THE GAMMA-GLUTAMYL TRANSPEPTIDASE CONTENTS IN SERUM AND URINE IN NEWBORN AS AN INDICATOR OF DISTURBANCE KIDNEY FUNCTION DUE TO ASPHYXIA

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The aim of the investigation was to evaluate the diagnostic value of the study serum and urinary contents of γ-glutamyl transpeptidase (GGTP) in newborns with impaired renal function due to asphyxia.

Materials and Methods. Investigation included 200 full-term newborns with disturbance kidney function: 100 infants who had severe asphyxia, and 100 — with moderate asphyxia. Comparison group consisted of 20 healthy children without asphyxia. The peripheral venous blood and urine of newborns were picked up by 1–2, 7–8 and 25–30 days of life. GGTP activity was determined by a standardized method of "endpoint" after rate of formation of 5-amino-2-nitrobenzoate at 405 nm and 37°C on the semi-automatic photometer using reagents of Olvex Company (Russia).

Results. Disturbance kidney function in neonates with asphyxia causes a significant increase the level of GGTP in serum and urine in the first 24–48 hours after birth. Enzyme concentration in biological fluids during this period in the groups of surveyed children was the highest. In the dynamics of the early neonatal period marked reduction of GGTP in serum and urine in children with kidney dysfunction due to asphyxia. By the end of the neonatal period the enzyme content in serum and urine in neonates with asphyxia and disturbance kidney function remained high. In infants with renal impairment due to moderate asphyxia during the first weeks of life ratio of serum/urine GGTP content decreased less than 2.5 relative to the comparison group, followed its raised to 25–30 days of life. At the same time, infants with severe renal impairment due to asphyxia were characterized by high rates ratio (greater than 4.0) during the whole neonatal period. In the examined groups of children during the early neonatal period indicated a statistically significant positive moderate correlation between the serum and urinary GGTP content.

Conclusion. Serum GGTP activity is characterized severity of cytolysis syndrome of proximal renal tubular epithelium in neonates with disturbance kidney function due to asphyxia. Noninvasive determination of GGTP levels in the urine in the early neonatal period, preferably for the screening of renal problems in neonates with asphyxia.

Key words: γ-glutamyl transpeptidase; disturbance kidney function due to asphyxia in newborns.

The assessment of organospecific enzyme content in biological fluids enables to determine localization and intensity of kidney damage [1]. This is of special importance in asphyxia in newborns as it may influence the tactics of managing the patients.

One of the enzymes specific for renal tissue is gamma-glutamyl transpeptidase (γ-glutamyl transferase, GGTP). This enzyme catalyses the transfer of γ-glutamyl group from a peptide or a compound, containing this group, to an acceptor peptide or amino acid [2].

GGTP is found in the cell membranes, possessing high secretory or absorption capability. The epithelium of proximal kidney tubules is known to have the highest activity. Despite of this fact, this enzyme is not practically identified in the urine of healthy people because of a high molecular weight. The increase of its concentration in urine is noted in patients with acute infectious diseases of the urine system and conditions, which are accompanied by a significant destruction of the renal tissue [2, 3].

The period of GGTP half-life in the blood serum is 7–10 days, and may increase to 28 days in case of enzyme clearance disturbance. Owing to this and a high organospecificity relative to the renal tissue, GGTP may be used for diagnosing kidney damage [4], including newborns.

Besides, this enzyme may be a marker of an oxidative stress. A high level of GGTP induces the reduction of glutathione provision, which enhances free radical production and provokes an oxidative stress [5]. The latter is one of the key elements of asphyxia at birth [6]. Nevertheless, a problem of diagnostic value of GGTP in kidney function impairment in neonates with asphyxia remains unstudied.

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The aim of the investigation is to assess a diagnostic value of γ-glutamyl transpeptidase content in the blood serum and urine of the neonates with kidney dysfunction due to asphyxia.

Materials and Methods. 200 full-term newborns with the signs of renal damage were investigated. Infants were divided into two groups: group 1 (n=100) — neonates suffered severe asphyxia, group 2 (n=100) — babies suffered moderate asphyxia. A comparison group comprised 20 infants without asphyxia at birth, which were being examined and treated at the in-patient department because of other problems.

