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"WEST KAZAKHSTAN MARAT OSPANOV MEDICAL UNIVERSITY"

ABSTRACT
Of the PhD DOCTORAL DISSERTATION

Topic title: "**Clinical and genetic markers of bone metabolism in children under one year old in the Kazakh population**"

According to the educational program 8D10102 "Medicine"
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Completion dates: 2020-2023

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ABSTRACT

Kim I.S. on the topic: "Clinical and genetic markers of bone metabolism in children under one year old in the Kazakh population" submitted for the degree of Doctor of Philosophy (PhD), specialty 8D10102 "Medicine".
Supervisor: Doctor of Medical Sciences, Professor Zhumalina A.K.
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Relevance of the study:

Today the problem of bone loss is an urgent medical and social problem. Therefore it is the object of increased attention for researchers in various fields. Osteoporosis is characterized by a decrease in bone mass and a violation of the structure of bone tissue, an increase in their fragility and an increased risk of fractures. A decrease in bone mass with no violation of the architectonics is considered "osteopenia" [1-3].

The condition of bone tissue is an indicator of the functional state and general health of a child [4]. In general, the dynamics of human bone mass goes though the increase in childhood, reaching its maximum at the age of 20, then stabilizes and undergoes a gradual decrease after 35 years, which ultimately leads to the development of osteoporosis [4].

The frequency of osteoporosis is high, for example, in the USA about 10 million people have osteoporosis and 18 million suffer from bone loss [5]. According to the Children's Health Center (Moscow), densitometry of 1000 children of 7-15 years old showed a decrease of bone density in 40% of those examined [6]. In a study conducted in Western Kazakhstan, osteopenia was detected in 70% of 396 healthy children of 12-17 years old [7,8].

In children development there are critical periods when high activity of biological processes is accompanied by an accelerated rate of skeletal bones growth with linear growth and differentiation of bone tissue. Critical periods include the first year of life, the age of 5-7 years and adolescence [9]. In this regard, it is very important to examine the age-related characteristics of bone metabolism, especially during critical periods of growth, especially in the first year of life [9,10].

The role of genetic factors in modulating the risk of developing bone metabolism disorders is discussed. Numerous studies have shown that the peak of bone mass and the rate of its loss are determined genetically for 60-80% [11,12]. According to the literature, genetic factors also affect the level of 25 (OH) D in the blood serum [13,14]. The contribution of the genotype to variations in the level of 25 (OH) D in the blood serum ranges from 23-43% to 77-80% [15]. Genes whose mutations affect 25(OH)D levels were identified as the main candidate genes. These include: CYP2R1, CYP27B1, GC fBP, CYP24A1, VDR, and DHCR7/NADSYN1 [16]. There is also an evidence that the RANKL gene is a biomarker for patients with metabolic disorders [17].

The role of genetic factors in osteopenic conditions has been proven in many studies. However, there are still many questions about the contribution of specific genes that control bone growth and development, depending on age and ethnicity.

Considering that similar studies have not been conducted in children of the Kazakh population, it seems prospecting to study the distribution of genotype and allele frequencies for molecular genetic testing of polymorphisms of the VDR gene (rs2228570, rs1544410) and RANKL (rs9594759, rs9594738), as well as an analysis of the relationship between molecular genetic markers and bone metabolism indicators with the development of diagnostic algorithms.

Purpose of the study: To study the polymorphism of the VDR genes (rs1544410, rs2228570), RANKL (rs9594738, rs9594759) and their contribution to bone metabolism in children under one year old in the Kazakh population.

Objectives of the study:

1. To study bone metabolism indicators (osteocalcin, vitamin D, Ca, P, calcitonin, PTH, deoxypyridinoline) in children under one year old in the Kazakh population.
2. To determine the frequency of allelic variants of the VDR genes (rs1544410, rs2228570), RANKL (rs 9594738, rs9594759) in children under one year old in the Kazakh population.
3. To examine the relationship between the presence of allelic polymorphism of the VDR genes (rs1544410, rs2228570), RANKL (rs 9594738, rs9594759) and bone metabolism in children under one year old in the Kazakh population.
4. To develop an algorithm for preclinical diagnostics of bone metabolism disorders in children under one year old in the Kazakh population.

