ABSTRACT

of the thesis on the topic"The state of mineral density and features of bone tissue metabolism in adolescents of Western Kazakhstan"

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of the thesis on the topic "The state of mineral density and features of bone tissue metabolism in adolescents of Western Kazakhstan" presented by A.Amanzholkzyzy for the degree of Doctor of Philosophy (PhD) on the specialty 6D110100 - "Medicine"

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Topicality

Strengthening and protecting the health of the people of Kazakhstan is an important priority strategy. The state program "Densaulyk" for 2016-2019 points to tasks solution that will contribute to improving the health potential of the country's adult and child population.

The level of physical and mental health of the younger generation is the guarantee of the health of the adult population and the nation as a whole. Studying and improving the health of children and adolescents is an urgent problem that requires constant research and evaluation of its facets.

In recent years, there has been a slight deterioration in the somatic health of children and adolescents in Kazakhstan, an increase in the number of children with disharmonious development (Dossimov Zh.B., Kulniyazova GM, Dolotova LV, 2015). Untimely diagnosis of metabolic bone disorders leads to the formation of pathological changes on the part of the skeleton. The condition of bone tissue is considered as an indicator reflecting the level and quality of the overall development of children (Dossimov Zh.B., Nurgalieva RE, 2016).

In modern conditions the violation of bone mineral density (BMD) in adults is associated with the features of formation and mineralization of bone tissue in childhood, in particular, in adolescence (Shcheplyagina LA, Kruglova IV, 2013; CooperC, WestlakeS., 2006; KerbageH, BahadoriS., 2014), although previously as a rule osteoporosis was considered a problem associated exclusively with representatives of the older age group.

The received current data of various population studies have shown that the variability of BMD by 60-80% is determined by genetic factors (LinsT.S., VieiraR.J., 2011). The results of sexual and racial differences in the frequency of its manifestations are in favor of the genetic nature of osteoporosis. It was studied that the manifestation of osteoporosis is caused by the effect of many functionally interconnected genes: calcium exchange genes, hormonal status, and bone remodeling genes, extracellular matrix component genes and many others (.....).

Among many candidate genes involved in the regulation of bone metabolism and the determinants of BMD an important role belongs to the polymorphism of the vitamin D receptor gene (VDR). A number of studies have been devoted to investigating the relationship of the VDR gene to BMD, but their relationship with bone metabolism is discussed only in single studies. The interest of most scientists and physicians working in the field of genetics is aimed at studying the genetic predictors of osteopathy.

It is known that bone metabolism is characterized by two opposite processes: synthesis of new with the participation of osteoblasts and degradation or resorption of old bone tissue with the participation of osteoclasts (Khramtsova SN, Shcheplyagina LA, 2007). In the adolescent period metabolic processes are most intense and the balance between synthesis and resorption affects the accumulation of peak bone mass, the lack of which causes the risks of osteopenia and osteoporosis development during the following stages of life cycle.

The majority of the country and foreign studies are devoted to the analysis of the mineralization of bone tissue in children with pathological processes in it (Kiseleva AL, Kilina O.Yu., Ogorodova LM 2010 Rakhimova, VR Shim, SR Namanova, ZA Vazhnova IA, Ilin AG, 2010; ShaikhMG, CrabtreeN, KirkJM., 2014; KatzmanDK, MisraM., 2013). Some sources refer to
the objective difficulties in assessing the etiopathogenetic aspects of reducing the mineralization of bone tissue in children who do not have a pronounced somatic pathology (Osipenko OV, 2010; Khrapova Yu.V., 2012). It should be noted that the results of scientific research on this issue are contradictory in character and various authors give an intricate interpretation (Ostrovskaya, I.M., 2013). In Kazakhstan, the experts of the Association of the Osteoporosis Fund set out the facts about the presence of osteopenia in preterm infants and cases of changes in BMD in schoolchildren (Shim VR 2013, Gabdulina G.H., Tokarev AG, Borzova SV 2010).

The experts of the Kazakhstan Association of Osteoporosis Foundation state the facts about the presence of osteopenia in preterm infants and cases of changes in BMD in schoolchildren (Gabdulina G.Kh., Tokarev A.G., Borzova S.V.2010; Shim V.R. 2013). However, up to the present time there are no data on the features of metabolic processes of bone tissue with the evaluation of synthesis / resorption markers, genetic markers, humoral maintenance of bone tissue remodeling in healthy adolescents.

