

ORIGINAL ARTICLE

CRYSTALLOGRAPHIC INVESTIGATION OF URINE IN NEWBORN WITH RENAL DISTURBANCE DUE TO ASPHYXIA

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ABSTRACT

The aim is to increase the efficiency of diagnosis of renal injury in neonates with asphyxia by identifying of structural markers according to research facies of urine in newborns of different gestational ages.

Materials and methods: The study involved 150 full-term with signs of kidney damage due to asphyxia: 75 babies with severe asphyxia, and 75 children with moderate asphyxia and 100 preterm infants: 50 children with severe asphyxia and 50 children with moderate. Comparison groups: group 1 consisted of 20 full-term infants, group 2 which included 20 preterm neonates. Material for the study – morning portion of urine, which was collected at 8-10 a.m. on 1-2 and 7-8 days of life.

Results: morphological picture of facies of newborns with asphyxia depends on the severity of pathological changes in the urine (proteinuria) and urine output. Structure of facies in babies with renal disturbance due to severe asphyxia indicates a significant loss of organic and mineral substances in the urine. The width of the peripheral zone facies, the amount of solid particles transferred depends on the severity of asphyxia, the difference in morphology facies is maintained even at the end of the early neonatal period.

Conclusions: Analysis of dried drops of urine in infants with renal impairment on the background of asphyxia can be used as one of the criteria for assessing kidney function and have prognostic value.

KEY WORDS: asphyxia, nephropathy, newborns, preterm infant

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INTRODUCTION

Growing interest of researchers for perinatal nephrology is due to increasing of number of congenital and hereditary nephropathy in infants, chronic kidney diseases and high level of disability due to children undergoing pathology in the neonatal period, latent lesions of the urinary tract in infants [1, 2]. The main factor in formation of pathology of infants is hypoxia. Hypoxia leads to redistribution of fetal blood flow with a primary blood supply to vital organs and peripheral vascular spasm, especially of renal vessels. [1]. Frequency of asphyxia in newborns ranges from 1 to 1.5% in different countries. Its presence and severity depends on gestational age and birth weight. Asphyxia damages all organs and tissues of a newborn baby, but most often affects the kidneys – 50%, CNS – 28%, cardio – vascular (25%) and respiratory system (23%) [3]. According to other researchers the frequency of kidney damage due to asphyxia can be up to 70% [2, 4]. The most common pathology in neonatal period is transient renal failure (ischemic nephropathy, toxic-ischemic nephropathy, kidney shock, transient renal failure), which under adverse conditions can lead to the development of acute renal failure. [1, 3]. Kidneys are very sensitive to the deficit of oxygen. Renal dysfunction can occur within 24 hours after an episode of ischemia and may provoke the development of cortical necrosis [3]. Early diagnosis of renal dysfunction is important for infants born in asphyxia as

allows to correct fluid and electrolyte disturbances in time. Diagnosis of renal neonatal asphyxia is difficult because of the lack of specific clinical symptoms and lack of informativeness of traditional survey methods [5, 6]. Relevance of the study determined the lack of highly sensitive and at the same time, the available non-invasive diagnostic methods for early detection of kidney damage in newborns.

THE AIM

The aim was to increase the efficiency of diagnosis of renal injury in neonates with asphyxia by identifying of structural markers according to research facies of urine in newborns of different gestational ages.

MATERIALS AND METHODS

The study involved 150 full-term infants with gestational age 38-41 weeks and signs of kidney damage due to asphyxia: 75 babies who have suffered from severe asphyxia, and 75 children with moderate asphyxia. Additionally, surveyed 100 preterm infants with gestational age $33,5 \pm 0,52$ (27-36) weeks with the signs of nephropathy due to asphyxia: 50 children who have suffered from severe asphyxia (gestational age $31,9 \pm 0,68$ (27-35) weeks.) and 50 children with moderate asphyxia (gestational age $35,1 \pm 0,31$ (34-36) weeks).

From the infants who had no asphyxia at birth, was formed two comparison groups: group 1 consisted of 20 full-term infants (gestational age $39,8 \pm 0,23$ (39-41) weeks.), group 2 which included 20 preterm infants (gestational age $35,7 \pm 0,26$ (35-37) weeks.).

Renal disease was diagnosed in case of significant renal dysfunction – the level of creatinine in plasma over 89 mmol / L, the level of urea in plasma more then 8 mmol / L, oliguria (urine output less than 1 mL / kg / h). The diagnosis of moderate and severe asphyxia established by the diagnostic criteria specified in the order of Ministry of Health of Ukraine from 08.06.2007 № 312 «On approval of clinical protocols for resuscitation and newborn care Postresuscitation.»