Scientific novelty of the study:

1. For the first time, the level of vitamin D provision for children under one year of age in the Kazakh population in Aktobe was determined. A high incidence of vitamin D deficiency with maximum severity in infants has been shown.
2. For the first time, the frequency of various allelic variants of the VDR (rs1544410, rs2228570), RANKL (rs9594738, rs9594759) genes in children under one year of the Kazakh population was studied.
3. For the first time, the relationship of polymorphisms of the VDR (rs1544410, rs2228570), RANKL (rs9594738, rs9594759) genes and indicators of bone metabolism in children under one year old in the Kazakh population was proved.
4. For the first time, an algorithm has been developed for the prenosological diagnosis of bone metabolism disorders in children under one year of age in the Kazakh population.

Theoretical and practical significance:

1. The results of the study allow us to recommend quantitative determination of serum vitamin D and osteocalcin as markers of preclinical diagnostics of bone metabolism disorders in children of the first year of life of the Kazakh population.

2. Genotyping of gene polymorphisms is recommended for use in practical medicine to predict the risk of bone metabolism disorders.
3. An algorithm for preclinical diagnostics of bone metabolism disorders in children under one year old of the Kazakh population is proposed for use. The results of the survey have been introduced into practical healthcare. There are acts of implementation of the "Algorithm for pre-clinical diagnostics of bone metabolism disorders in children under one year": (No. 7 dated 10.10.2023 in the State Enterprise "City Polyclinic No. 2" at the Right of Economic Management of the State Healthcare Institution of the Aktobe Region; No. 8 dated 10.10.2023 in LLP TT&K).

Main provisions submitted for defense

1. Reliable differences in the levels of bone metabolism indicators such as Total Calcium ($p < 0.001$), Phosphorus ($p < 0.01$), Calcitonin ($p < 0.001$), Osteocalcin ($p < 0.01$) and Vitamin D ($p < 0.001$) in different age groups were revealed.
2. It was established that the frequency of the GG rs1544410 genotype of the polymorphic variant of the VDR gene in children under one year of the Kazakh population is 59%. While the frequency of the CC genotype of the rs9594759 polymorphism of the RANKL gene varies within 43%. A possible marker of an increased risk of bone metabolism disorders in children under one year old of the Kazakh population is the presence of the GG genotype ($p<0.05$) of the rs1544410 polymorphism of the VDR gene.
3. A reliable average relationship was revealed between the carriage of the GG genotype ($p<0.05$) rs1544410 of the polymorphic variant of the VDR gene with a reduced level of Vitamin D and Phosphorus in children under one year old of the Kazakh population. A statistically significant average correlation was found between the presence of the CT genotype of the rs9594759 polymorphism of the RANKL gene and the concentration of Calcitonin in children of the same group.

Approbation of the work

The main provisions of the dissertation were presented at an extended meeting of the Scientific Problem Commission of the West Kazakhstan Marat Ospanov Medical University.
The results of the study were reported on:

1. International scientific and practical conference "Modern Medicine: New Approach and Current Research" among medical educational organizations of Kazakhstan, near and far abroad countries, dedicated to the World Osteoporosis Day, Aktobe, 10.20.2021. The report: "Gene polymorphism associated with the risk of developing Vitamin D hypovitaminosis in young children of the Kazakh population."
2. IV Interregional scientific and practical conference with international participation within the information project of the Ministry of Health of the Saratov Region and the Federal State Budgetary Educational Institution of Higher Education Saratov V.I. Razumovsky State Medical University "Current Problems of Modern Pediatrics", Saratov, 10.23.2021. Report: "Genetic polymorphism of Vitamin D deficiency in children of the Kazakh population."

3. VII International Scientific and Practical Conference of the Caspian States "Topical Issues of Modern Medicine", Astrakhan, 24-25.11.2022. Report: "Markers of Bone Metabolic Disorders in Children of the Kazakh Population".
4. Clinical Conference with International Participation, Marat Ospanov West Caucasian Medical University "Functional Disorders of the Gastrointestinal Tract in Children Under One Year", Aktobe, 31.01.2023. Report: "Vitamin D Status in Children Under One Year with Different Types of Feeding".
5. 1st International Forum "Asfen. Forum, New Generation-2023", Almaty, 05.06.2023. Report: "Diagnostical Value of Osteocalcin as a Marker of Bone Metabolic Disorders in Children Under One Year of the Kazakh Population".
6. XVI International Scientific and Practical Conference named after B.A. Atchabarov "Ecology. Radiation. Health", dedicated to the 70th anniversary of the non-commercial joint-stock company "Semey Medical University", Semey, August 28-29, 2023. Report: "Vitamin D level and frequency of allelic variants of the RANKL gene in children under one year of age in the Kazakh population."

