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Original Article

Blood and urine 8-iso-PGF2α levels in babies of different gestational ages

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Abstract: Objective: We measured cord blood and urine 8-iso-prostaglandin F2α (8-iso-PGF2α) levels in babies of different gestational ages to determine lipid peroxidation status. Methods: Babies at gestational ages of 28-43 weeks were divided into group A (28-32 weeks), group B (33-36 weeks), group C (37-41 weeks), and group D (42-43 weeks). 8-iso-PGF2α in umbilical cord blood (UCB) at birth and urine at 6 hours after birth was and tested by ELISA. Results: UCB and urine 8-iso-PGF2α levels in group C were 130.09 ± 31.73 pg/ml and 27.14 ± 6.73 pg/ml, respectively. UCB 8-iso-PGF2α levels in group A and B were 188.42 ± 59.34 pg/ml and 189.37 ± 68.46 pg/ml, and urine 8-iso-PGF2α were 32.14 ± 7.32 pg/ml and 30.46 ± 8.83 pg/ml, respectively. Blood and urine 8-iso-PGF2α levels in group D (post-term) were 252.01 ± 46.42 pg/ml and 44.00 ± 8.50 pg/ml. For all babies, UCB and urine iso-PGF2α levels were significantly correlated ($r = 0.65$, $P < 0.01$). Conclusions: We established blood and urine iso-PGF2α levels in normal full-term babies. Urine 8-iso-PGF2α levels may reflect the extent of lipid peroxidation in babies. In pre-term and post-term babies, there was evidence for increased lipid peroxidation.

Keywords: Babies, lipid peroxidation, 8-iso-prostaglandin F2α, cord blood, urine

Introduction

There is strong lipid peroxidation activity in many babies that have various diseases concomitant with dramatically increased levels of serum 8-iso-PGF2α. This causes lipid peroxidation injuries that play important roles in the pathogenesis of various diseases, especially in children [1-7]. However, the relationship between 8-iso-PGF2α levels and childhood diseases remains unknown. Therefore, establishing the normal reference values of lipid peroxide products in babies is necessary in order to clarify the mechanism(s) of lipid peroxidation in childhood diseases, which should provide evidence for clinical antioxidant treatments for these diseases of children.

8-iso-PGF2α is a prostaglandin-like material with biological activity that was recently discovered [3]. 8-iso-PGF2α is the specific metabolic product of cell membrane arachidonic acid due to the actions of free radicals and lipid peroxidation, and which does not depend on enzyme catalytic processes. 8-iso-PGF2α is very stable in body fluids and tissues. Thus, it is now considered as the most ideal biochemical index for evaluating the extent of free radical oxidation *in vivo* for clinical applications [3].

Thus, in this study, we determined umbilical cord blood and urine 8-iso-PGF2α levels in babies with gestational ages of 37-41 weeks to establish the normal reference values of 8-iso-PGF2α for full-term babies. We also measured these levels in other groups of babies (both pretem and post-term) to assess possible correlations between umbilical cord blood and urine 8-iso-PGF2α levels and to determine whether these reflected lipid peroxidation status based on 8-iso-PGF2α levels.

Material and methods

Clinical data and study groups

Our research subjects were babies whose gestational ages were 28-43 weeks and who were

Table 1. Body weights and gestational ages of babies

Group	Numbers	Body weight (g)	Gestational age (weeks)
A	50	2050.3 ± 88.2	29.08 ± 0.95
B	62	2260.7 ± 95.0	34.92 ± 1.32
C	85	3161.2 ± 265.8	38.73 ± 1.87
D	25	2990.1 ± 252.1	42.65 ± 0.55

Values are means ± standard deviations.

Table 2. Apgar scores of babies at 1 min, 5 min, and 10 min after birth

Group	Numbers	1 min		5 min		10 min	
		4~7	8~10	4~7	8~10	3~7	8~10
A	50	26	24	6	42	0	50
B	62	17	45	6	56	0	60
C	85	0	85	0	85	0	85
D	25	3	22	1	25	0	25

Table 3. General data for the mothers of the babies

Group	Numbers	Age (years)	Gravidity	Number of births
A	50	29.20 ± 2.53	1.75 ± 0.85	1.24 ± 0.35
B	62	28.46 ± 2.90	1.77 ± 0.94	1.28 ± 0.36
C	85	27.95 ± 2.87	1.73 ± 0.87	1.46 ± 0.28
D	25	29.10 ± 2.10	1.63 ± 0.95	1.31 ± 0.30

Values are means ± standard deviations.

born between January 2008 and September 2009 in The Sixth Affiliated Hospital, Sun Yat-Sen University, Baoan Women and Children Hospital, Shenzhen City, Foshan Women and Children Hospital, Foshan City, The causes for premature birth include hypertension in pregnancy, placental dysfunction and premature rupture of membranes. For experiments involving human subjects, our study was approved by the Institutional Review Board of The First Affiliated Hospital, Sun Yat-sen University. Informed consent was provided according to the Declaration of Helsinki. Fresh blood collection and preparation were done as previously described [3].

