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DIAGNOSTIC VALUE FOR DETERMINATION OF ENZYMURIA IN NEONATES WITH IMPAIRED RENAL FUNCTION DUE TO ASPHYXIA

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Abstract

The article investigates the diagnostic value of the determination of enzymes lactate dehydrogenase and γ-glutamyl transpeptidase in the urine of infants with impaired renal function due to birth asphyxia.

Investigation included 200 full-term newborns with impaired kidney function: 100 infants who had severe asphyxia and 100 infants with moderate asphyxia.

The urine of newborns was collected by 1-2, 7-8 and 25-30 days of life. Activity of lactate dehydrogenase was determined using a kinetic spectrophotometric technique. Gamma-glutamyl transpeptidase activity was determined via "endpoint" standardized technique.

Enzyme content in the urine of newborns with asphyxia can be used as one of the early non-invasive markers of kidney damage, because the levels of lactate dehydrogenase and γ-glutamyl transpeptidase increased significantly already on 1-2 days of life. At the same time, the enzymes showed statistical correlation associated with severe lesions of the tubular epithelium. Due to such lesions a significant amount of brush border and cytoplasmic enzymes got into urine. Enzymes levels in urine throughout the neonatal period may also reflect the degree of severity of the asphyxia.

Analysis of prognostic significance of early urine enzymes determination showed that lactate dehydrogenase and γ-glutamyl transpeptidase are important predictors of renal dysfunction in neonates with asphyxia. Gamma-glutamyl transpeptidase has greater prognostic value reflecting both cystatin C (with lactate dehydrogenase), and the diuresis.

The non-invasive study of urine enzymes is highly informative method; it should be preferred for the diagnosis of renal injury in neonates with asphyxia.

Keywords: *kidney, asphyxia, enzyme, newborn, prognosis.*

Introduction

In accordance with the data of various research centers the prevalence of neonatal asphyxia in the world is 1-1.5% [*Kurinczuk J et al., 2010*]. Lack of oxygen promotes the development of multiple organ dysfunctions. The most exposed target organs in this case are kidneys, whose frequency of involvement varies from 47.1% to 70% [*Shah P et al., 2004; Gupta B et al., 2005*]. Renal dysfunction can be formed on the first day of life in the presence of tissue hypoxia due to metabolic, hemodynamic and microcirculation disorders [*Gupta B et al., 2005*]. However, diagnosis of renal injury in newborns is difficult because of the lack of specific clinical symptoms and low informative value of traditional survey methods [*Askenazi D et al., 2009*].

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The clinical examination of newborns with asphyxia, especially in critical condition, during the first hours of life can not identify kidney injury. This is a serious disadvantage of existing diagnostic methods. For example, adequate diuresis in the newborn does not always reflect normal kidney function (so-called non-oliguric acute kidney injury). At the same time, oliguria may be as a manifestation of transient features of renal function after birth, even in healthy children.

Traditional laboratory parameters are not sufficiently specific and sensitive for early detection of acute kidney injury. Serum creatinine in acute kidney damage increases slowly, moreover there isn't direct correlation between its content and glomerular filtration rate [*Salgado J et al., 2010*].

At the same time, some biochemical parameters may be more effective for the diagnosis of renal injury in neonates with asphyxia. Disorder of energy metabolism and dysfunction of biological mem-

branes due to kidney ischemia lead to cell damage. The enzymes getting in the urine due to the instability of cell membranes can be the markers of such damage [*Herget-Rosental S et al., 2004*]. Furthermore, elevated enzymes in urine are the earliest indicators of kidney damage, rather than tubular proteinuria [*Fauconneau B et al., 1997*].

One of the important biochemical markers is lactate dehydrogenase (LDH), the level of which increases due to the intensification of glycolysis during asphyxia. The enzyme catalyzes the reverse reaction of recovery of pyruvic acid (pyruvate) to the lactate, and is a key enzyme in the tissues with anaerobic metabolism [*Champe P, Harvey R, 2005*]. Its high serum level 10-12 hours after birth reflects the predominant involvement of organs with aerobic metabolism, including kidneys [*Karlsson M, Hirot af Ornas S, 2008*].

Second important renal enzyme is γ-glutamyl transpeptidase (GGT) that catalyzes the movement of γ-glutamyl group from a peptide or a compound containing the group to the acceptor peptide or amino acid. The highest GGT activity is typical for proximal renal tubular epithelium. Despite this, due to the high molecular weight in the urine of healthy humans enzyme isn't detected substantially [*Whitfield J, 2001*].

