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Calcium, creatinine and urinary phosphate/creatinine ratio concentrations in neonates of various gestational ages

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Objective: specify peculiarities of calcium and phosphates excretion in neonates of various gestational ages and types of feeding in neonatal period. **Patients and methods**. Calcium-creatinine (Ca/Cr) and phosphate-creatinine (P/Cr) ratio concentrations were determined in 96 healthy neonates of 38-40 weeks of gestational age and 146 premature infants of 28-37 weeks of gestational age of various types of feeding. **Results.** The Ca/Cr ratio concentration in healthy term infants in the early neonatal period amounted to 0.9-2.2 (median – 1.8), the P/Cr ratio concentration – 0.8-2.1 (median – 1.6). The Ca/Cr ratio concentration in premature infants (28-37 weeks of gestational age) amounted to 0.9-2.4 (median – 1.9), which is comparable to this parameter's value in term infants. The P/Cr ratio concentration amounted to 0.7-3.1 (median – 2.4), which exceeds this parameter's value in term infants. The lesser the gestational age and birth weight, the higher the Ca/Cr and P/Cr ratio concentrations. The authors revealed hypercalciuria and hyperphosphaturia in premature infants with a very low body weight fed with specialized formulas. **Conclusions.** Use of specialized formulas in small premature infants (gestational age < 33 weeks) with VLBW results in excessive calcium and phosphates excretion. It is reasonable to monitor their concentrations using a non-invasive and informative method of determining Ca/Cr and P/Cr ratios. Feeding of premature infants with BW > 1,500 g with breast milk only (in case of the mother's adequate lactation) allows avoiding hypercalciuria and hyperphosphaturia and preventing risk of a renal pathology.
Keywords*: neonates, premature infants, calcium excretion, phosphorus excretion, calcium-creatinine (Ca/Cr) ratio, phosphate-creatinine (P/Cr) ratio.*

Introduction

Rational infant feeding in the neonatal period is a pledge of a neonate's adequate growth and development not only in the small, but also in the mature age. Shift to artificial feeding is a "metabolic stress" for a child, as even the most modern artificial formula cannot fully replace breast milk [1].

Breast feeding is especially important in premature infants due to morphofunctional immaturity of organs and systems resulting in metabolic disorders, such as calcium and phosphorus imbalance.

A relatively high need of nutrient materials contradicts limited digestive capacity of premature infants: excessive delivery of proteins, inorganic salts and fluids may lead to renal functional disorders [2].

After premature delivery, breast milk features a distinctive composition, especially in terms of protein (1.86 ± 0.19 g/ml), phosphorus (15 mg / 100 ml) and calcium (30 mg / 100 ml) content, which is more adequate for premature infants' needs and their capacity for digestion, assimilation and homeostasis maintenance [3]. According to the current recommendations on feeding of premature infants, specialized formulas with high protein (2.03-2.5 g / 100 ml), calcium (87-120 mg / 100 ml) and phosphorus (47-73 mg / 100 ml) content ought to be used to ensure the body weight gain rate similar to the intrauterine one [1]. However, increase in the load on the functionally immature kidneys may result in a metabolic disorder, e.g., phosphorus-calcium imbalance. Such disorders may not feature any marked clinical manifestations in the neonatal period; this requires searching for methods of diagnosing imbalance of these electrolytes, which would be informative and non-traumatic for infants. The method of determining calcium-creatinine (Ca/Cr) and phosphate-creatinine (P/Cr) ratios in spot urine meets these requirements.

According to foreign researchers, Ca/Cr for 0-6-month-old children is 0.1-2.6; P/Cr – 1.4-20.0 [4]. According to G.P. Grushetskaya (2003), Ca/Cr ratio in term infants is 0.64-0.68, P/Cr ratio – 0.28-0.3 [5].

This study is aimed at specifying peculiarities of calcium and phosphates excretion in premature neonates depending on the type of feeding and regional distinctions.

PATIENTS AND METHODS

Calcium-creatinine and phosphate-creatinine ratios were determined in 242 neonates of various gestational ages.

Group I inclusion criteria:

Gestational age – 38-40 weeks (96 term infants), Apgar score – 7-9, breast feeding (BF). Morning urine was collected in the 4^{th}-7^{th} postnatal day to determine Ca/Cr and P/Cr ratios taking into consideration transient conditions of neonates, particularly of the urinary system.