All of the above mentioned determined the aim of this research.

**Aim of the research:**

To reveal the peculiarities of the state of mineral density and metabolic processes of bone tissue in adolescents of Western Kazakhstan.

**Objectives of the research:**

1. To assess the state of mineral density of bone tissue in adolescents of different sex and ethnic group according to the results of ultrasonic osteodensitometry;
2. To study the features of the process of bone tissue synthesis and its humoral maintenance at various parameters of BMD in adolescents taking into account gender;
3. To study the state of 25-OH vitamin D in adolescents with different BMD indicators taking into account gender and ethnicity;
4. To study the polymorphism of the VDR gene of vitamin D receptor in adolescents taking into account ethnic group;
5. To study the peculiarities of the process of bone tissue resorption and its humoral maintenance with different parameters of BMD in adolescents taking into account gender.

**Scientific novelty of the study:**

For the first time in the Republic of Kazakhstan:

- densitometric studies were carried out in a certain age group of relatively healthy adolescents (12-17 years);
- a comparison of the densitometric index of BMD and biochemical markers of bone tissue synthesis / resorption and their humoral maintenance in adolescents taking into account gender was done;
- sensitive markers of bone tissue remodeling were revealed under various conditions of BMD;
- D-vitamin status was evaluated in adolescents with different BMD and its connection with the polymorphism of the VDR gene of the vitamin D receptor, taking into account gender and ethnic group.
1. The condition of osteopenia (69% of girls and 72% of boys) was found in 70% of the relatively healthy adolescents of Western Kazakhstan, and 18% had osteoporosis (15% of girls and 21% of boys); there were no differences in BMD on ethnic grounds.

2. In adolescents with a BMD index of osteopenia and osteoporosis from markers of bone tissue synthesis, the PINP marker is sensitive, while in boys it is 3 times higher in comparison with the girls. Humoral maintenance is due to the activity of insulin, more pronounced in girls.

3. In all tested relatively healthy adolescents a moderate deficit of 25-OH vitamin D is observed with different BMD indices; in representatives of the Kazakh ethnic group, compared with the Russian markedly significantly lower values of 25-OH vitamin D.

4. Reduction of bone density in the adolescents surveyed is characterized by the presence of polymorphisms of the VDR gene of the vitamin D receptor due to the mutant T / C allele and to a lesser extent C / C; the occurrence in the examined adolescents of two ethnic groups of polymorphisms of the VDR gene of the vitamin D receptor does not affect the content of 25-OH vitamin D.

5. In adolescents with a BMD index osteopenia and osteoporosis from bone resorption markers are β-CrossLaps and diploxyipyridinoline (DPID) sensitive, while the participation of β-CrossLaps is more pronounced in boys and DPID in girls. Humoral maintenance is due to the activity of PTH without gender-specific characteristics.

Theoretical and practical significance of the work

The obtained results of scientific research can be used in the educational process (lectures and practical lessons) in mastering the disciplines of normal and pathological physiology, pharmacology, pediatrics, traumatology and orthopedics, therapy, general practitioner practice. The significant prevalence of osteopenia / osteoporosis in relatively healthy adolescents makes it possible to justify the need to include ultrasonic osteodensitometry in the program of planned specialized prophylactic examinations in secondary schools. In detecting significant violations of bone mineral density in adolescents, it is necessary to evaluate the state of remodeling processes using significantly sensitive markers - PINP, β-CrossLaps, DPID; the definition of sensitive markers can be recommended to assess the effectiveness of preventive and curative measures. According to the results of scientific research there are Acts of implementation into the teaching process and practical health care.

Publication data

On the topic of the thesis research 13 scientific works were published, including: 1 publication in the international scientific issue included in the international database Scopus "GeorgianMedicalNews"; a reference was received on the review and admission to the press in 2017, 1 publication in the international scientific issue included in the international database Thomson Reuters "Journal of the National Medical Association"; 6 - in scientific publications recommended by the Committee for Control in Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan, 5 - in the materials of international scientific conferences, including 3 foreign ones.
List of publications on the doctoral thesis

- A. Amanzholkyzy., A.Zh. Dossimov. "The state of bone tissue density in healthy adolescents in the Western region of Kazakhstan" Fundamental science and clinical medicine — man and health XVIII international biomedical conference of young researchers on the twentieth anniversary of the medical faculty of St. Petersburg state University. 18 April 2015. The conference proceedings Page.29-30
Materials and methods of the research:

The research was carried out within the framework of grant financing of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan on the topic "Development of new technologies for protecting children's health and reproductive health". Registration in KazGOSINTI №0114PK00485.