The *objective of this study* was to assess the diagnostic value of enzymuria detection in neonates with impaired renal function due to birth asphyxia.

Materials and Methods

Investigation included 200 full-term newborns with disturbance kidney function: 100 infants who had severe asphyxia and 100 infants with moderate asphyxia. Comparison group included 20 neonates without birth asphyxia, who were at the hospital examination and treatment for other reasons.

The study was approved by the Commission on Bioethics of the Medical Institute of Sumy State University and consistent with the principles outlined in the Declaration of Helsinki (Br. Med. J., 1964; p. 177), with the subsequent supplements.

Impaired kidney function was diagnosed if blood creatinine level increased more than 89 *μmol/l*, blood urea more than 8 *mmol/l*, and urine output decreased less than 1 *ml/kg/h* [*Kulikova N, 2011*]. Moderate or severe asphyxia in newborns were diagnosed in accordance with the "Protocol on primary neonatal resuscitation and post-resuscitation care" (order of the Ministry of Health of Ukraine, No. 312, 08.06.2007).

The actual material used for the tests was urine samples from the neonates, collected in a sterile drainage bag. In order to eliminate the effect of circadian rhythms on urine enzymes excretion only morning urine sample was tested, which was collected between 8 am and 10 am. The study was conducted on the 1-2 days of life, at the end of early neonatal period (7-8 days of life), and the end of the first month of life (25-30 days).

The activity of LDH was determined via kinetic spectrophotometric technique by the optical density decrease rate of nicotinamide adenine dinucleotide (NADH) at the wavelength of 340 *nm* and 37°*C* using reagents manufactured by Olvex Diagnosticum Company (Russian Federation). The technique is based on the difference in spectral absorption of reduced (NADH) and oxidized (NAD) forms of nicotinamide adenine dinucleotide. NADH has a maximum absorption at 340 *nm* in contrast to NAD. Therefore, the conversion of NADH to NAD causes decrease in optical density of the substrate at 340 *nm*.

GGT activity was determined with "endpoint" standardized method on semiautomatic photometer using reagents manufactured by Olvex Diagnosticum Company (Russian Federation). Enzyme activity is proportional to the amount of the formed 5-amino-2-nitrobenzoate at the wavelength of 405 *nm* and 37°*C*.

Statistica 6.0 software was used for statistical processing of the results of the research. The arithmetic mean (m), the arithmetic mean error (M), confidence interval (CI) were determined. To assess the link between the variables Spearman's rank correlation coefficient was used. Our results were non-normally distributed, and therefore non-parametric testing was used such as Wilcoxon's test. P values < 0.05 were considered significant. To assess the prognostic significance, the multiple regression method was used, which allows to analyze the relationship between several independent factors (so-called regressors or predictors) and the dependent variable.

Results and Discussions

Increase of LDH levels in the urine of healthy newborns is associated with its partial reabsorption in the renal tubule due to imperfect kidney function after birth (Table 1). Physiological proteinuria may include trace amounts of proteins in urine of both low and high molecular weight [*Davison A et al., 2005*].

Proteins with molecular weight under 70 *kDa* are usually filtered into urine [*Davison A et al., 2005*]. LDH has a weight of about 135 *kDa* [*Champe P, Harvey R, 2005*], which is why the main source of its entry into urine is the epithelium of the renal tubules.

In neonates with renal impairment due to moderate asphyxia in the 1-2nd day life urinary LDH content increased 2.5 times ($p < 0.001$) relative to healthy babies. By the end of the first week of life LDH levels in urine in this group increased significantly ($p < 0.001$) and then remained stable until the end of the neonatal period.

Urinary LDH levels in newborns with impaired kidney function due to severe asphyxia, was seven times higher than in healthy newborns. Subsequently, the enzyme activity reached their maximum level on the 7-8th days of life, reflecting the rupture of membranes of epithelial cells of the renal tubules. About 70% of LDH enters urine from the proximal and distal parts of the nephron, that's why a significant damage of these areas is accompanied with its increased levels [*Davison A et al., 2005*]. Due to cytosolic location of the enzyme, its high activity in the urine testifies to significant damage of epithelial cells of the renal tubules.

At the end of the first month of life there was a significant decrease ($p < 0.001$) in LDH levels in the urine in infants with impaired renal function due to severe asphyxia, but its contents remained statistically higher relative to neonates with moderate asphyxia and healthy children.

Thus, as early as the 1-2nd day of life urinary LDH content in children with renal impairment due to asphyxia is significantly different from the comparison group, and can be considered as an early

Table 1.