Material for the study was the morning portion of urine, which was collected at 8-10 a.m. on 1-2 and 7-8 days of life.

Facies of urine obtained by the following procedure. On the horizontal slide-fat deposited drop of biological fluid volume of 0.01 ml. In this volume are given the necessary parameters: the angle of curvature of the drop surface is 25-30°, the diameter of the droplets is 3-5 mm, the average thickness – about 1 mm. Within 18-24 hours at 20-25 °C and relative humidity of 65-70% a sample was drying and then examined under the microscop. In the process of

dehydration observed a number of processes that lead to the formation of facies with a certain structure.

Detecting of morphological changes was performed in dried drops by microscopic examination using a light microscope in ordinary light and dark field at 40-fold magnification. Photography was performed with a digital system image selection «SEO SCAN Lab IEX 285AK-F IEE – 1394» (Ukraine). For further image processing we used program AxioVision LE 4.8.2.0 (Carl Zeiss MicroImaging GmbH) and PhotoM 1.21 (A. Chernigovskij).

In addition to the description of the morphological characteristics of facies we performed mathematical calculations of dried drops – determined the total number of crystals and their area, which allowed to apply statistical methods in the analysis of facies.

Statistical analysis of results carried out with a program Microsoft Excel. We used statistics variation methods which are suitable for medical and biological research. For all parameters measured the average (M), the average error (m). Using the Student's criterion (t) determined reliability index (R). The difference was considered probable at $p < 0.05$.

This study was approved by the ethics committee of the Sumy State University, Sumy Ukraine. All procedures were carried out in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration (6th, rev., 2008, Seoul) and the Universal Declaration on Bioethics an Human Rights (2006) as key documentation considering ethical decision making and conceptualization for the article's scientific content.

RESULTS

CRYSTALLOGRAPHIC FEATURES OF URINE OF TERM INFANTS WITH RENAL IMPAIRMENT DUE TO ASPHYXIA

In the study of urine of healthy term infants revealed a tendency to amorfyzation of facies (low salt crystals) and lack of distribution on the boundary and central zones are represented in Fig. 1.