Group II inclusion criteria:

Average gestational age – 33.3 ± 2.4 (28-37) weeks (146 premature infants), birth weight (BW) – more than 1,000 g. Random distribution of children by types of feeding: 29 children with BF (19.8%), 55 – with artificial feeding (AF) (37.7%), 52 – mixed feeding (MF) (35.6%); 10 children received fortified breast milk (FBM) (6.8%). Morning urine was collected in the 2^{nd}-3^{rd} postnatal week to determine Ca/Cr and P/Cr ratios in these children. Urine was collected in this period, as the most active development of canaliculi is observed in the 32^{nd}-36^{th} gestational week; therefore, it allows comparing the determined ratios with the ratios in term infants [6].

Exclusion criteria: hemodynamically significant fetal communications (clinical and instrumental test methods), malformations of organs and systems, genetic or endocrine disorder, organic CNS lesion, TORCH syndrome symptoms, grade II-III intraventricular hemorrhage, occlusive hydrocephalus, severe infectious diseases (sepsis, necrotizing enterocolitis) and hemolytic disease of the newborn.

Clinical description of the examined premature infants is given in tb. 1: very low birth weight (VLBW) – 36 (24.6%), low birth weight (LBW) – 95 (65.1%), weight of 2,500 g or more – 15 (10.3%).

The primary factor of the condition severity was cerebral ischemia; grade III ischemia was detected in children with VLBW, grade II ischemia – in children with LBW. Respiratory distress syndrome, bronchopulmonary dysplasia or intrauterine infection as the primary pathology were most often detected in children with VLBW.

Concurrent nosological entities included minor cardiac abnormalities (MCAs), hemodynamically insignificant functioning fetal communication (patent foramen oval, open arterial duct), conjugated jaundice (73-100% of cases), early anemia of prematurity and retinopathy.

In accordance with the developmental care standards, all the premature infants underwent complex treatment consistent with severity and character of the pathological process, including antibacterial, ethiopathogenetic, symptomatic and metabolic therapy and rehabilitation procedures. All the premature infants had been receiving preventive doses of vitamin D (1,000 IU) since the age of 3-4 weeks (depending on the gestational age at birth) (after urine collection for the purpose of determining Ca/Cr and P/Cr ratios).

Thus, analysis of clinical-anamnestic data and neonatal period course peculiarities demonstrated that the pathology, that would significantly affect calcium-phosphorus metabolism, was not present in the group of the examined premature infants.

Methods. We determined Ca/Cr and P/Cr ratios in order to assess calcium and phosphates excretion levels.

We determined levels of calciuria and phosphaturia in children of various gestational ages depending on the type of feeding (given higher calcium and phosphorus concentration in formulas than in breast milk) in order to assess advantages of breast milk over specialized formulas.

It ought to be mentioned that we did not achieve the normal (Gaussian) distribution at analysis of Ca/Cr and P/Cr ratios, which is why we excluded the cases (outliers) inconsistent with the representative sample. Ca/Cr was determined in 142 premature infants, P/Cr – in 138.

The 75th percentile of the calciuria level of a breast-fed premature infant (2.5) was taken as a provisional norm for hypercalciuria assessment on the basis of the BW and the gestational age.

The 75th percentile of the phosphaturia level in a breast-fed premature infant (2.1) was taken as a provisional norm for hyperphosphaturia assessment on the basis of the BW and the gestational age.

Urinary levels of natural metabolites were determined as follows: calcium – arsenazo method (reagent arsenazo III); phospates – reagent ammonium orthomolybdate kit; creatinine – Jaffe reaction with picric acid (apparatus Sapphire-400 [Tokyo Boeti Ltd, Japan]). Calcium-creatinine and phosphate-creatinine ratios were calculated by dividing Ca and P values (mmol/l) by the Cr value (mmol/l).

Statistical data manipulation was performed using a standard software package Statistica 6.0. Significance of differences between the analyzed groups of patients was assessed using parametric Student's t-test; significance of differences in parameter distribution between the groups (p) was assessed using χ^2 test. Histograms were constructed in order to determine 25th-75th percentiles of Ca/Cr and P/Cr; they were considered representative if each cell contained at least 5 cases. In order to eliminate random errors in the initial data, we performed local 7-point smoothing of histograms with even cell distribution using the ordinary least squares method with a polynomial of degree 3. After that, we calculated the cumulative curve containing 25th-75th percentiles of Ca/Cr and P/Cr. In order to assess correlation of Ca/Cr and P/Cr with gestational age, BW, calcium and phosphorus concentration in breast milk and specialized artificial formulas, we performed Pearson's linear correlation analysis and calculated the corresponding coefficient (r). $R > 0.13$ was significant for total sample ($n = 242$) at $p < 0.05$. $R > 0.17$ was significant for the group of premature infants ($n = 146$) at $p < 0.05$ [7]. Differences in parameters were considered significant at probability level $p < 0.05$.

