ., English abstract). DOI:10.25207/1608-6228-2017-2-73-82.
16. Bykov I.M., Ivchenko L.G., Domenyuk D.A., Kostyukova N.Y., Storozhuk A.P., Ilijev D.M. Features of free radical oxidation and antioxidant protection in children with sugar diabetes of the first type. Kuban Scientific Medical Bulletin. 2017; (4): 27-38. (In Russ., English abstract). DOI: DOI:10.25207/1608-6228-2017-24-4-27-38.
17. Davydov B.N., Gilmiyarova F.N., Domenyuk D.A., Ivchenko L.G. Optimization of diagnostics of type I diabetes in children according to the

results of cytomorphological studies of buccal epithelium and processes of oxidative stress in the oral cavity. Children's dentistry and prevention. 2017; Vol. XVI; 3(62): 9-18. (In Russ.).
18. Gilmiyarova F.N., Davydov B.N., Domenyuk D.A., Ivchenko L.G. Influence of severity of type I diabetes mellitus in children on dental status and immunological, biochemical parameters of blood serum and oral fluid. Part II. Periodontology. 2017; Vol. XXII; 3 (84): 36-41. (In Russ.).
19. Karslieva A.G., Domenyuk D.A., Zelensky V.A. Mixed saliva trace element composition in children with dentoalveolar anomalies through apparatus-involved treatment. Archiv EuroMedica, 2014; 4(1): 29-35.
20. Domenyuk D.A., Davydov B.N., Vedeshina E.G., Dmitrienko S.V. Perfection of diagnostic methods of dentoalveolar anomalies by results of studying functional shifts in the system of oral homeostasis (Part II). The Dental Institute. 2016; 72(3): 58-60. (In Russ.).
21. Shcheplyagina LA, Moiseeva T.Yu., Kruglova I.V. Clinical evaluation of bone mass in children. Scientific and practical rheumatology. 2005; 1: 79-84. (In Russ.).
22. Domenyuk D.A., Davydov B.N., Gilmiyarova F.N., Ivchenko L.G., Vedeshina E.G. Diagnostic and prognostic value of crystalline structures of the oral fluid in children with anomalies of occlusion. Children's dentistry and prevention. 2017; Том XXI; 2(61): 9-16. (In Russ.).
23. Benevolenskaya L.I., Lesnyak O.M. Osteoporoz. Diagnostika, profilaktika i lecheniye: Klinicheskiye rekomendatsii. M.: GEOTAR-Media. 2005; 271 p. (In Russ.).
24. Diane L. Chau, Steven V. Edelman. Osteoporosis and diabetes. Clin. Diabetes, 2002; Vol. 20; 3: 153-157.
25. Gilmiyarova F.N., Davydov B.N., Domenyuk D.A., Ivchenko L.G. Influence of severity of type I diabetes mellitus in children on dental status and immunological, biochemical parameters of blood serum and oral fluid. Part I. Periodontology. 2017; Vol. XXII; 2 (83): 53-60. (In Russ.).
26. Alexopoulou O. Bone density and markers of bone remodeling in type 1 male diabetic patients. Diabetes Metab, 2006; Vol. 32; 5: 453-458.
27. Domenyuk D.A., F.N. Gilmiyarova, N.I. Bykova. Metabolicheskiye i mikrobiologicheskiye osobennosti biotopov polosti rta u detey s zubochelyustnoy patologiyey. Stavropol: Stavropolskii Gos.Univ., 2017. 312 p. (In Russ.).
28. Domenyuk D.A., Bazikov I.A., Gevandova M.G., Dmitrienko S.V. Mikroekologiya polosti rta detey s vrozhdonnym nesrashcheniyem noba. Stavropol: Stavropolskii Gos.Univ., 2016. 160 p. (In Russ.).
29. Karslieva A.G., Domenyuk D.A., Zelensky V.A. Mixed saliva trace element composition in children with dentoalveolar anomalies through apparatus-involved treatment. Archiv EuroMedica, 2014; 4(1): 29-35.
30. Davydov B.N., Gilmiyarova F.N., Domenyuk D.A., Ivchenko L.G. Clinical and diagnostic significance of the activity of matrix metalloproteinase and their tissue inhibitors in assessing the condition of periodontal tissues in children with type 1 diabetes mellitus. Part II. Children's dentistry and prevention. 2018; Vol. XVII; 1 (64): 37-46. (In Russ.).
31. Gunczker P. Decreased bone mineral density and bone formation markers shortly after diagnosis of clinical type 1 diabetes mellitus. J Pediatr Endocrinol Metab, 2001; Vol. 14: 525-528.
32. Nekrasova M.R., Suplotova L.A. Predictors of the development of osteopenia in type 1 diabetes mellitus. Diabetes. 2006; 1: 58-61. (In Russ.).
33. Belykh O.A., Kochetkova E.A. The state of calcium-phosphorus metabolism in patients with type 1 diabetes mellitus. Osteoporosis and osteopathy. 2005; 1: 12-15. (In Russ.).