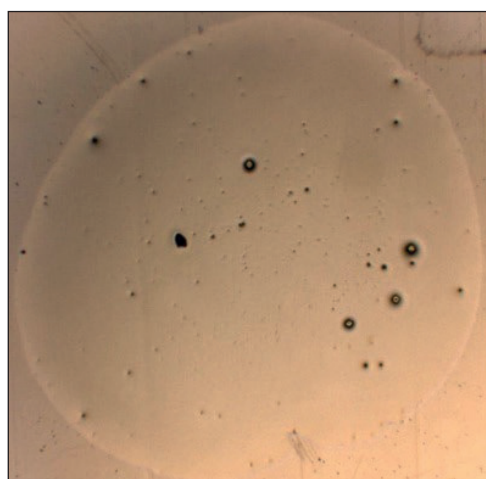


Fig 1. Facies of urine of term infant without asphyxia

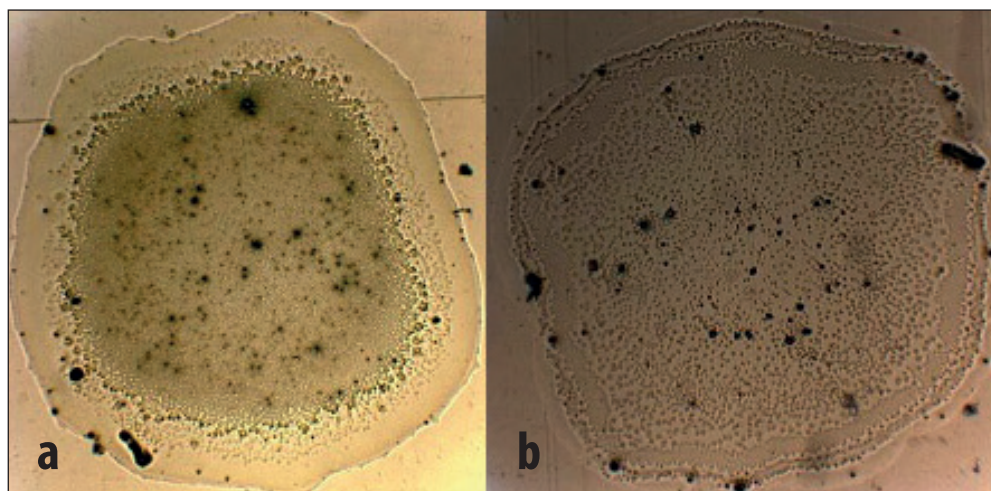
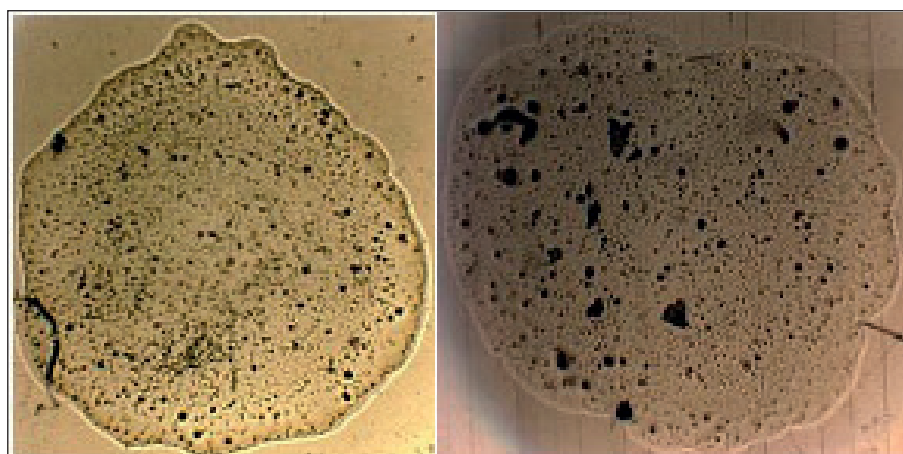
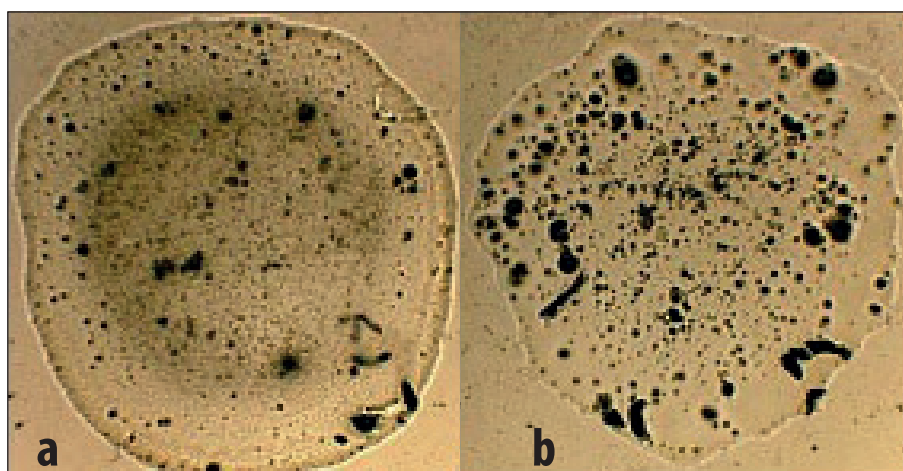


Fig2. Facies of urine of infants with renal disturbance due to moderate asphyxia

Table I. Dynamics of morphological picture of urine facies in term infants with renal impairment due to asphyxia

	1-2 day		7-8 day	
	Number of inclusions	The total area of inclusions, %	Number of inclusions	The total area of inclusions, %
Newborns with kidney disturbance due to moderate asphyxia, n= 75	52,7±3,32 p	3,1±0,47 p	48,3 ± 3,54 p	2,98 ± 0,63 p
Newborns with kidney disturbance due to severe asphyxia, n= 75	102,17±8,2 p, p ₁	6,42±0,54 p, p ₁	87,81±7,36 p, p ₁	5,03±0,68 p, p ₁
Comparison group, n= 20	11,9 ± 1,75	0,81 ± 0,11	9,22 ± 0,89	0,61 ± 0,09

Notes: p – significantly difference of indexes relative to the comparison group;
p₁ – significantly difference of indexes relative to the newborns with moderate asphyxia;

**Fig 3.** Facies of urine of infants with renal disturbance due to severe asphyxia at 1-2 days of life.**Fig 4.** Facies of urine of infants with renal disturbance due to severe asphyxia at 7-8 days of life.

