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Value of urine 8-*iso*-$PGF_{2\alpha}$ in the assessment of the severity of neonatal hypoxic-ischemic encephalopathy

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Abstract: Objective It was aimed to determine the concentration of urine 8-*iso*-$PGF_{2\alpha}$ in normal neonates and neonates with hypoxic-ischemic encephalopathy (HIE) and to study the value of urine 8-*iso*-$PGF_{2\alpha}$ in the assessment of the severity of neonatal HIE. **Methods** Urine samples from normal ($n = 126$) and HIE ($n = 151$) neonates were collected on the 1st, 3rd and 7th days after birth. ELISA was used to determine the urine 8-*iso*-$PGF_{2\alpha}$ contents. **Results** 1) Urine 8-*iso*-$PGF_{2\alpha}$ contents from normal neonates were 29.9 ± 7.9, 27.7 ± 9.8 and 27.5 ± 10.5 ng/mmol · Cr on the 1st, 3rd and 7th days after birth, respectively. There was no significant difference among the three days for the urine 8-*iso*-$PGF_{2\alpha}$ contents. 2) On the 1st day of HIE onset, neonates with mild, moderate and severe HIE had higher levels of urine 8-*iso*-$PGF_{2\alpha}$ (65.3 ± 13.2, 154.6 ± 31.6 and 241.7 ± 41.0 ng/mmol · Cr, respectively) compared with the normal neonates ($P < 0.001$). 3) On the 3rd day, the urine 8-*iso*-$PGF_{2\alpha}$ content in neonates with moderate and severe HIE remained higher (34.2 ± 10.3 and 50.8 ± 12.8 ng/mmol · Cr, respectively) than the normal neonates ($P < 0.001$), while that of the neonates with mild HIE regressed to normal. 4) On the 7th day, there was no significant difference in the urine 8-*iso*-$PGF_{2\alpha}$ level between all HIE and normal neonates. 5) The boundaries of 45.5, 89.9 and 217.5 ng/mmol · Cr of urine 8-*iso*-$PGF_{2\alpha}$ were defined to distinguish from normal to mild HIE, from mild to moderate HIE and from moderate to severe HIE. As defined, the corresponding sensitivity and specificity were 95.2% and 99.2%, 100% and 95.2%, 65.8% and 100%. **Conclusions** The urine 8-*iso*-$PGF_{2\alpha}$ levels in neonates were stable within 7 days after birth. The urine 8-*iso*-$PGF_{2\alpha}$ contents in HIE neonates reached the peak within 24 hours after onset. There was a correlation between the urine 8-*iso*-$PGF_{2\alpha}$ contents and the severity of HIE, suggesting that urine 8-*iso*-$PGF_{2\alpha}$ may be a reliable and convenient index for the assessment of the severity of HIE. [**Chin J Contemp Pediatr, 2005, 7(1):103-106**]

Key words: 8-*iso*-$PGF_{2\alpha}$, urine; Hypoxia-ischemia, brain; Infant, newborn

尿 8－异前列腺素 $F_{2\alpha}$ 测定在新生儿缺氧缺血性脑病病情评估中的价值

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[**摘 要**] **目的** 测定新生儿尿 8-异前列腺素 $F_{2\alpha}$(8-*iso*-$PGF_{2\alpha}$)水平,分析其在缺氧缺血性脑病(HIE)患儿病情评估中的价值。**方法** 分别于生后 1 d、3 d 和 7 d 收集正常($n = 126$)和 HIE($n = 151$)新生儿尿液,应用 ELISA 法检测其 8-*iso*-$PGF_{2\alpha}$含量。**结果** ①正常新生儿生后 1、3 和 7 d 的尿 8-*iso*-$PGF_{2\alpha}$水平分别为 29.9 ± 7.9、27.7 ± 9.8 和 27.5 ± 10.5 ng/mmol · Cr,差异无统计学意义;②病程第 1 d,轻、中和重度 HIE 患儿尿 8-*iso*-$PGF_{2\alpha}$水平分别为 65.3 ± 13.2、154.6 ± 31.6 和 241.7 ± 41.0 ng/mmol · Cr,差异具有显著性意义,且均高于正常新生儿水平($P < 0.001$);③病程第 3 d,中度和重度 HIE 患儿 8-*iso*-$PGF_{2\alpha}$水平(34.2 ± 10.3 ng/mmol · Cr、50.8 ± 12.8 ng/mmol · Cr)仍高于正常新生儿($P < 0.001$),而轻度 HIE 患儿尿 8-*iso*-$PGF_{2\alpha}$水平下降至正常新生儿水平;④病程第 7 d,轻、中和重度 HIE 及正常新生儿之间的 8-*iso*-$PGF_{2\alpha}$水平无明显差异;⑤以 8-*iso*-$PGF_{2\alpha}$水平 45.5、89.9 和 217.5 ng/mmol · Cr 作为区分正常与轻度 HIE、轻度与中度 HIE、中度与重度的界限,其敏感性和特异性分别为