Publications on the topic of the dissertation

17 scientific works have been published:

3 articles in journals indexed in the Scopus database.

- The Importance of Determining the Level of Bone Metabolism Markers and Vitamin D in the First Year of Life in the Kazakh Population// Akmaral Zhumalina, Balash Tusupkaliev, Anna Mania, Irina Kim, Mairamkul Zharlykasinova //J Pediatr Pharmacol Ther 2024 Vol. 29 No. 4, p. 410-416
- Features of d-vitamin status in young children in the Kazakh population// Akmaral Zhumalina, Irina Kim, Balash Tusupkaliev, Mairamkul Zharlykasinova, Botagoz Zhekeyeva// Polski Merkuriusz Lekarski, volume LII, issue 2, march -april 2024, p. 161-170
- Vitamin D level and indicators of bone tissue metabolism in Kazakh infants //Zhumalina, A.K., Kim, I.S., Delyagin, W.M.//Russian Family Doctor, 2023, 27(3), pages 23–29.

3 articles in journals recommended by the Committee for Quality Assurance in Science and Higher Education of the Ministry of Education and Science of the Republic of Kazakhstan:

- Vitamin D receptor gene polymorphism among children//Zhumalina A., Tusupkaliev B., Kim I., Zharlykasinova M.//Astana Medical Journal No. 4(106), 2020, pp. 97-106
- The frequency of allelic variants of the VDR gene and the level of vitamin D in children under one year old in the Kazakh population//Zhumalina A., Tusupkaliev B., Kim I., Sakhanova S., Zharlykasinova M.//Journal of Clinical Medicine of Kazakhstan, 2023 Volume 20, Issue 1, P.61-64
- Frequency of allelic variants of the Rankl gene and the risks of bone metabolism disorders in children under one year of age in the Kazakh population//A.K. Zhumalina, S.K. Sakhanova, I.S. Kim, B. Tusupkaliev,

M.B. Zharlykasinova//“Clinical Medicine and Pharmacology” No. 6 (251), 2023, p. 55-59

1 patent No. 6968 dated 14.10.2022 “Method for preclinical diagnosis of vitamin D deficiency in children under one year of age” Zhumalina Akmaral Kanashevna (KZ), Tusupkaliev Balash (KZ), Kim Irina Sergeevna (KZ), Anna Marta Mania (PL), Zharlykasinova Mairamkul Burkutbaevna (KZ)

1 methodical recommendation “Vitamin D and bone metabolism in children under one year of age” // ISBN 9788-601-80845-8-4 //A.K. Zhumalina, B.T. Tusupkaliev, I.S. Kim, M.B. Zharlykasinova

3 abstracts - in collections of International scientific and practical conferences

2 author's certificates:

• No. 20379 dated 09.22.2021 "Vitamin D receptor gene polymorphism among children" Zhumalina A.K., Tusupkaliev B.T., Kim I.S., Zharlykasinova M.B.

• No. 28675 dated 09/07/2022 "Vitamin D and bone metabolism in children under one year" Zhumalina A.K., Tusupkaliev B.T., Kim I.S., Zharlykasinova M.B.

4 acts of implementation:

In practical healthcare:

• "Algorithm for pre-clinical diagnostics of bone metabolism disorders in children under one year" No. 7 dated 10/10/2023 in the State Enterprise "City Polyclinic No. 2" on the Right of Economic Management of the State Healthcare Institution of the Aktobe Region

• "Algorithm for pre-clinical diagnostics of bone metabolism disorders in children under one year" No. 8 dated 10/10/2023 in LLP TT&K

In the educational process:

• "Use of the vitamin D indicator in blood serum in assessing the level of metabolic disorders in newborns" Zhumalina A.K., Tusupkaliev B.T., Kim I.S. No. 178 dated 10.25.2021

• "Polymorphism of bone metabolism genes in children under one year" Zhumalina A.K., Tusupkaliev B.T., Zame Yu.A., Kim I.S. No. 295 dated 03.06.2023

Personal contribution of the author

The author conducted a thorough analysis of scientific sources related to the topic of the dissertation. All parts of this research, including the goals, objectives, research program, processing of statistical data, interpretation of the results, conclusions and practical recommendations, were completed by the author independently.