We found small, medium-sized and large circular shape crystals. There was a special distribution of crystals: at the periphery facies they met frequently and were of small size, approaching the center drop number of crystals decreased and their size increased. At 1-2 days of life the number of particles ranged from 3 to 25 per facies ($11,9 \pm 1,75$), and their total area was $0,81 \pm 0,11\%$. During the early neonatal period significant changes in the above quantitative indicators were not observed.

A small number of crystals in drops of urine in term infants without asphyxia is due to the low loss of salts and

organic molecules (proteins) in physiological conditions, leading to a reduced ability of urine to the formation of organic-mineral textures (Fig. 2).

In neonates with renal impairment due to moderate asphyxia we revealed a clear division of facies into zones, central, transitional and peripheral. The width of the peripheral zone was $8,2 \pm 1,15\%$ of the radius of the nodules, the width of the transition zone was $11,1 \pm 0,95\%$. The central zone was close-grained.

Facies contained small, medium-sized and large rounded, elongated or irregular shape crystals. Most inclusions

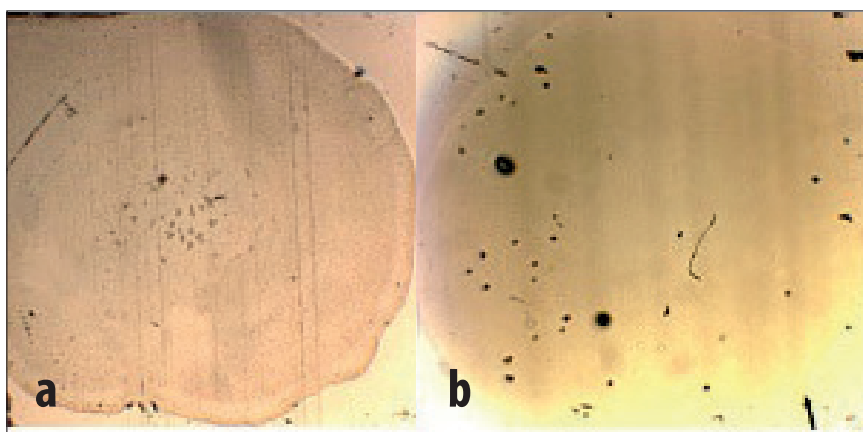


Fig 5. Facies of urine of premature newborns without asphyxia at 1-2 and 7-8 days of life

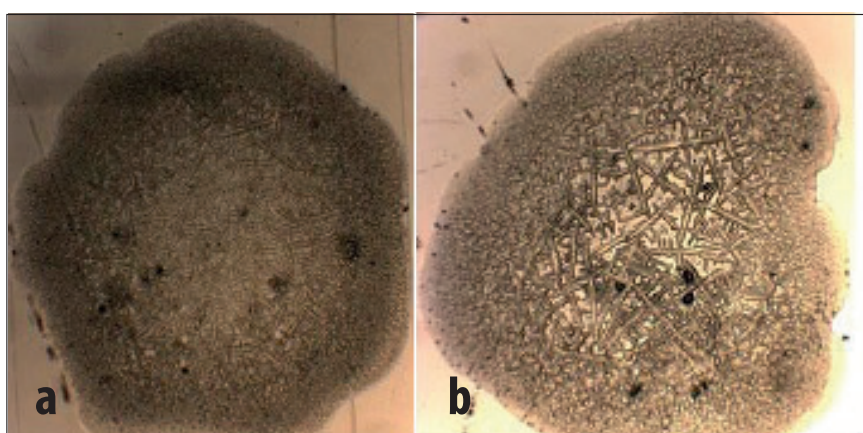


Fig 6. Facies of urine of premature infants with renal disturbance due to moderate asphyxia at 1-2 and 7-8 (6) days of life

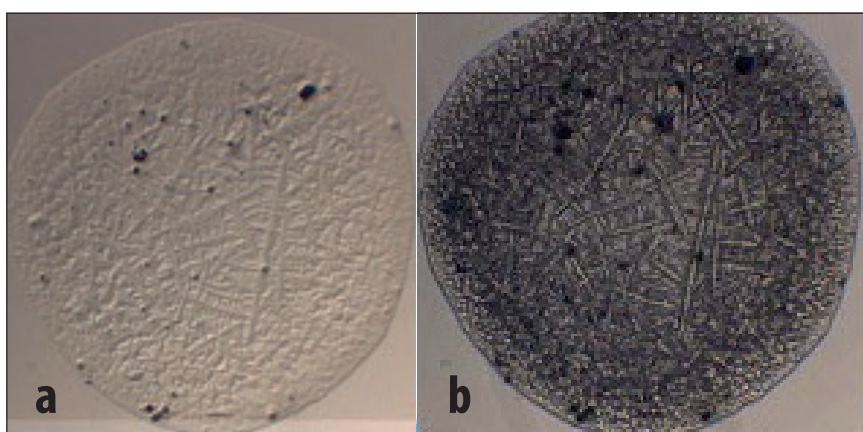


Fig 7. Facies of urine of premature infants with renal disturbance due to severe asphyxia at 1-2 days of life