It ought to be mentioned that we did not achieve the normal (Gaussian) distribution at analysis of Ca/Cr and P/Cr ratios, which is why we excluded the cases (outliers) inconsistent with the representative sample. Ca/Cr was determined in 142 premature infants, P/Cr – in 138.

STUDY RESULTS AND DISCUSSION

The data on mean values (medians) and interquartile range (IQR) between 25th-75th percentiles of Ca/Cr and P/Cr ratios in the group of breast-fed term infants (gestational age – 38-40 weeks) are given in tb. 2.

Ca/Cr and P/Cr rates were close, i.e. calcium and phosphorus excretion levels are almost the same in healthy term infants. The obtained parameters are consistent with the data given in foreign literature on 0-6-month-old children and are different from the data obtained by G.P. Grushetskaya (2003); in our opinion, these differences are connected with regional

peculiarities – Orel Region is endemic in terms of urolithiasis due to the increased iron concentration in water and water hardness [8].
The data on mean values (medians) and the IQR between 25^{th}-75^{th} percentiles of Ca/Cr and P/Cr ratios in the group of premature infants are given in tb. 3. The obtained Ca/Cr and P/Cr ratios are similar to such ratios in the group of term infants (pic. 1, 2).
Significant differences regarding Ca/Cr ratios between the groups of term and premature infants were not revealed (see pic. 1). Comparison of P/Cr ratios helped to reveal a higher phosphaturia level in the group of premature infants (see pic. 2).
Calciuria levels – both mean values and the IQR between 25^{th}-75^{th} percentiles – were almost the same at different types of feeding ($p_{1, 2, 3} > 0.05$; pic. 3). P/Cr ratios – both mean values and the IQR between 25^{th}-75^{th} percentiles – were higher when specialized formulas were used instead of breast feeding ($p_{1, 2} = 0.008$, $p_{1, 3} = 0.02$, $p_{2, 3} = 0.03$; pic. 4).
Correlation analysis revealed the following statistically significant correlations of Ca/Cr ration with P/Cr ratio regarding gestational age, BW and calcium and phosphorus concentration in breast milk and specialized artificial formulas for premature infants:
1) negative correlation of Ca/Cr and P/Cr ratios with gestational age ($r = -0.20$, $p = 0.002$ and $r = -0.20$, $p = 0.002$, respectively) and BW ($r = -0.14$, $p = 0.03$ and $r = -0.24$, $p = 0.0002$), i.e. the lower the gestational age and BW, the higher the calcium and phosphates excretion;
2) positive correlation of calcium/phosphorus concentration in specialized formulas with *gestational age and P/Cr in the event of joint correlation thereof* ($r = 0.18$, $p < 0.05$), i.e. high phosphaturia rates were observed in the premature infants fed with specialized formulas with higher calcium ad phosphorus concentration than in breast milk.
No significant difference was observed in hypercalciuria rate depending on the BW; however, a tendency to hypercalciuria reduction is observed in the children with BW > 1,500 g, especially if these children are fed artificially (tb. 4).
Hypercalciuria rate in the group of small premature infants is significantly higher at AF than at BF (tb. 5).
Hyperphosphaturia rate in the group of breast-fed children > 1,500 g was higher than in the group of artificially- ($p_{1, 2} = 0.008$) and mixed-fed ($p_{1, 3} = 0.007$) children. Hyperphosphaturia rate in the group of VLBW breast-fed children cannot be reliably assessed due to the small size of the sample ($n = 2$; tb. 6).
The hyperphosphaturia rate analysis revealed the following with regard to gestational age: hyperphosphaturia rate in small premature infants is significantly less often at BF than at AF and MF; a tendency to hyperphosphaturia rate reduction as gestational age increases is observed in the premature infants fed with specialized formulas (tb. 7).

Conclusion

Use of specialized formulas in VLBW small premature infants (gestational age < 33 weeks) results in the increased excretion of calcium and phosphates, the level whereof is reasonable to monitor using a non-invasive and informative method of determining Ca/Cr and P/Cr ratios. It has been proven that premature infants with BW > 1,500 g may be fed with breast milk only (in case of the mother's adequate lactation); it allows avoiding hypercalciuria and hyperphosphaturia and preventing risk of a renal pathology.