Urinary lactate dehydrogenase and γ-glutamyl transpeptidase content in neonates with impaired kidney function,

Study groups		Days of life		
		1-2	7-8	25-30
lactate dehydrogenase *u/l*				
Comparison group, n=20		2.29±0.44 CI 1.29 - 3.28	2.54±0.48 CI 1.46 - 3.62	2.44±0.43 CI 1.47- 3.41
Asphyxia	moderate, n=100	5.83±0.35 CI 5.14 - 6.53 p*	8.82±0.55 CI 7.71 - 9.92 p*, p*$_2$	7.61±0.65 CI 6.32 - 8.91 p*, p$_2$**
	severe, n=100	15.86±0.88 CI 14.09 - 17.63 p*, p$_1$*	24.61±0.95 CI 22.70 - 26.51 p*, p$_1$*, p$_2$*	10.37±0.62 CI 9.13 - 11.60 p*, p$_1$***, p$_2$*, p$_3$*
γ-glutamyl transpeptidase *nmol/(sec·l)*				
Study groups n=20		5.87±1.10 CI 3.56 - 8.17	4.21±0.77 CI 2.60 - 5.82	4.13±0.70 CI 2.66- 5.60
Asphyxia	moderate, n=100	52.94±2.95 CI 47.03 - 58.86 p*	40.80±2.47 CI 35.84 - 45.77 p*, p$_2$***	16.06±0.52 CI 5.57 - 7.64 p*, p$_2$*, p$_3$*
	severe, n=100	65.38±4.47 CI 56.39 - 74.36 p*, p$_1$**	55.37±4.89 CI 45.55 - 65.19 p*, p$_1$***	25.28±2.69 CI 19.88 - 30.68 p*, p$_1$***, p$_2$*, p$_3$*

Notes: CI - confidence interval, p – reliability relative to comparison group; p$_1$ – reliability relative to newborns with moderate asphyxia; p$_2$ – reliability relative to 1-2nd day of life; p$_3$ – reliability relative to 7-8th days of life.*<0.001, **<0.05, ***<0.01.

marker of kidney damage. Also, during the neonatal period LDH activity in the examined newborns varies depending on the severity of the asphyxia.

Among newborns of comparison group GGT levels in urine was determined in only 75% of infants on the 1-2nd day of life, in 80% on the 7-8th days of life, and in 85% by the end of the neonatal period. The average content of the enzyme in the urine of healthy children has remained stable during the 1st month of life.

Maximum enzyme content in urine of neonates with renal impairment due to asphyxia was observed on the 1-2nd day of life (Table 1). The highest rate is typical for infants with severe asphyxia. Later there was a significant decrease in enzyme levels in the urine in all observed newborns, and the difference between the groups of neonates with impaired kidney function due to moderate and severe asphyxia retained. It should be noted that by the end of the neonatal period GGT levels in the urine of newborns of examined groups remained high, exceeding those of the comparison group by the 4 and 6 times in case of moderate and severe asphyxia, respectively.

High levels of enzyme in urine indicate renal dysfunction [*Herget-Rosental S et al., 2004*]. About 2/3 of GGT is localized in the brush border of the proximal convoluted tubules of the nephron, the damage of this part is accompanied by the appearance of enzymuria [*Guder WG, Ross BD, 1984; Whitfield JB, 2001*]. Approximately 1/3 of the enzyme is localized intracellularly in the Golgi complex and lysosomes, that is why high GGT activity in urine may be the manifestation of severe damage of the epithelial cells of the renal tubules [*Whitfield JB, 2001*].

Urinary glutamyl transpeptidase is an early marker of damage – its levels increase on the 1-2 day of life. As in the case of determining LDH in the urine, the method has a significant advantage – noninvasive, that is important in the newborns in critical condition [*Li Y et al., 2012*].

Probably high enzymuria during early neonatal period in newborns with renal impairment due to asphyxia caused by renal ischemia, dysfunction of cell membranes and entry into urine of not only the brush border enzymes, but also intracellular ones. This explains the presence of high levels of both LDH and GGT in urine of examined newborns. The preservation of increased enzyme content at the end of the neonatal period in the urine of newborns with asphyxia and renal disorder is predominantly provoked by continued instability of the brush border.

The correlation analysis showed that all newborns are characterized by a positive relationship between the level of LDH and GGT in the urine on the 1-2nd day after birth. In neonates with renal disorder due to asphyxia the correlation was significant; its power was dependent on severity of the asphyxia. Statistical relationship is explained by significant damage of tubular epithelium, which is why a lot of both brush border and cytoplasmic enzymes enter the urine.