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95.2% 和 99.2%，100% 和 95.2%，65.8% 和 100%。**结论** 生后 7 d 内正常新生儿尿 8-*iso*-$PGF_{2\alpha}$ 水平相对稳定；HIE 患儿尿中 8-*iso*-$PGF_{2\alpha}$ 峰值于病程第 1 d 出现，其升高程度与病情严重程度相关，是一项评估患儿病情可靠而简便的生化指标。

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[关 键 词] 8-异前列腺素 F2α，尿；缺氧缺血，脑；婴儿，新生

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Free radical-induced peroxidation injury plays an important role in the development of hypoxic-ischemic encephalopathy (HIE). Recent studies have identified some biochemical indexes that can reflect free radicals injury, such as malonaldehyde, superoxide dismutase (SOD) and glomerular-stimulating hormone (GSH). However, these indexes are significantly affected by factors in vitro and in vivo, therefore they cannot correctly reflect the actual levels of free radicals in vivo[1]. In 1994, Morrow and his colleagues[2] tested a kind of the prostaglandin-like compound with nagatron chemi-ionization. The compound was named as isoprostanes which are formed through the cyclo-oxygenase independent process and related to 8-iso-prostaglandin F2α (8-*iso*-$PGF_{2\alpha}$) is a stable constituent of isoprostanes. It has been demonstrated that 8-*iso*-$PGF_{2\alpha}$ can specially reflect lipid peroxidation after reperfusion and therefore this metabolite provides a reliable marker of oxidative stress[1,3,4]. The purpose of this study was to determinate urine 8-*iso*-$PGF_{2\alpha}$ levels in neonates and to investigate its value in the assessment of the severity of neonatal HIE.

Materials and methods

Subjects and grouping

A total of 151 neonates with HIE were enrolled in this study from the First Affiliated Hospital of Jinan University, the Second Affiliated Hospital of Guangzhou Medical College, Zhanjiang Maternal and Children's Health Hospital, and Bao'an Maternal and Children's Health Hospital from January 2001 to June 2004. Clinical diagnosis along with the severity of HIE was made based on the criteria described by the Subspecialty Group of Neonatology, Pediatric Society, Chinese Medical Association at the 1996 Hangzhou conference[5]. HIE was diagnosed in all 151 patients within 48 hours after delivery. The patients were divided into three groups according the HIE degree: Mild ($n = 62$, 37 males and 25 females), Moderate ($n = 51$, 33 males and 18 females) and Severe ($n = 38$, 20 males and 18 females). One hundred and twenty six normal neonates (76 males and 50 females) were served as the Control group. The following neonates were excluded in this study: those who had perinatal asphyxia but not diagnosed with HIE; those who were diagnosed with congenital disease and infection such as TORCH infection; and those born from the mother with cardiovascular disease, respiratory disease, diabetes, hepatitis, pregnancy-induced hypertension (PIH) or malignant tumor, or from smoking / drinking mothers. There were no statistical differences in the mother's age, neonate's gender constituent ratio, gestational age (GA) and birth weight (BW) between the HIE and the Control groups (Table 1).

Table 1 Population characteristics

Group	n	Mother's age	Male/Female	GA (week)	BW (Kg)
Control	126	27 ± 3	76:50	39 ± 1	3.32 ± 0.40
HIE	151	26 ± 3	90:61	39 ± 2	3.22 ± 0.78
t		0.569		0.772	0.535
P		0.575		0.450	0.598

Collection of the sample

One mL urine was collected in a disposable plastic tube in 1st, 3rd and 7th days after birth for determining creatinine (Cr) and 8-*iso*-$PGF_{2\alpha}$ levels.

Determination of the 8-*iso*-$PGF_{2\alpha}$ concentration

8-*iso*-$PGF_{2\alpha}$ concentration was determined using EIA kit (Cayman Chemical Company, USA). OD values were read on Microplate analyzer (Auslab Company, Switzerland). The low detectable limit of kit was 4 pg/mL. Intra- and inter- variation coefficients were 5.6% and 7.8%, respectively.