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Table 1. Somatic and neurological status of premature infants (n = 146)

Diseases / **Birth weight**	**1,000-1500 g (n = 36)**		**1,501-2500 g (n = 95)**		**> 2,501 g (n = 15)**	
	Abs.	%	Abs.	%	Abs.	%
Cerebral ischemia:						
Grade I	-	-	28	29.5	3	20
Grade II	14	38.9	58	61.1	12	80
Grade III	22	61.1	9	9.5	-	-
Respiratory distress syndrome	17	47.2	9	9.5	2	13.3
Bronchopulmonary dysplasia, pneumonia, obstructive syndrome	5	13.9	8	8.4	-	-
Intrauterine infections of undefined origin	5	13.9	5	5.3	-	-
MCAs, functioning fetal communication	36	100	88	92.6	14	86.7
Conjugated jaundice	29	80.6	86	90.5	11	73.3
Neonatal retinopathy	19	52.8	7	7.4	-	-
Grade II-III early anemia of the newborn	26	72.2	14	14.7	-	-
Congenital dacryocystitis, conjunctivitis	1	2.8	6	6.3	4	26.6
Omphalocele, bubonocele	9	25.0	7	7.4	-	-
Hydrocele	4	11.1	4	4.2	-	-

Note. MCAs – minor cardiac abnormalities.

Table 2. Ca/Cr and P/Cr ratios in the group of term infants (n = 96)

Parameters	**Ca/Cr**	**P/Cr**
Mean values (medians)	1.8	1.6
Interquartile range	0.9-2.2	0.8-2.1

Table 3. Ca/Cr and P/Cr ratios in the group of premature infants

Parameters	**Ca/Cr**	**P/Cr**
Mean values (medians)	1.9	2.4
Interquartile range	0.9-2.4	0.7-3.1

Table 4. Hypercalciuria rate depending on birth weight and type of feeding

Type of feeding	Birth weight	1,000-1500 g (n = 24)		1,501-2500 g (n = 98)		> 2,500 g (n = 14)	
		Abs.	%	Abs.	%	Abs.	%
Breast feeding, n = 29 (p_1)		-/2*	-	6/19	31.6	2/8	25.0
Artificial feeding, n = 54 (p_2)		9/17	53.0	6/36	16.7	-/2	-
Mixed feeding, n = 52 (p_3)		1/5	20.0	11/43	25.6	1/4	25.0

Note. * - number of hypercalciuria cases / number of children with specific type of feeding and BW; $p_{1, 2, 3} > 0.05$.

Table 5. Hypercalciuria rate depending on gestational age and type of feeding

Type of feeding	Gestational age	28-32 months (n = 44)		33-34 months (n = 37)		> 35 months (n = 55)	
		Abs.	%	Abs.	%	Abs.	%
Breast feeding, n = 29 (p_1)		1/8*	**12.5**	3/5	60.0	4/16	25.0
Artificial feeding, n = 54 (p_2)		11/21	**52.4** **$p_{1.2} = 0.03$**	4/15	26.7	-/19	-
Mixed feeding, n = 52 (p_3)		4/15	26.7	4/17	23.5	5/20	25.0

Note. * - number of hypercalciuria cases / number of children with specific type of feeding and gestational age; $p_{1, 2, 3} > 0.05$. Explanatory notes present in tables 5-7 to the values in bold are given in the text.

Table 6. Hyperphosphaturia rate depending on birth weight and type of feeding

Type of feeding	Birth weight	1,000-1500 g (n = 24)		1,501-2500 g (n = 98)		> 2,500 g (n = 14)	
		Abs.	%	Abs.	%	Abs.	%
Breast feeding, n = 28 (p_1)		1/2*	50.0	2/19	**10.5**	1/8	12.5
Artificial feeding, n = 50 (p_2)		10/17	58.8	12/36	**33.3** **$p_{1.2} = 0.008$**	-/2	-
Mixed feeding, n = 51 (p_3)		2/5	40.0	16/43	**37.2** **$p_{1.3} = 0.007$**	2/4	50.0