Materials and methods of the study:

The work complies with the principles of the Helsinki Declaration of the World Medical Association (1964, updated in October 2013 at the 64th General Assembly of the WMA, Fortaleza, Brazil). The dissertation was reviewed by the local ethics committee at the Marat Ospanov West Kazakhstan Medical University of Aktobe on 04.12.2020, protocol No. 10. The study protocol was registered on ClinicalTrials.gov, which is managed by the US National Library of Medicine (NLM) at the National Institutes of Health and is the largest database of clinical trials. Identification number: NCT05375331.

In accordance with the purpose and objectives of the study 250 children under 1 year old of the Kazakh population born in Aktobe were examined. The sample size was calculated using the EpiInfo program:
(N-13500, p = 16% n = 208 + 20% (42) = 250 children.
N is the total birth rate of children in Aktobe;
p is the frequency of occurrence;
n is the number of children to be taken + 20% losses (sample size).

The work was based on the analysis of the results of a comprehensive clinical and laboratory examination of 250 children. Parents or legal representatives of patients included in the study signed informed consent with permission to process personal data for scientific purposes.

Children were recruited using the probability sampling method in Aktobe city. A total of 94 boys (37.6%) and 156 girls (62.4%) were examined. All examined children were divided into 3 age groups: Group 1 - children from 0 to 28 days (newborns); Group 2 - from 1 month to 6 months, group 3 - from 6 to 12 months.

The age groups of the examined children were formed by taking into account the characteristics of exogenous intake of vitamin D. In particular, in group 1, a direct dependence of the vitamin D content on a similar maternal indicator is assumed. The quantitative content of vitamin D in group 2 does not depend on exogenous intake, since children of this age group do not receive complementary foods. In group 3, additional intake of vitamin D with complementary foods is assumed, in accordance with the content of the National Program for Optimizing Feeding of Children in the First Year of Life.

The group of children examined as part of the scientific study was formed in accordance with the inclusion and exclusion criteria.

Inclusion criteria: "practically healthy" children under one year (0-12 months) of the Kazakh population; children without organic pathology and genetic syndromes; satisfactory condition at the time of the study; the possibility of taking blood from children for examination; the presence of informed consent signed by parents or legal representatives.

Determination of belonging to the Kazakh population was carried out by assessing the genealogy in three generations with filling out a questionnaire.

Exclusion criteria: hereditary diseases of the musculoskeletal system; severe chronic somatic diseases; disability due to other diseases; the fact of taking vitamin D in a therapeutic dose; refusal of parents or legal representatives to participate in the study; premature babies; age of children over 1 year.

Study design: descriptive one-stage cross-sectional study

Stage I. Clinical and anamnestic study: questionnaires for parents; analysis of children's anamnestic data; assessment of the child's health

Stage II. Assessment of bone metabolism. Biochemical blood test: Calcium, Phosphorus, Parathyroid hormone, Osteocalcin, Vitamin D, Calcitonin. Urine test: deoxypyridinoline.

Stage III. Research on genetic markers: VDR (rs1544410, rs2228570), RANKL (rs 9594738, rs9594759).

Stage IV. Development of an algorithm for preclinical diagnostics of bone metabolism disorders in children.

Clinical and anamnestic examination:

All parents of the examined children gave voluntary consent for the study. Taking into account the set goals and objectives, an individual registration card was developed, which was filled out during the study for each child. The examination began with the collection of questionnaire data (date of birth of a mother and a child, gender of the child, residential address, contact information for further monitoring of the child). The questioning of mothers was carried out according to an individual registration card, including age, the presence of chronic diseases and bad habits of the mother, medication intake during pregnancy, number of pregnancies and births, information about the current pregnancy and birth, the presence of additional intake of Vitamin D and Calcium. The clinical and anamnestic data of the child included: weight and height indicators at birth, the type of feeding in the first year of life, the stages of the introduction of complementary foods, the prevention of the Rickets. The assessment of physical development consisted of measuring the child's body weight, body length and a comparative analysis of the measured indicators with development standards using the centile scales developed by the WHO.