Statistical analysis

To eliminate the influence of urine volume and dilution, 8-*iso*-$PGF_{2\alpha}$ concentration was expressed with ng/mmol · Cr ($\bar{x} \pm 2s$). Statistical analysis was performed using the statistical package SPSS 10.0. One-way ANOVA was used to analyze differences between groups. The Dunnett t-test was used to assess differences between individual means. Differences were considered significant at $P < 0.05$.

Results

Urine 8-*iso*-$PGF_{2\alpha}$ levels of the HIE and Control groups on the 1st, 3rd and 7th days after birth are shown in Table 2.

Table 2 Urine 8-*iso*-$PGF_{2\alpha}$ levels

(ng/mmol · Cr, $\bar{x} \pm s$)

Group	1st day	3rd day	7th day
Control	30 ± 8 (126)	28 ± 10 (92)	27 ± 11 (84)
Mild HIE	65 ± 13 (62)	27 ± 10 (56)	24 ± 10 (52)
Moderate HIE	155 ± 32 (51)	35 ± 10 (50)	27 ± 11 (48)
Severe HIE	242 ± 41 (38)	51 ± 13 (38)	29 ± 13 (37)

( ) denotes the case number included in each group.

Urine 8-*iso*-$PGF_{2\alpha}$ concentrations in the normal neonates

As shown in Table 2, urine 8-*iso*-PGF2α levels in the normal neonates on the 1st, 3rd and 7th days had no statistical differences ($F = 2.262$, $P > 0.05$), suggesting that the urine 8-*iso*-$PGF_{2\alpha}$ levels were stable within the first week of life in the normal neonate. The average urine 8-*iso*-$PGF_{2\alpha}$ level was 28 ng/mmol · Cr.

Urine 8-*iso*-$PGF_{2\alpha}$ concentration in neonates with HIE

On the 1st day after birth, urine 8-*iso*-$PGF_{2\alpha}$ levels in mild, moderate or severe HIE neonates were higher than those in normal neonates ($t = 23.05$, 41.43, 55.11, $P < 0.001$). On the 3rd day after birth, the urine 8-*iso*-$PGF_{2\alpha}$ levels in mild HIE neonates regressed to normal, but the levels remained higher in moderate and severe HIE neonates than in the normal controls ($t = 3.66$, $P < 0.001$; $t = 10.59$, $P < 0.001$). On the 7th day after birth, the urine 8-*iso*-$PGF_{2\alpha}$ levels in mild, moderate and severe HIE neonates regressed to normal ($t = 1.75$, $P > 0.05$; $t = 0.53$, $P > 0.05$; $t = 0.80$, $P > 0.05$).

The sensitivity and specificity of urine 8-*iso*-$PGF_{2\alpha}$ determination in assessing the severity of HIE

$\bar{x} \pm 2S$ of urine 8-*iso*-$PGF_{2\alpha}$ level in normal neonates (45.5 ng/mmol · Cr) was defined as the first boundary between the normal and mild HIE. One hundred and twenty-five cases of 126 normal neonates had 8-*iso*-$PGF_{2\alpha}$ levels lower than the first boundary, while 59 of 62 mild HIE neonates had the levels higher than the boundary. $\bar{\chi} \pm 2S$ of urine 8-*iso*-$PGF_{2\alpha}$ level in mild HIE neonates (89.9 ng/mmol · Cr) was defined as the second boundary between the mild and moderate HIE. The urine 8-*iso*-$PGF_{2\alpha}$ levels of 59 cases in 62 mild HIE neonates were within the first and second boundaries, while that of all moderate HIE neonates was higher than the second boundary. $\bar{x} \pm 2S$ of urine 8-*iso*-$PGF_{2\alpha}$ level in moderate HIE neonates (217.5 ng/mmol · Cr) was defined as the third boundary between the moderate and severe HIE. The urine 8-*iso*-$PGF_{2\alpha}$ levels of all 51 moderate HIE cases were within the second and third boundaries, while that of 25 cases in 38 severe HIE neonates was higher than the third boundary. The sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of urine 8-*iso*-$PGF_{2\alpha}$ in the assessment of HIE severity are listed in Table 3.