A sampling method was used in the investigation. Newborns with other diseases, aggravating their condition, were excluded from it, e.g. polycythemia, hemolytic and hemorrhagic diseases, acute surgical pathology, requiring correction in the neonatal period, purulent meningitis, neonatal pneumonia, urinary tract infection, congenital abnormalities of the heart, gastrointestinal tract or kidneys, arrhythmias in neonatal period, multiple congenital defects, chromosome abnormality. Additionally, babies with conjugated jaundice, hepatic pathology, as well as infants born from the parents consuming alcohol, as the level of enzyme in the serum in this case might not be caused by renal reasons, were also excluded from the study [2]. The examined groups did not contained infants with hypoxic myocardial damage in order to exclude the influence of cardiac causes on the GGTP values [1, 2].

The study complies with the Declaration of Helsinki (the Declaration was passed in Helsinki, Finland, June, 1964, and revised in October, 2000, Edinburg, Scotland) and was performed following approval by the Ethic Committee of Sumy State University. Written informed consent was obtained from the parents of the newborns.

Kidney dysfunction was diagnosed, if the level of blood creatinine was higher than 89 μmol/L, blood urea — higher than 8 mmol/L, urine output dropped lower than 1 ml/kg/h [7]. The diagnosis of moderate and severe asphyxia was established according to the "Protocol of initial resuscitation and post-resuscitation aid to newborns" (Order of the Ministry of Health of Ukraine of June 08, 2007, No.312).

Diagnostic criteria of severe asphyxia at birth were: Apgar assessment lower than 4 scores during the first 5 minutes of life; clinical symptoms of heavy CNS damage, which appeared in the first 72 hours of life; signs of impairment of one more vital organ or system (respiratory, cardio-vascular, urinary, gastro-intestinal and so on) during the first 3 days of life; metabolic or mixed acidosis (pH<7.0 and/or base deficiency more than 12 mmol/L) in the cord blood.

Diagnostic criteria of moderate asphyxia at birth were the following: less than 7 scores according to Apgar scale during the first 5 minutes; clinical symptoms of moderate CNS damage, which occurred in the first 72 hours of life; signs of transitory disturbance of one of the vital organ or system (respiratory, cardio-vascular, urinary, gastro-intestinal and so on) during the first 3 days of life; metabolic or mixed acidosis (pH<7.15 and/or base deficiency more than 12 mmol/L) in the cord blood. If it was technically impossible to estimate an acid-base balance of the neonate's blood, the diagnosis of asphyxia at birth was based on the first three signs.

The material for investigation was a peripheral venous blood of the newborns with the signs of kidney impairment caused by asphyxia. Blood was taken fasting in the morning to avoid the influence of daily fluctuations and food intake on the enzymemia level. Blood was collected on the 1–2 day of life, at the end of the early neonatal period (7–8 day of life), and at the end of the first month (25–30 day). Peripheral venous blood, remained after laboratory test necessary for evaluating infant condition after asphyxia, was used for the investigation.

To determine the activity of enzymes in the urine, it was collected into sterile urinals after the cleaning of external genital organs. In order to exclude the influence of diurnal rhythms on the excretion of urinal enzymes, an early urine portion, collected between 8 and 10 o'clock, was examined

GGTP activity was determined by a standard "endpoint" method, considering the rate of forming 5-amino-2-nitrobenzoate at 405 nm wavelength and 37°C, using a semi-automatic photometer and reagents, produced by Olvex Company (Russia).

Statistical processing of the investigation findings was performed by means of Excel and Statistica 6.0 programs. Methods of variation statistics suitable for medico-biological investigations were used here [8]. Data were presented as the mean (M), standard error of the mean (m), and confidence interval (CI). A calculation of ratio coefficients of GGTP content in the blood serum and urine in newborns was made to make a diagnostic value of determining enzyme levels in the biological fluids higher. Spearman rank correlation coefficient was used to estimate the correlation between the variables. As the data did not correspond to the normal law of distribution, index of confidence (p) was found using Wilcoxon criterion.

Results. In the examined infants of the comparison group the value of GGTP in blood serum was within the reference intervals (0–23 nmol/(s·L) during the whole first month of life. Besides, it was zero in 25, 15, and 30% of healthy newborns on the 1–2, 7–8, and 25–30 day of life, respectively.

In infants with kidney damage asphyxia causes significant elevation of GGTP level in blood serum in the first 24–48 hours after birth. Concentration of the enzyme in the given time interval was the highest in the groups of the examined infants over the period of observation, and exceeded this parameter 7 times or more in newborns with renal dysfunction after moderate asphyxia and over 17 times in severe asphyxia and kidney damage in comparison with healthy neonates (Table 1).