Fig 8. Facies of urine of premature infants with renal disturbance due to severe asphyxia at 7-8 days of life.

located in the central zone of drop, transition and peripheral zones had only a few inclusions. Their number at 1-2 days of life ranged from 40 to 70 per facies ($52,7 \pm 3,32$), and their total area was $3,1 \pm 0,47\%$. Within 1 week of life there were no significant changes of these parameters.

Thus asphyxia leads to the disturbance of kidney function and the process of filtration, reabsorption and secretion. This leads to falling of organic components and minerals into urine with their crystallization and formation of specific picture (Table I).

In infants who had signs of renal impairment on the background of severe asphyxia facies can be divided into central and peripheral zones only conditionally. The width of the peripheral zone was $5,2 \pm 0,57\%$ of the radius of the nodules. The structure of the central zone in most cases had

Table II. Dynamics of morphological picture of urine facies in preterm infants with renal impairment due to asphyxia

	1-2 days of life		7-8 days of life	
	Number of inclusions	The total area of inclusions, %	Number of inclusions	The total area of inclusions, %
Premature infants with renal disturbance due to moderate asphyxia, n= 50	184,2±10,3 p, p ₁	50,35±2,71 p, p ₁	79,7 ± 5,42 p, p ₁ , p ₃	25,63 ± 2,03 p, p ₁ , p ₃
Premature infants with renal disturbance due to severe asphyxia, n= 50	159,7±12,8 p, p ₁	33,38±2,45 p, p ₁ , p ₂	149,15±9,06 p, p ₁ , p ₂	37,23±3,11 p, p ₁ , p ₂
Comparison group 1, n= 20	11,9 ± 1,75	0,81 ± 0,11	9,22 ± 0,89	0,61 ± 0,09
Comparison group 2, n= 20	15,35±0,62	0,74± 0,02	17,43±1,04 p	0,87± 0,07 p

Notes: p – significantly difference of indexes relative to the comparison group 1;

p₁ – significantly difference of indexes relative to the comparison group 2;

p₂ – significantly difference of indexes relative to the newborns with moderate asphyxia;

p₃ – significantly difference of indexes relative to the 1-2 days of life.

close-grained character, but we met plot gap of facies. A number of children had pigment in the central part of facies, which could be formed during the passage of unconjugated bilirubin through the kidney filter on the background of hyperbilirubinemia and imperfect renal function (Fig. 3).

We revealed two main types of distribution of inclusions in this group of patients. The symmetrical radial distribution of inclusions was formed at low proteinuria (Fig. 3a) with a predominance of small and medium-sized round or elongated crystals. Asymmetric distribution type was formed at significant proteinuria, especially in combination with oliguria (Fig. 3b), in which the central area contained the inclusion of various sizes and shapes, which concentrated mainly near one of the poles of the drop.

The number of inclusions in lesions of the kidneys due to severe asphyxia at 1-2 days of life ranged from 50 to 150 per facies (102,17 ± 8,2), and their total area was 6,2 ± 0,54%.

By the end of the early neonatal period facies structure of some children approached to that at moderate asphyxia – division into zones was appeared, decreased the number of inclusions, etc. (Fig. 4a). The remaining infants were kept pathological changes indicated by 1-2 days of life (Fig. 4b). The number of particles ranged from 40 to 150 per facies (87,81 ± 7,36), and their total area was 5,03 ± 0,68% (Fig.4).

CRYSTALLOGRAPHIC FEATURES OF URINE OF PRETERM INFANTS WITH RENAL IMPAIRMENT DUE TO ASPHYXIA

Preterm infants of comparison group, as opposed to full-term, had the distribution of facies into the marginal and central zones. Drops contained cruciate form crystalline inclusion only in the central zone. At 1-2 days of life the number of particles ranged from 10 to 19 per facies (12,35 ± 0,62), and their total area was 0,74 ± 0,02% (Fig.5).