The design of the study is a one-stage transverse, descriptive study.

To determine the quantitative sample of the osteodensitometric study, a calculation was made using a special formula in accordance with the design of the study. The established number for our study allows to take 384 adolescents.

Inclusion criteria: local residents, by ethnicity identical to the third generation having an average unambiguous consumer basket, age 12-17 years, are relatively healthy.

Exclusion criteria: adolescents with somatic diseases, athletes, age 18 years or older.

The research work consisted of two stages. At the first stage in the clinical and diagnostic center of Aktobe, the surveyed adolescents were assessed anthropometric data and bone mineral density (BMD) by ultrasonic osteodensitometry. The method of ultrasonic osteodensitometry was performed using the SONOST-3000 South Korea apparatus (the main device was registered on 07.12.2009 with the certificate number RK-MT No. 007207) which measured the ultrasound velocity in the calcaneus.

The BMD index was studied by the Z-score criterion which is the value of the standard deviation of the actual bone density in relation to the corresponding average age. Based on this criterion, the entire surveyed population is distributed according to the bone density: 1st group (Zscore > -1 SD) or normal BMD; 2nd group (Z score from -1 to -2.5 SD) or osteopenia; 3rd group (Z score < -2, 5 SD) or osteoporosis.

At the second stage 110 adolescents who gave informed consent, blood and urine tests were performed (hormones, a panel of the genetic marker and biomarkers of bone tissue metabolism were determined), and only 13 indicators in the two specified biological media. In the blood serum, the basal levels of PINP (N-terminal propeptidprocollagen 1 type - marker of collagen synthesis), calcitonin (CT) were determined by the method of immunological analysis on the automatic analyzer IMMULITE 2000, Siemens (Germany). The level of osteocalcin (OK), 25-OH Vitamin D and parathyroid hormone (PTH) was determined by electrochemoluminescent immunoassay analysis of Gobas-e601 (Roche). In the target blood (with EDTA), the level of B-CrossLaps (C-terminal telopeptide - a marker for the destruction of collagen type 1) was determined by the method of immunological analysis.

In urine, the indices of deoxypyridinolone (DPID) were determined, in the serum of the blood - somatotropic hormone (STH) by the method of solid phase chemiluminescent immunoassay.

The analysis was carried out by the polymorphism of the VDR gene of vitamin D. The genetic marker was determined by polymerase chain reaction. The genes of single nucleotide polymorphisms (SNP) of the vitamin D receptor have been identified. The genes of single nucleotide polymorphisms (SNP) of the vitamin D receptor, which in allelic variants represented genotypes - T / T, T / C and C / C; Of these, T / T is a normal variant of polymorphism; The T / S mutation is found in a heterozygous form associated with a risk of osteoporosis; C / C - mutation is found in both paired genes, otherwise the homozygous form associated with the expressed risk of development of osteoporosis.

Statistical processing of the data was carried out using the STATISTICA 10.0 software package from StatSoft, Inc. USA. Verification of the null hypothesis that there is no difference between the observed distribution of the trait and the theoretical expected normal distribution was performed using the Kolmogorov-Smirnov test. The differences between the samples were estimated:

- under the normal distribution of paired variables using t-Student's test and ANOVA in the case of multiple independent data;
- in the absence of a normal distribution and in the case of paired independent sets using the Mann-Whitney U-criteria (Mann - Whitney);
- in the case of multiple independent populations, the Kruskal-Walis criterion was used (Kruskal-Walis).

The arithmetic mean values of the quantitative indicators represented in the text in the form $M \pm SD$, where $M$ is the arithmetic mean, $SD$ - standard deviation are used. In the distribution of indicators that differ from normal, medians (Me) with lower and upper quartiles (Q1 and Q2) are taken. Qualitative indicators are presented in the form of a share (percent) and confidence intervals (CI). To determine the dependencies between the studied parameters, a correlation analysis was performed using the Spearman rank correlation coefficient ($r$). In all statistical analysis procedures, the confidence level was taken at $p \leq 0.05$.