Table 1

GGTP content in blood serum of full-term newborns (M±m) and confidence interval (CI), nmol/(s·L)

Newborns	Life period		
	1–2 day	7–8 day	25–30 day
Comparison group (n=20)	16.44±2.96 10.24–22.64	15.16±2.04 10.88–19.43	13.06±2.74 7.31–18.81
With kidney damage due to moderate asphyxia (n=100)	129.14±5.27 118.56–139.73 p	100.17±6.63 86.85–113.49 p, p_2	67.04±5.93 55.13–78.95 p, p_2, p_3
With kidney damage due to severe asphyxia (n=100)	282.11±12.09 257.82–306.39 p, p_1	226.09±8.57 208.88–243.31 p, p_1, p_2	163.45±5.73 151.93–174.98 p, p_1, p_2, p_3

Notes: p — statistical significance of the value difference relative to the comparison group; p_1 — relative to newborns with moderate asphyxia; p_2 — relative to 1–2 days of life; p_3 — relative to 7–8 days of life. M±m values are given in the numerator, CI values — in the denominator.

Table 2

GGTP content in the urine of the full-term newborns (M±m) and confidence interval (CI), nmol/(s·L)

Newborns	Life period		
	1–2 day	7–8 day	25–30 day
Comparison group (n=20)	5.87±1.10 3.56–8.17	4.21±0.77 2.60–5.82	4.13±0.70 2.66–5.60
With kidney damage due to moderate asphyxia (n=100)	52.94±2.95 47.03–58.86 p	40.80±2.47 35.84–45.77 p, p_2	16.06±0.52 5.57–7.64 p, p_2, p_3
With kidney damage due to severe asphyxia (n=100)	65.38±4.47 56.39–74.36 p, p_1	55.37±4.89 45.55–65.19 p, p_1	25.28±2.69 19.88–30.68 p, p_1, p_2, p_3

Notes: p — statistical significance of the value difference relative to the comparison group; p_1 — relative to newborns with moderate asphyxia; p_2 — relative to 1–2 days of life; p_3 — relative to 7–8 days of life. M±m values are given in the numerator, CI values — in the denominator.

Table 3

Ratio coefficients of GGTP content in the blood serum and urine in the newborns

Newborns	Life period		
	1–2 day	7–8 day	25–30 day
Comparison group (n=20)	2.80	3.60	3.16
With kidney damage due to moderate asphyxia (n=100)	2.44	2.46	4.17
With kidney damage due to severe asphyxia (n=100)	4.31	4.08	6.47

During the early neonatal period reduction of serum GGTP content in both groups of infants by 20% was noted, though statistical difference in enzyme concentration between the groups remained.

By the end of the neonatal period further statistically significant decrease of GGTP in the serum continued, however its content remained high compared to the healthy babies. Besides, GGTP concentration was 2.5 times higher in infants with renal impairment due to severe asphyxia than in those with moderate asphyxia.

Among the neonates of the comparison group GGTP content in the urine was found only in 75% on the 1–2 day of life, in 80% — on the 7–8 day, and in 85% — by the end of the neonatal period. An average enzyme level in the urine of the healthy newborns remained stable during the first month of life.

Maximum enzymuria in the newborns with kidney damage due to asphyxia was noted on the 1–2 day of life in both groups (Table 2). The highest value was in those infants who suffered severe asphyxia. Later on statistically significant decrease of enzyme concentration in the urine of all infants took place, but the difference among the groups of newborns with kidney damage due to severe and moderate asphyxia remained. Notably, that by the end of the neonatal period the level of GGTP in the urine of both examined groups remained high, being 4 and 6 times the value of the comparison group in moderate and severe asphyxia, respectively.

In the newborns with renal impairment due to moderate asphyxia decrease of ratio coefficients of GGTP levels in the blood serum and urine is noted during the first week of life relative to the comparison group with the following rise on the 25–30 day of life (Table 3). At the same

time, high coefficients during the whole neonatal period are characteristic of the neonates with renal impairment due to severe asphyxia.

A statistically significant positive moderate correlation between serum and urinary GGTP content is noted in the examined groups of infants as well as in the healthy neonates during the early neonatal period (Table 4). A significant relationship between these values disappears in all infants by the end of the first month of life.

Table 4

Spearman's correlation coefficient of GGTP content in blood serum and urine during the neonatal period

Period of life	Newborns with kidney damage due to severe asphyxia	Newborns with kidney damage due to moderate asphyxia	Comparison group
1–2 day	0.610 p=0.000	0.423 p=0.002	0.660 p=0.002
7–8 day	0.499 p=0.0002	0.544 p=0.013	0.526 p=0.017
25–30 day	0.187	0.149	0.247

Discussion. Revealing GGTP in the blood serum of the healthy newborns is likely to be connected with some extrarenal sources [1, 2, 9]. Presence of this enzyme in the urine of the healthy babies can be explained by its localization in the brush border of the tubular epithelium. And in the process of cytoplasmatic membrane regeneration it may appear in the urine [10].