By the end of the early neonatal period morphological picture of facies of premature infants approached to the full-term infants. We noted amorfization of facies, lossing

of separation of the boundary and central zones, form crystals became round or oval, but were located predominantly in the periphery. The total number of inclusions in facies and their area was significantly higher than rates in term infants. Higher propensity for crystal formation in premature infants may be associated with an increased loss of salt concentration on the background of the imperfections of renal function (Fig.6).

Preterm infants with renal disturbance due to moderate asphyxia at 1-2 days of life had division of facies into zones: central, transitional and peripheral. The width of the peripheral zone was 11,4 ± 0,95% of the radius of the drop, the width of the transition zone was – 17,4 ± 0,82%. Peripheral and transition zones had close-grained character, while a typical crystal formation was observed in the central zone. The crystals had the shape of a cross and occupied the area about half of facies. Against the background of a homogeneous structure of drops we noted the presence of a dense rounded form structures that were located mainly in the central area, the average number of them was 11,8 ± 0,75.

Thus, the renal tissue hypoxia is able to deepen morphological immaturity of kidney function of preterm infants, and leads to greater loss of salts and organic molecules with urine and increasing of crystal formation in facies.

At the end of the early neonatal period structure drops was slightly changed. The facies division into zones was present, but the width of the peripheral zone decreased by 2 times to 5,9 ± 0,27% of the radius of the drop, the transition zone became wider and reached 28,6 ± 1,9%. Cruciate form crystals localized in the central zone. They were larger than at birth, and formed a general picture of the type «fern leaf». Due to the larger size of crystal total area of particles significantly decreased to 25,63 ± 2,03%. Number of denser structures in drops also decreased (7,6 ± 0,23).

Thus, at moderate asphyxia at the end of the 1st week of life we marked decreasing of crystal formation, which may indicate the early resumption of reabsorption and secretion in the epithelium of the renal tubules (Tabl. II).

The facies of premature infants with the signs of kidney disturbance due to severe asphyxia at 1-2 days of life can be divided into central, transitional and peripheral zones. The width of the peripheral zone ($16,1 \pm 1,1\%$ of the radius of the drop) is almost equal to the width of the transition ($16,7 \pm 0,9\%$ of the radius of the drop). The peripheral zone had close-grained character, and transitive – cryptocrystalline. The central part of large cross-drops contained crystals with ramifications that formed a picture «fern leaf». Crystals occupied area over a third facies. The formation of dense rounded form was present in the central and peripheral zones, the average of them was $19,2 \pm 1,37$. During the 1st week of life premature infants in this group had no significant changes in morphometric parameters (Fig.7).

Thus, the development of nephropathy in the background of severe asphyxia accompanied by proteinuria, revealed by the widest peripheral zone facies in infants as organic compounds dehydration displaced to the periphery. A large number of solid crystals round shape indicates a significant loss of salts in the group on the background premature disruption of reabsorption and secretion in the renal tubule (Fig.8).

Significant stress of kidney function in premature infants on the background of anatomy – functional immaturity causes differences in the structure of facies of term newborns.

DISCUSSION

So, morphological picture of facies of newborns with asphyxia depends on the severity of pathological changes in the urine (proteinuria) and urine output. The morphology of facies of urine for 1-2 days can predict the presence of proteinuria and oliguria in the newborn in serious condition.

Asphyxia has a negative effect on the kidney so urine facies picture in premature infants with renal impairment significantly different from the pattern of premature who are not exposed to asphyxia. Structure of facies in preterm babies with renal disturbance due to severe asphyxia indicates a significant loss of organic and mineral substances in the urine. The width of the peripheral zone facies, the amount of solid particles transferred depends on the severity of asphyxia, the difference in morphology facies is maintained even at the end of the early neonatal period.

Thus, the morphology of facies depends on the gestational age of the child and the severity of asphyxia. Analysis of dried drops of urine in infants with renal impairment on the background of asphyxia can be used as one of the criteria for assessing kidney function and have prognostic value.

CONCLUSIONS

1. Significant saturation of urine salts and organic molecules due to the development of nephropathy in the background asphyxia causes the formation of certain organic and mineral structures in dried drops of urine, morphology differs in children of different ages hestatsynho.
2. Morphology of facies of urine on 1-2 days of life give possible to estimate renal function in term and preterm newborns with

asphyxia. Number of inclusions in facies, the total area and distribution depend on the severity of the transferred asphyxia.

3. Structural changes in urine is stored long enough. The difference in morphology facies in the examined groups of children exists even at the end of the early neonatal period.

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Conflict of interest:

The Authors declare no conflict of interest

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