**Results of the research**

When performing ultrasound ostendensitometry among the contingent of the examined relatively healthy children, $Z$ score $-2.0 \pm 0.9$ or osteopenia in 70% was revealed. Based on the study, the entire contingent of tested conditionally healthy adolescents is ranked into three groups according to the BMD index. Among the examined relatively healthy adolescents ostodensitometry revealed a percentage distribution of the BMD state to the value of $Z$-score: normal - in 12%, osteopenia in 70%, and osteoporosis in 18% of cases. The sign of osteopenia was almost identical in boys and girls (69% and 72%); the sign of osteoporosis was 1.5 times higher among boys than in girls; and there was no distinction in the distribution of BMD characteristics between representatives of two ethnic groups.

To assess the process of bone tissue synthesis, quantitative data of markers in the serum of the examined adolescents were studied: CT, OK, PINP, APF, as well as hormones of STH and insulin. In comparison with the reference values from all markers, the PINP indicator was 1.9 times higher. The number of PINPs was significantly different in schoolchildren when compared with normal MIPC with osteopenia / osteoporosis: 99.1 \([78.2-183.5]\) and 189.7 \([121.9-351.4]\) (ng / ml); 99.1 \([78.2-183.5]\) and 210.1 \([131.9-450.6]\) (ng / ml) ($H = 12.66, p = 0.001$).

In the state of normal BMD in boys and girls, the markers of PINP, OK and APF differed, respectively: 202.3 \([183.5-329.8]\) and 89.0 \([74.1-102.3]\) (ng / ml); 38.0 \([35.0-64.0]\) and 27.5 \([18.0-31.0]\) (ng / ml); 109.0 \([100.0-137.0]\) and 78.0 \([68.5 - 92.0]\) (ng / ml) ($p < 0.02$). A similar analysis for osteopenia revealed: 332.1 \([202.2-605.7]\) and 151.9 \([98.3-183.8]\) (ng / ml); 41.0 \([32.0-61.0]\) and 25.5 \([22.0-34.0]\) (ng / ml); 131.0 \([107.0-165.0]\) and 95.0 \([79.0-113.0]\) (ng / ml) ($p < 0.01$). The difference in hormone insulin was found on the basis of gender: in boys and girls - 4.0 \([3.0-5.7]\) and 6.3 \([5.1-8.9]\) $\mu U / l$ ($p < 0.01$). In adolescents with osteoporosis, the same trend, but only by the markers of PINP, OK, and the values of hormones are without special features. Analysis of osteosynthesis in each separate sexual subgroup, taking into account BMD, did not reveal distinctive parameters in boys. The difference in BMD between normal BMD and osteopenia 89.0 \([76.2, 100.7]\) and 151.9 \([98.3, 183.8]\) (ng / ml) was obtained in girls on the PINP marker; ($p = 0.02$).

The values of 25-OH vitamin D and the polymorphism of the VDR gene of the vitamin D receptor were studied. In adolescents, a moderate deficit of 25-OH vitamin D was detected. The range of reference values for a moderate deficit of 25-OH vitamin D was from 10 to 24 ng / ml. While the average the value of the contingent of the subjects corresponded to 20.5 ± 6.5 ng / l. Without taking into account the BMD, the value of 25-OH vitamin D differed in gender: in boys was more than in girls - 23.0 ± 7.6 ng / ml and 18.7 ± 5.1ng / ml ($p = 0.002$); According to the ethnic group by analogy in Russian and Kazakh adolescents it was 22.9 ± 7.1 ng / ml and 18.9 ± 5.6 ng / ml ($p = 0.004$). The study of the gender difference was due to the MBT which revealed the difference in 25-OH vitamin D in adolescents with osteopenia: in boys 23.7 ± 7.8 ng / ml and in girls 18.9 ± 4.7 ng / ml ($p = 0.007$).

The study of polymorphisms of the VDR gene of the vitamin D receptor among all examined without taking into account the BMD values revealed the distribution of the occurrence of variants
of alleles: T / T in 48% of adolescents; adverse hetero- and homozygous - T / C in 44% and C / C in 8%. We investigated the interethnic difference in the occurrence of polymorphisms of the VDR gene of the vitamin D receptor. The number of cases of the detected T / T allele in adolescents of Kazakh and Russian nationality, respectively: 56% and 36%; on the contrary, the T / S "mirror" 36% and 55% and the C / C almost unambiguous - 8% and 9%. This polar distribution of normal T/T and the negative variant of the T/S allele in representatives of ethnic groups did not correspond to the ethnic difference in the value of 25-OH vitamin D in adolescents. The analysis indicated that in adolescents with normal BMD, the presence of a normal T / T allele was in 63% of adolescents; mutant allele of the heterozygous type T / C in 37% and a mutant homozygous C / C allele was absent. The same methodology revealed almost the same ratio of alleles in the examined with signs of osteopenia and osteoporosis: T / T - in 46% and 40%; T / C - in 44% and 55% and C / C -y 10% and 8%. The revealed negative genotypes indicated the significance of the heterozygous T / C allele as compared to the homozygous C / C. Genetic determination of bone mineralization reduction according to the obtained results is revealed by the presence of negative variants of T / C and C / C alleles in adolescents of the Russian ethnic group with BMD of osteopenia and osteoporosis.