The increase of GGTP level in blood serum is a characteristic feature of acute renal damage and oxidative stress [6, 11]. In the course of the investigation it was established, that growth of GGTP serum activity is noted rather early in the newborns with kidney damage with underlying asphyxia. Already in the first 24–48 hours after birth a high serum level of GGTP is characteristic for all newborns with asphyxia and renal dysfunction. It reaches the highest values in severe asphyxia.

Significant enzymemia is associated with a high rate of enzyme release from the cells of proximal renal tubules due to the damage of the cellular membrane caused by ischemia — reperfusion [12]. Taking into account a cytosolic localization of the enzyme [2, 10], it should be understood, that only significant destruction of the membrane or cell death will cause the increase of its concentration in blood serum.

Determination of the given enzyme activity in serum may characterize not only the presence of oxidative stress, but the degree of intensity of nephrothelium cytolysis syndrome [10]. The findings of the given investigation allow to state, that cytolysis syndrome is most intensive in neonates with kidney damage caused by severe asphyxia. In the dynamics of the neonatal period the reduction of its manifestation is noted, however GGTP level in the serum does not reach normal values even by the end of the first month of life.

Thus, a high GGTP level in the serum has been proved by the authors to be an early indicator of the injury of the proximal tubular epithelium in newborns with kidney damage due to asphyxia.

Enzymuria is also a marker of renal dysfunction [13]. As about 2/3 of GGTP are localized in the brush border of the nephron proximal convoluted tubules, injury of these particular parts is accompanied by the appearance of enzymuria [2, 14]. Nearly 1/3 of the enzyme has intracellular localization in the Golgi complex and lysosomes [2, 10], therefore, to the author's opinion, a high activity of GGTP in the urine is supposed to be an evidence of the marked damage of the epithelial cells of the renal tubules due to asphyxia at birth.

High enzymuria in the early neonatal period in newborns with kidney damage caused by asphyxia is most likely to be caused by ischemia of the renal tissue, dysfunction of the cellular membranes, and passage into the urine the enzymes not only of the brush border but intracellular ones as well. By the end of the neonatal period the content of GGTP in the urine becomes lower in the neonates with asphyxia and kidney impairment and this, most probably, is connected with the brush border remaining unstable.

Urine GGTP, in addition, is an early marker of the damage as its level increases already on the 1–2 day. But as opposed to the serum enzyme its identification in the urine has a significant advantage — noninvasiveness, which is of special importance in newborns in the critical state [15, 16]. Therefore, the assessment of GGTP activity in the urine may be used in the neonatal intensive therapy units for laboratory confirmation of kidney impairment in newborns with asphyxia.

A combination of high enzymemia and enzymuria in the majority of infants with kidney damage due to asphyxia is associated with a significant dysfunction of the apical and basal membranes of renal tubular epytheliocytes, which results in the passage of the enzyme not only in the urine but in the serum as well.

Calculation of ratio coefficients of serum GGTP/urine GGTP allows to raise the diagnostic value of neonatal examination (See Table 3). The coefficient below 2.5 in the early neonatal period typical for the newborns with kidney dysfunction due to moderate asphyxia seems to reflect relative predominance of enzymuria with underlying structural, functional and metabolic disorders of the epithelium brush border of the nephron proximal part. The coefficient value of more than 4.0 characteristic of the newborns with kidney damage caused by severe asphyxia is the result of relatively high enzymemia due to instability of the basal membrane of the tubular epithelium.

Availability of the significant positive correlation between the values of GGTP activity in the serum and

urine in the newborns with kidney damage caused by asphyxia in the early neonatal period (See Table 4) speaks of the fact, that GGTP of the urine can with a great degree of confidence reflect serum enzyme level in an infant. As the level of GGTP in the urine increases prior to proteinuria and elevation of serum creatinine content [17, 18], preference should be given to noninvasive identification of GGTP level in the urine in diagnosing kidney damage in newborns with asphyxia. This method may be used as a screening technique, as the investigation is simple, inexpensive and reproducible.

Conclusion. Noninvasive identification of GGTP in the urine in the early neonatal period is a preferable method for screening kidney damage in newborns with asphyxia. Increase of GGTP serum activity is a marker, enabling to assess the intensity of epithelium cytolysis syndrome of the proximal kidney tubules in newborns with kidney damage caused by asphyxia.

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