Laboratory examination of children:

During the work, to assess the state of bone metabolism, children underwent a biochemical blood test to determine the serum concentration of Calcium, Phosphorus, PTH, Osteocalcin, Vitamin D, Calcitonin, and Deoxypyridinoline in the urine.

In 2020, laboratory tests of patients were performed in the Invivo Clinical Diagnostic Laboratory at the address: Abylkhaiyr Khan Ave., 62/3, Aktobe city. The study was conducted as part of the intra-university project: "Clinical and genetic markers of Vitamin D status in newborns and young children in the Kazakh population" Order No. 13 / 2-18-124 dated 02/26/2020.

In 2021 laboratory tests were conducted at the Beauty and Health Center of the Aigerim Clinics, located at Maresyeva Street 87, as part of the intra-university project: “Clinical and genetic markers of bone metabolism in children of the Kazakh population” Order No. 13/2-18-303н/к dated 05/24/2021. Blood was collected in the treatment room of the relevant medical institution, in compliance with the generally accepted rules for collecting blood from a vein for its biochemical analysis.

During the study, biochemical studies of bone metabolism were carried out:

- Serum Calcium concentration was determined by the colorimetric photometric method on the BA 400 automatic biochemical analyzer, 2021. Reference values - 2.2-2.7 mmol / l.
- Serum Phosphorus concentration was estimated by a colorimetric method using ammonium molybdate on the BA 400 automatic biochemical analyzer, 2021. Reference values for Phosphorus in children under 12 years of age are 1.45–2.16 mmol/l.

- Osteocalcin content - by an immunochromatographic method on the Siemens Immulite 2000 Xpi analyzer. 2013. Reference values for osteocalcin are 2.8-41 ng/ml.
- Serum 25(OH)D content was determined using an immunochemiluminescent method on the Iflash analyzer/2022. Vitamin D status was assessed in accordance with the criteria established by the Institute of Medicine, 2011, USA [17]. 25(OH)D concentration (<21 ng/ml) - deficiency; (21-30 ng/ml) – insufficiency; (>30-75 ng/ml) – adequate concentration.
- Serum Calcitonin content by an immunochromatographic method on the Immulite 2000 Xpi analyzer. 2013. Reference values: less than 50 ng/l [18].
- Parathyroid hormone (PTH) concentration was determined using an immunochromatographic method on the Immulite 2000 Xpi analyzer. 2013. Reference values 15-65 pg/ml [18].
- Deoxypyridinoline concentration – by an immunochromatographic method on the Immulite 2000 Xpi analyzer. 2013. Reference values: 13.7-41.0 nmol/mmol [18].
- Genotyping of the VDR polymorphism (rs1544410, rs2228570), RANKL (rs 9594738, rs9594759) was carried out by real-time PCR on the DT-prime amplifier (DNA-technologies, Russia) using commercial reagent kits from TestGen LLC (Russia) by the fluorescence detection method based on degradable oligonucleotide probes using synthetic analogs of oligonucleotides [19,20].

Statistical methods of the study:

The data obtained during the study were combined into a single table in the Microsoft Excel 2016 program, which included information from individual registration cards and laboratory test results. The distribution pattern of quantitative variables was assessed using the Shapiro-Wilk test. To describe quantitative variables with a normal distribution, the arithmetic mean values of the indicators presented in the text were calculated as M±SD, where M is the arithmetic mean and SD is the standard deviation. In the absence of a normal distribution, the median and interquartile range were used; qualitative variables were presented as absolute frequencies and percentages. In the case of multiple independent differences in groups of quantitative variables, they were assessed using the Mann-Whitney U test; Kruskal-Walis rank analysis of variance and the median test. The Pearson Chi-square test was used to analyze qualitative variables, the measure of association was estimated using the Cramer V test, the odds ratio (OR) and 95% confidence interval (CI) were calculated. For all types of analysis, the $p<0.05$ level was considered statistically significant. Statistical data processing was performed using the calculator for genetic calculations using the Gen Expert program (http://gen-exp.ru/calculator_or.php). To study the risks of bone metabolism disorders with a combination of several factors, the Decision Tree method was used (construction method: exhaustive chaid, allowing the use of variables in nodes of several levels). The program used is IBMSPSS "STATISTICA 10.0" by StatSoft, Inc. and SPSS 25.