The process of osteorheal resection was assessed by KF markers, β-Cross Laps, DPID, PTH, leptin. The obtained marker data were compared with the reference ones and revealed a 2-fold difference only on the β-Cross Laps marker: 0.95 and 0.58 ng / ml. The same marker was studied in the serum of adolescents with a difference in BMD. The dynamics was obtained between those examined on the DPID marker with normal BMD and osteoporosis: 8.3 [7.3-10.4] ng / ml and 11.9 [10.5-14.3] ng / ml (p = 0.003). In adolescents with normal BMD, the β-Cross Laps marker was detected in a larger number in boys when compared with girls: 1.2 [1.1-2.4] ng / ml and 0.9 [0.7-1.1] ng / ml (p = 0.005), by analogy it was in adolescents with osteopenia. In boys and girls with osteoporosis, the difference was obtained for β-Cross Laps and DPID, according to the indicated sequence: 2.2 [2.1-2.7] -0.9 [0.7-1.1] ng / ml; 13.4 [12.1-17.2] ng / ml (p <0.03). When studying bone resorption markers in adolescents in each sexual subgroup, a significant increase in the number of β-CrossLaps marker was found in boys with osteopenia and osteoporosis, and in girls with normal BMD and osteoporosis of the DPID marker (p <0.03).

Correlation analysis between the parameters of the markers OK, CT, PINP, OA of the osteosynthesis and hormones of STH and insulin revealed single weak correlation links (r = 0.2, (p> 0.05) or their absence in osteoporosis, whereas between the markers of osteorectomy of CF, Cross Laps, DPID and hormones of PTH, leptin, reliable mean correlation correlations (r = 0.4, p <0.05) were obtained.

Conclusions

1 Results of ultrasonic osteodensitometry among relatively healthy adolescents revealed the condition of BMD: norm in 12% (Z-score -0.3 ± 0.4); osteopenia in 70% (Z-score -2.0 ± 0.5); osteoporosis in 18% (Z-score -3.2 ± 0.3); without a significant difference in gender and ethnicity.

2 In the examined relatively healthy adolescents, the process of bone tissue remodeling (osteosynthesis) in osteopenia and osteoporosis is caused by the sensitivity of the PINP marker (p≤ 0.003); the number of the PINP marker in boys is 2 times greater than that of girls with different BMD conditions; humoral maintenance is equally identified by the hormones of STH and insulin, however, for girls the activity of insulin is significantly higher in comparison with boys (p <0.01).
3 In adolescents with different BMD, the average value of 25-OH vitamin D is $20.5 \pm 6.5$ ng/l, which corresponds to a moderate deficit; in boys the content of 25-OH vitamin D is significantly higher in comparison with girls ($p = 0.0007$), in representatives of the Russian ethnic group is significantly higher in comparison with the Kazakh ($p = 0.001$).

4 Among the polymorphisms of the VDR gene of the vitamin D receptor in adolescents in the states of osteopenia and osteoporosis, a heterozygous mutant allele $T/C$ is more common than in homozygous $C/C$, respectively: $T/C$ - in 44% and 55; $C/C$ - in 10% and 8%. Genetic determinism of the condition of BMD is characteristic of the adolescents of the Russian ethnic group.

5 In the examined adolescents, the process of bone tissue remodeling (resorption) in osteopenia and osteoporosis is caused by the sensitivity of the DPID marker; in boys, the participation of $\beta$-Cross Laps is more pronounced, in girls - DPID ($p < 0.05$). Humoral maintenance is due to the activity of PTH ($p < 0.05$) with no gender differences.

6 The peculiarities of bone tissue metabolism with its reduced density in relatively healthy adolescents indicate a slight discoordination of remodeling (the prevalence of osteosorption over osteosynthesis), which can be assessed as an active physiological process, not excluding the borderline state.