Later the direction of correlation changed in the comparison group newborns, and the end of the first month of life the correlation was strong and reliable. In newborns with renal disorder due to asphyxia in the early neonatal period the strength of correlation decreased and then changed its direction. Such changes are due to recovery processes in the tubular epithelial function and decreased loss of intracellular enzymes (Table 2).

The analysis of the prognostic significance of early determination of enzymes in urine and their impact on the level of the main indicators reflecting the impairment of renal function (serum levels of cystatin C, creatinine, urea and urine output) [*Roos J et al., 2007; Kulikova N, 2011*] was performed in infants with impaired renal function due to asphyxia on the 1-2nd day of life. As independent predictors are levels of enzymes in urine: X1, X2 representing the content of LDH, GGT, respec-

Table 2.

Spearman's correlation between lactate dehydrogenase and γ-glutamyl transpeptidase content in the urine during neonatal period

Study groups		Days of life		
		1-2	7-8	25-30
Comparison group		–0.205	–0.274	–0.639 p=0.046
Asphyxia	servere	0.507 p=0.022	0.791 p=0.006	0.395
	moderate	0,495 p=0.027	0.411	–0.547

Note: p – reliability of the correlation coefficient (p<0.05).

Table 3.

Characteristics of predictors used for the analysis

	Variables	β	Standard error β	B	Standard error B	t	p	Partial correlation
Cystatin C	X1	0.398	0.162	33.441	13.626	2.454	0.022	0.398*
	X2	0.503	0.162	8.317	2.682	3.101	0.005	0.543*
Diuresis	X1	–0.083	0.143	–0.004	0.007	–0.578	0.569	–0.119
	X2	–0.812	0.143	–0.008	0.001	–5.679	0.0009	–0.764*
Creatinine	X1	0.275	0.238	0.992	0.860	1.153	0.261	0.234
	X2	0.347	0.239	0.246	0.169	1.453	0.159	0.290
Urea	X1	–0.119	0.263	–0.085	0.190	–0.445	0.660	–0.092
	X2	0.481	0.266	0.067	0.037	1.806	0.084	0.352

Note: * – reliability of the partial correlation index ($p<0.05$).

tively. Their influence on the dependent variables (Y) (serum levels of cystatin C, creatinine, urea and urine output) was determined.

Table 3 shows the descriptive statistics of variables X1, X2 and their characteristics.

Analysis of the impact urinary enzyme content on serum cystatin C level showed a significant partial correlation value for both enzymes, which characterize the allowable level of interaction between the independent variables. Standardized regression coefficient index (β) indicates that GGT is a more important predictor of influence on serum cystatin C. Furthermore, GGT level in urine is an important negative regressor for diuresis, unlike LDH. The influence of independent regressors (LDH and GGT) on serum urea and creatinine levels was not found.

Thus, urinary LDH and GGT are important predictors of renal dysfunction in neonates with asphyxia. GGT has greater prognostic value, which has an effect on both cystatin C (together with LDH) and the diuresis.

Despite the high renal perfusion and adequate supply of oxygen, its activity in renal tissue is relatively low, which makes kidneys sensitive to hypoxic injury [*Eckardt K et al., 2003*]. Renal tissue hypoxia due to asphyxia is accompanied by damage of the glomerular capillaries [*Eckardt K et al., 2003*], which is manifested in filtration dysfunction, decreased urine output and increased levels of marker molecules, such as cystatin C, and later – creatinine [*Roos J et al., 2007; Li Y et al., 2012*].

Impaired function of the renal tubules in case of asphyxia is accompanied by the onset of enzymuria [*Herget-Rosental S et al., 2004*]. Increased levels LDH and GGT in urine reflect the predominant localization of lesions (proximal convoluted tubules), its depth (damage of the brush border or more serious tubular epithelium damage with the advent of cytosolic enzymes), and, moreover, may be a predictor of renal dysfunction in neonates with asphyxia. This method is non-invasive (which is important for infants in critical condition) and informative, which is why it should be preferred for the diagnosis of renal damage in neonates with asphyxia.

Thus, activity test for the enzymes LDH and GGT in the urine should be used as a noninvasive marker for early diagnosis of kidney injury in newborns with asphyxia. GGT level in the urine on the 1-2nd day of life is an important predictor of renal dysfunction in neonates with asphyxia.

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