Main results of the study

Analysis of the anamnesis and the state of pre- and postnatal vitamin and mineral prevention in children of the surveyed groups revealed deviations in the

health of 80.4% of mothers. In the structure of maternal pathology, gestosis of the first half of pregnancy and anemia prevailed in 42.4% and 46.8%, respectively. Infectious and inflammatory diseases are in second place (37.6%). Prenatal prevention was carried out with vitamin-mineral complexes and Calcium preparations in 29.6% of cases. The high percentage of women - 55.6% (n = 139), who did not receive antenatal prophylaxis, is explained by its absence in clinical protocols. Postnatal prophylaxis with vitamin D was carried out in children in 40.4% (n = 101) of cases. Physical development: 69.2% of children corresponded to age; 19.6% - ahead; 11.2% - lagged behind the passport age. Physical development was harmonious in 75.6%, disharmonious - in 24.4%. According to the results of the study, the bone metabolism indices (Calcium, Phosphorus, Calcitonin, Osteocalcin) were distributed within the reference values. When comparing the indices using the Kruskal-Wallis method, reliable statistical differences were revealed between age groups 1 and 2, as well as 2 and 3 for Calcium, and between groups 1 and 2 for Phosphorus ($p<0.01$), statistical differences between groups 2 and 3 ($p<0.01$) for Calcitonin, as well as for Osteocalcin - between groups 1 and 2 ($p<0.01$) and 1 and 3 ($p<0.01$).

Vitamin D levels were determined in 250 children and the results revealed that in 78% of cases (n=195) (95% CI:72.9-83.1) vitamin D deficiency or insufficiency was detected. In the study, the average level was 24.23 ±1.6 ng/ml. The best indicators of Vitamin D provision were observed in children aged 6-12 months (29.22±1.9 ng/ml); the lowest indicators were in neonatal children (14.23±2.8 ng/ml).

The study included an analysis using the rank correlation coefficient to establish the relationship between bone metabolism parameters and Vitamin D levels. The results showed a "weak positive relationship" between Vitamin D and Calcium concentration (r=0.22), a "weak positive relationship" between Osteocalcin and Vitamin D (r=0.261), a "weak negative relationship between Parathyroid hormone levels and Vitamin D (r=-0.185), a "weak negative relationship between Calcitonin levels and Vitamin D (r=-0.175).

In accordance with the detected relationships between routine bone metabolism parameters and Vitamin D levels, we put forward a null hypothesis suggesting the possibility of an effect of Vitamin D levels on bone metabolism parameters. A comparative analysis was conducted between bone metabolism parameters (Ca, P, Calcitonin, Osteocalcin) and Vitamin D levels using the nonparametric Mann-Whitney method. Reliable statistical differences were obtained in the group of children with reduced Vitamin D levels in terms of Osteocalcin ($p \leq 0.01$), Phosphorus ($p \leq 0.01$), Calcium ($p \leq 0.01$) and PTH ($p \leq 0.01$), which confirms the effect of Vitamin D on metabolic processes in the skeletal system. A decrease in Calcitonin and Deoxypyridinoline (statistically insignificant) was established. Along with the effect of Vitamin D on mineral metabolism, its effect on bone tissue formation processes is also confirmed. It was established that the Osteocalcin content is a statistically significant sensitive indicator that responds to changes in Vitamin D levels. Thus, the obtained results

indicate that the Vitamin D level affects both mineral metabolism and bone tissue formation.

The second stage was to study the frequency distribution of allelic variants of the VDR, RANKL gene in children under one year old in the Kazakh population.