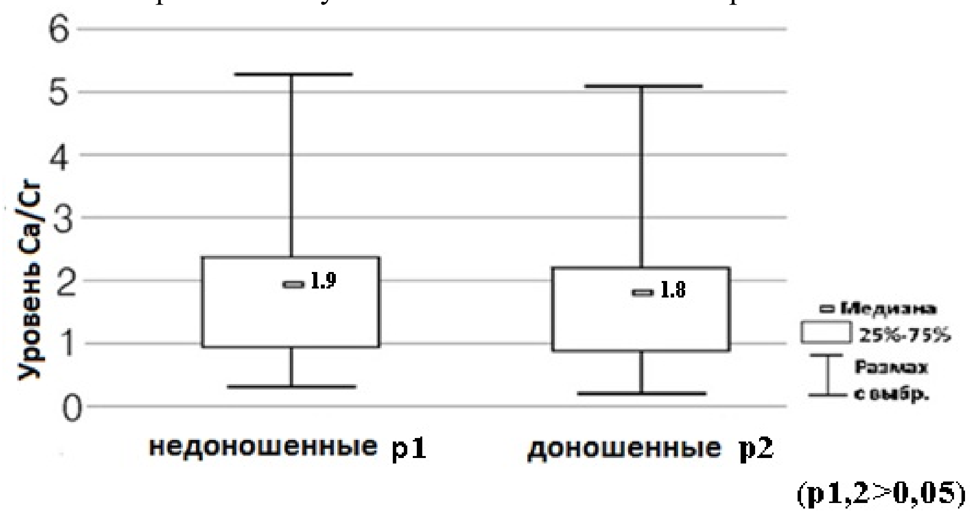
Note. * - number of hyperphosphaturia cases / number of children with specific type of feeding and BW.

Table 7. Hyperphosphaturia rate depending on gestational age and type of feeding

Type of feeding	Gestational age	28-32 months (n = 44)		33-34 months (n = 37)		> 35 months (n = 55)	
		Abs.	%	Abs.	%	Abs.	%
Breast feeding, n = 28 (p_1)		1/8*	**12.5**	1/5	20.0	2/16	**12.5**
Artificial feeding, n = 50 (p_2)		14/21	**66.7** **$p_{1.2} = 0.003$**	4/15	26.7	4/19	**26.7** **$p_{1.2} = 0.04$**
Mixed feeding, n = 51 (p_3)		9/15	**60.0** **$p_{1.3} = 0.005$**	5/17	29.4	6/20	**30.0** **$p_{1.3} = 0.03$**

Note. * - number of hyperphosphaturia cases / number of children with specific type of feeding and gestational age.

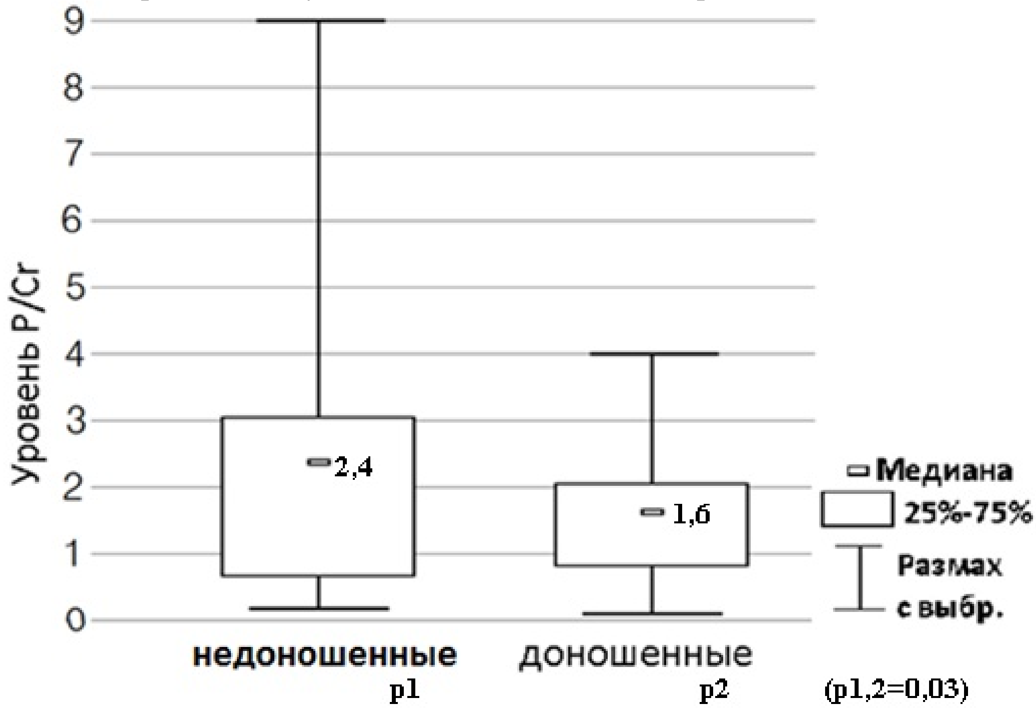
Pic. 1. Comparative analysis of Ca/Cr ratios in term and premature infants*



Уровень Ca/Cr	*Ca/Cr ratio*
недоношенные p1	*Premature infants p1*
доношенные p2	*Term infants p2*
Медиана	*Median*
Размах с выбр.	*Sample range*

Note. * - the number of outliers for Ca/Cr among premature infants was 4.