Table 3 Validity of urine 8-*iso*-$PGF_{2\alpha}$ in the assessment of HIE severity

Boundary of 8-*iso*-$PGF_{2\alpha}$	Sensitivity (%)	Specificity (%)	Accuracy(%)	PPV(%)	NPV(%)
45.5 ng/mmol · Cr	95.2	99.2	97.9	98.3	97.7
89.9 ng/mmol · Cr	100	95.2	97.3	94.4	100
217.5 ng/mmol · Cr	65.8	100	85.4	100	79.7

Discussion

Up to now, the pathologic mechanism of HIE in neonates has not been very clear, but it has been shown that free radical-induced lipid peroxidation plays an important role in neuronal injury after asphyxia[5,6]. The primary target of free radicals in cells is unsaturated fatty acid in lecithoid membrane which results in lipid peroxidation and induces membrane damage. Human neonates nerve cell membrane abounds in unsaturated fatty acid and lacks of SOD and GSH peroxidase(anti-peroxidation system), so it can be attacked by free radicals which can induce oxidation damage[7].

8-*iso*-$PGF_{2\alpha}$ is a kind of prostaglandin-like compound with biological activities. Chemically, it is an alkene, a product of free radical attack on membrane lipids, and this process is enzyme independent. 8-*iso*-

$PGF_{2\alpha}$ is very stable in body fluid and organs, so it can be used as a reliable and accurate index to assess the oxidation of free radicals in vivo and the therapeutic effect of anti-oxidant in clinical trial[4,8]. It has been shown that urine 8-*iso*-$PGF_{2\alpha}$ concentrations in patients with cardiovascular disease, respiratory disease, nervous disease, cirrhosis and diabetes increased and had a positive correlation with the severity of the disease[1,3]. No matter whether in vivo or in vitro, there is a positive correlation between 8-*iso*-$PGF_{2\alpha}$ concentrations and the conditions such as the sustained anoxemia and ischemia or ischemic reperfusion. Up to date, there are some relevant reports about neonatal umbilical blood 8-*iso*-$PGF_{2\alpha}$[9,10], but there are no reports about testing normal and abnormal neonatal urine 8-*iso*-$PGF_{2\alpha}$.

In this study, urine 8-*iso*-$PGF_{2\alpha}$ levels of normal neonates were stable within the first week after birth (28.3 ± 9.5 ng/mmol · Cr), suggesting that it may be suitable to use 8-*iso*-$PGF_{2\alpha}$ as an index to assess lipid peroxidation of neonates in an early stage. The above urine 8-*iso*-$PGF_{2\alpha}$ values should be considered as a reference for normal 1-week neonates because these values were derived from limited samples (126 cases) and repeats (302 measurements). To obtain reliable normal values of urine 8-*iso*-$PGF_{2\alpha}$ during the whole neonatal stage, the sample size should be extended and the collection period should last for 28 days. In this study, an other important result was that urine 8-*iso*-$PGF_{2\alpha}$ of HIE neonates is higher than that of normal neonates. The elevated values had a positive correlation with the severity of the disease in the 1st day, suggesting that HIE neonates has remarkable lipid peroxidation and free radical damage; and the severer the HIE condition, the stronger the peroxidation. It is necessary for HIE neonates to use anti-oxidant to alleviate the lipid peroxidation and the produce of free radicals. In addition, the peak of the urine 8-*iso*-$PGF_{2\alpha}$ level in HIE neonates appeared earlier (within 24 hours) and decreased with day-ages; urine 8-*iso*-$PGF_{2\alpha}$ levels in mild HIE neonates decreased to normal in 3 days; and that in all other HIE neonates decreased to normal in 7 days. The possible explaintions for these results could be that the blood reperfusion occurs and oxygen supply recovers immediately after resuscitation, and that the over-produced free radical induces lipid peroxidation and blood 8-*iso*-$PGF_{2\alpha}$ increases rapidly in a short time. With consumption of free radicals and recovery of enzyme activities, the free radicals are eliminated, so that the urine 8-*iso*-$PGF_{2\alpha}$ levels of the mild HIE neonates decrease to a normal level. Continuous existence of reperfusion after anoxemia and ischemia induces sustained free radical damage and lipid peroxidation in moderate and severe HIE neonates, so that higher urine 8-*iso*-$PGF_{2\alpha}$ contents last for a longer time.

Based on higher sensitivity, specificity, accuracy, positive and negative predictive value, it is suitable to use 45.5 ng/mmol · Cr and 89.9 ng/mmol · Cr of urine 8-*iso*-$PGF_{2\alpha}$ as the boundaries between normal and mild HIE as well as between mild and moderate HIE; while the urine 8-*iso*-$PGF_{2\alpha}$ boundary between moderate and severe HIE, 217.5 ng/mmol · Cr, may be too high so that the sensitivity, accuracy and negative predictive value are low. Therefore, it is necessary to increase the sample size of moderate and severe HIE neonates in order to revise this boundary value.

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