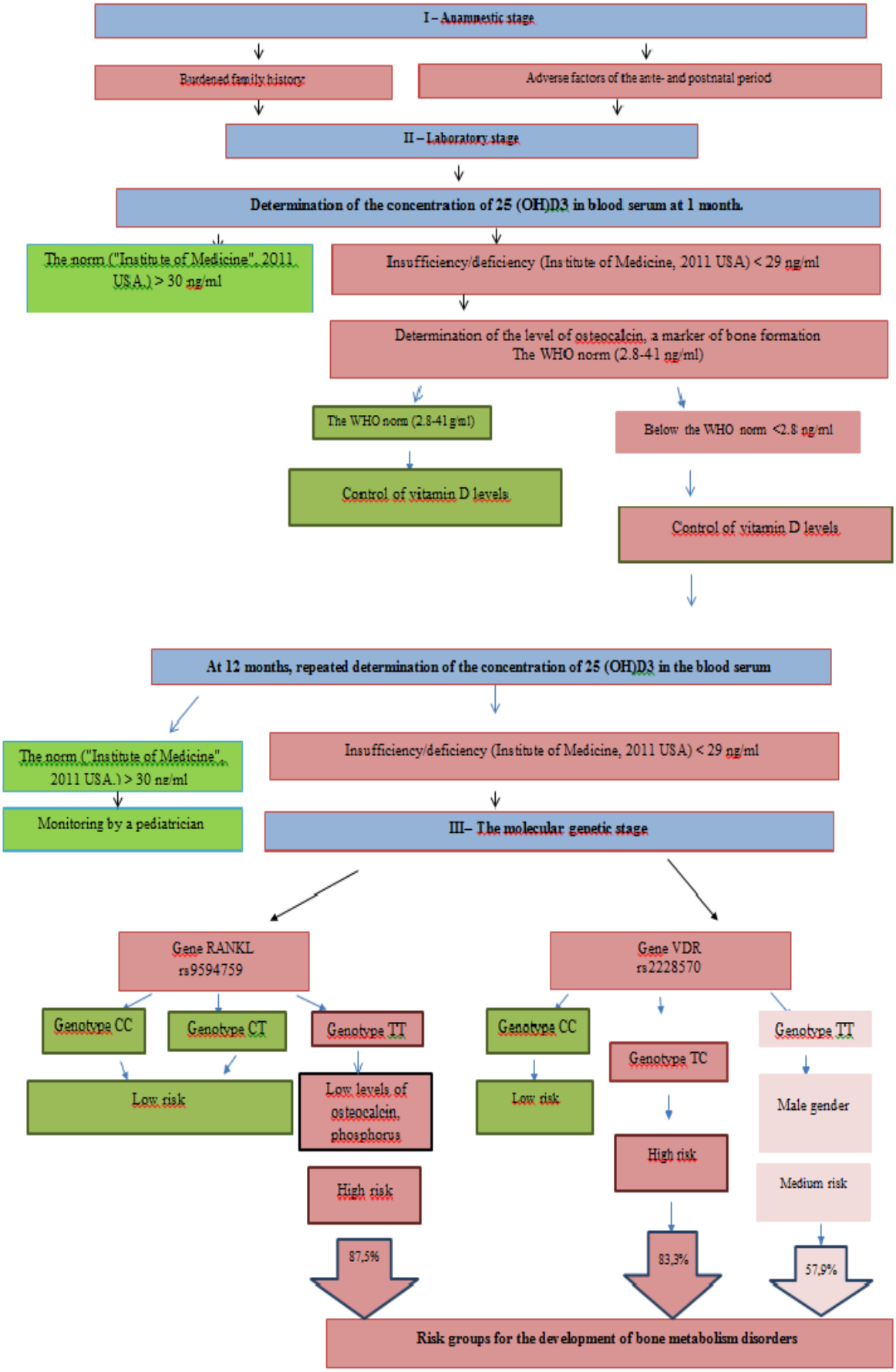
Distribution frequency of genotypes and alleles of the VDR rs2228570 gene polymorphism: TT-0.26, TC-0.28, CC-0.46, allele frequency T-0.51, C-0.68. In children under one year old of the Kazakh population, the CC genotype (46%) and the C allele (68%) of the VDR rs2228570 gene predominated. According to literatue sources, this polymorphism affects bone mineral density in children and often varies depending on race and nationality, which is confirmed by the results of the study.

Distribution frequency of genotypes and alleles of the VDR rs1544410 gene polymorphism: GG-0.59, GA-0.38, AA-0.03, allele frequency G-0.77, A-0.23. It was revealed that the GG genotype in children of the Kazakh population occurred in 59% of cases compared to the GA and AA genotypes, according to which the frequency of the G allele is more common than the A allele. The obtained results do not contradict the literature data, which indicate that the GG genotype is responsible for reducing the risk of low bone mineral density disorders, GA-intermediate risk, AA-increased risk.