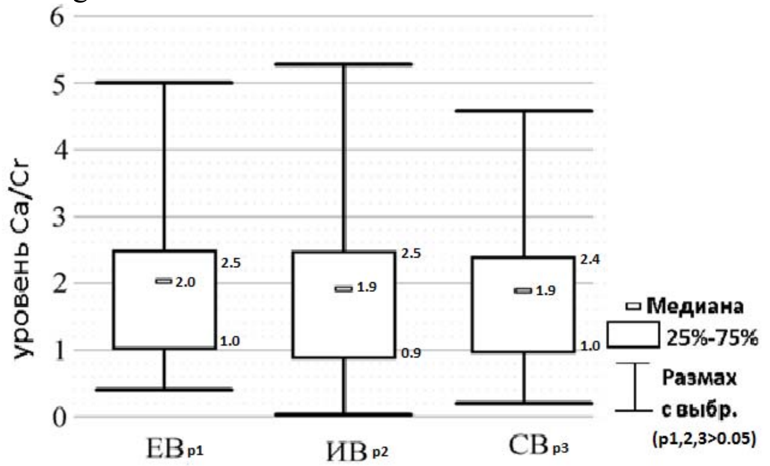
Pic. 2. Comparative analysis of P/Cr ratios in term and premature infants



Уровень P/Cr	*P/Cr ratio*
недоношенные p1	*Premature infants p1*
доношенные p2	*Term infants p2*
Медиана	*Median*
Размах с выбр.	*Sample range*

Note. * - the number of outliers for P/Cr among premature infants was 8.

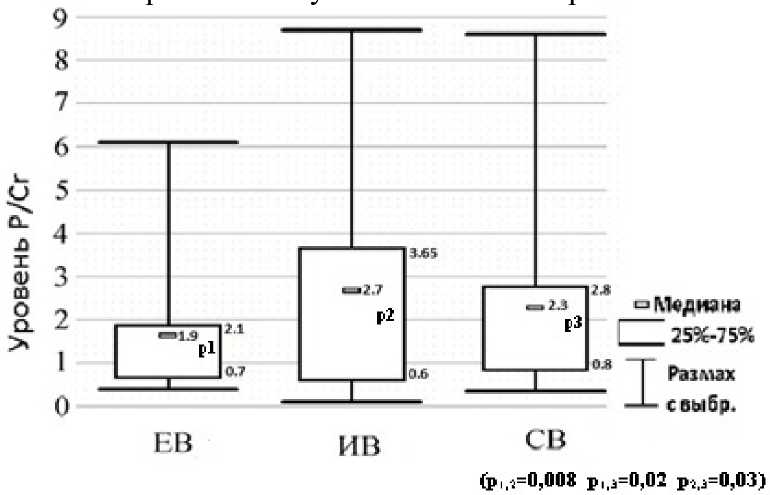
Pic. 3. Comparative analysis of Ca/Cr ratio in premature infants depending on the type of feeding*



Уровень Ca/Cr	Ca/Cr ratio
$ЕВ_{p1}$	*BF_{p1}*
$ИВ_{p2}$	*AF_{p2}*
$СВ_{p3}$	*MF_{p3}*
Медиана	*Median*
Размах с выбр.	*Sample range*

Note. * - the number of outliers for Ca/Cr at breast feeding (BF) – 0; at artificial feeding (AF) - 1; at mixed feeding (MF) – 1.

Pic. 4. Comparative analysis of P/Cr ratio in premature infants depending on the type of feeding*



Уровень P/Cr	*P/Cr ratio*
ЕВ	*BF*
ИВ	*AF*
СВ	*MF*
Медиана	*Median*
Размах с выбр.	*Sample range*

Note. * - the number of outliers for P/Cr at breast feeding (BF) – 1; at artificial feeding (AF) - 5; at mixed feeding (MF) – 1.