During further examination of children under one year of the Kazakh population, the frequency of distribution of genotypes and alleles of the RANKL gene polymorphisms rs9594738 and rs9594759 was determined and the following results were obtained: CC-0.52, CT-0.18, TT-0.3, the frequency of alleles C-0.72, T-0.55 rs9594738 and CC-0.43, CT-0.31, TT-0.26, the frequency of alleles C-0.66, T-0.34. In children under one year of the Kazakh population, the CC genotype (52%) and the C allele (72%) of the RANKL rs9594738 gene prevailed. With the rs9594759 polymorphism, the CC genotype (43%) and the C allele (66%) were found in the same ratio. The results of the study are comparable with the literature data, since it is known that the rs 9594738 and rs 9594759 polymorphism of the RANKL gene increases susceptibility to changes in bone mineral density.

Next, the relationships between the levels of Vitamin D, Total Calcium, Phosphorus, Parathyroid hormone, Calcitonin, Osteocalcin, Deoxypyridinoline and the distribution of genotype frequencies of the VDR and RANKL genes were studied using the Pearson Chi-square test and the measure of the relationship was assessed using the CramerV criteria. A statistically significant relationship was found between the carriage of the GG genotype ($p<0.05$) of the VDRrs1544410 gene with a decrease in vitamin D content and phosphorus levels in children under one year of age in the Kazakh population. A statistically significant relationship was established between the presence of the CT genotype of the RANKL rs9594759 gene and the concentration of calcitonin in children under one year of age of the Kazakh population (the strength of the relationship according to Cramer V is 0.167).

One of the objectives of the survey was to develop an algorithm for preclinical diagnostics of bone metabolism disorders in children.


I – Anamnestic stage
Burdened family history
Adverse factors of the ante- and postnatal period
II – Laboratory stage
Determination of the concentration of 25 (OH)D3 in blood serum at 1 month.
The norm ("Institute of Medicine", 2011 USA.) > 30 ng/ml
Insufficiency/deficiency (Institute of Medicine, 2011 USA) < 29 ng/ml
Determination of the level of osteocalcin, a marker of bone formation
The WHO norm (2.8-41 g/ml)
The WHO norm (2.8-41 g/ml)
Below the WHO norm <2.8 ng/ml
Control of vitamin D levels
Control of vitamin D levels
At 12 months, repeated determination of the concentration of 25 (OH)D3 in the blood serum
The norm ("Institute of Medicine", 2011 USA.) > 30 ng/ml
Monitoring by a pediatrician
Insufficiency/deficiency (Institute of Medicine, 2011 USA) < 29 ng/ml
III– The molecular genetic stage
Gene RANKL rs9594759
Genotype CC
Genotype CT
Genotype TT
Low risk
Low levels of osteocalcin, phosphorus
High risk
87,5%
Gene VDR rs2228570
Genotype CC
Low risk
Genotype TC
High risk
83,3%
Genotype TT
Male gender
Medium risk
57,9%
Risk groups for the development of bone metabolism disorders

Conclusions:

1. Significant differences in the levels of bone metabolism indicators such as Total Calcium ($p < 0.001$), Phosphorus ($p < 0.01$), Calcitonin ($p < 0.001$), Osteocalcin ($p < 0.01$) and Vitamin D ($p < 0.001$) in different age groups were revealed.

2. It was found that the frequency of the GG genotype rs1544410 polymorphic variant of the VDR gene in children under one year of the Kazakh population is 59%. While, the frequency of the CC genotype rs9594759 polymorphism of the RANKL gene varies within 43%. A possible marker of an increased risk of developing bone metabolism disorders in children under one year of the Kazakh population is the presence of the GG genotype ($p < 0.05$) rs1544410 of polymorphism of the VDR gene.

3. A reliable average association was found between the carriage of the GG genotype ($p<0.05$) rs1544410 polymorphic variant of the VDR gene with reduced levels of Vitamin D and Phosphorus in children under one year old of the Kazakh population. A statistically significant average correlation was found between the presence of the CT genotype rs9594759 polymorphism of the RANKL gene and the concentration of Calcitonin in children of the same group.