Our inclusion criteria were the following. A baby's gestational age was 28-43 weeks. There was no evidence of significant complications, such as RDS, hyperbilirubinemia, hypoxic ischemic encephalopathy, infection, or hemolytic disease, by 6 hours after birth. There was no prolonged use of a nasal cannula with a high oxygen concentration at 6 hours after birth. There was no enforcement of oxygen by hoods, CPAP, or by mechanical ventilation. No antioxidant treatments were used, such as VitC, VitE, or others. Mothers of the babies had no previous history of hypertension, cardiovascular disease, kidney disease, or diabetes, and no history of drinking, smoking, or drug abuse. There were no complications for the babies by the ages of more than one month, and Apgar scores were ≥ 8. The mothers of the babies had no complications of pregnancy.

Based on their gestational ages, the babies were divided into the following four groups: Group A (N = 50) was at 28-32 weeks; Group B (N = 62) was at ages of 33-36 weeks; Group C (N = 85) was at ages of 37-41 weeks; and Group D (N = 25) was ages of ≥ 42 weeks.

Sample collection and processing

Immediately after birth, 2 ml of umbilical cord blood was taken from the fetal end and placed in a dry tube without any anticoagulant. The blood was allowed to set for 2 hours at -4°C, and then centrifuged at 3000 rpm for 10 min. The upper plasma layer was collected and stored at -80°C for subsequent analysis.

In addition, 2 ml of urine was collected at 6 hours after birth and stored at -80°C for subsequent analysis.

8-iso-PGF2α determinations

Blood and urine 8-iso-PGF2α levels were determined by ELISA using kits from Assay Designs Company.

Statistical analysis

Results for continuous variables are given as means ± standard deviations. Paired t-tests were used to compare the results of two groups. Single factor analysis of variance (ANOVA) was used to compare the results of more than two groups. Associations between variables were assessed using simple linear regression analysis. A *P*-value of < 0.05 was considered significant. Statistical analysis was done using SPSS13.0 statistics software.

Table 4. Prenatal and intrapartum complications of the babies*

Group	Numbers	Hypertensive disorders in pregnancy	Placental dysfunction/calcification	Premature rupture of fetal membranes
A	5 0	38	3	7
B	62	42	8	8
C	85	0	0	0
D	25	1	18	3

*Two or three complications could exist in the same baby's mother.

Table 5. Cord blood and urine 8-iso-PGF2α levels in full term babies

	Numbers	8-iso-PGF2α (pg/ml)	95% CI
umbilical cord blood	85	130.09 ± 31.73	123.25~136.94
urine	85	27.14 ± 6.73	25.69~28.59
t		35.0637	
p		0.0000	

Values are means ± standard deviations or ranges.

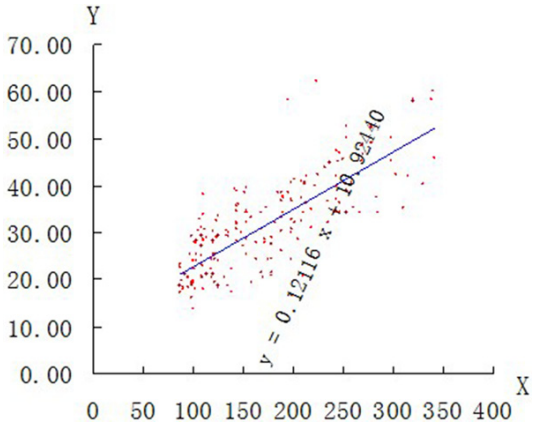

Figure 1. Correlation between umbilical cord blood and urine 8-iso-PGF2α levels (pg/ml).

Results

Clinical characteristics of the babies and mothers

Table 1 shows the body weights (g) and the gestational ages (weeks) of the babies in the four groups (A-D). There were significant differences in body weights and the gestational ages of the babies in the four groups (both $P < 0.01$).

The Apgar scores of the babies in the four groups at 1 min, 5 min, and 10 min after birth are shown in **Table 2**. We excluded babies who had Apgar scores at 1 min of < 4 points (asphyxia) and those with Apgar scores at 10 min of < 8 points. The Apgar scores of the babies in Group C (full term) at 1 min, 5 min, and 10 min were all 8-10 points.

The ages, gravidity, and parity conditions of the mothers of the babies are shown in **Table 3**. For the mothers of the babies in the four groups, there were no significant differences in age ($P = 0.260$), gravidity ($P = 0.315$), or parity ($P = 0.297$).

There were no pregnancy complications for the mothers of the babies in Group C, but there were prenatal and intrapartum complications, including hypertension of pregnancy, placental dysfunction or calcification, or premature rupture of membranes in Group A (N = 41), in Group B (N = 50), and in Group D (N = 19) (**Table 4**).

Cord blood and urine 8-iso-PGF2α levels in normal full-term babies

As shown in **Table 5**, the umbilical cord blood levels of 8-iso-PGF2α for the 85 babies with gestational ages of 37-41 weeks when the babies were born and the urine levels of 8-iso-PGF2α at 6 hours after birth were 130.09 ± 31.73 pg/ml and 27.14 ± 6.73 pg/ml, respectively. These were significantly different ($P < 0.01$).

Correlation between umbilical cord blood and urine 8-iso-PGF2α levels in normal term babies

As shown in **Figure 1**, there was a positive correlation between the umbilical cord blood levels of 8-iso-PGF2α of the 85 babies with gestational ages of 37-41 weeks when the babies were born and the urine levels of 8-iso-PGF2α at 6 hours after birth ($P < 0.01$). The regression equation was Y = 0.12116X + 10.92440 (Y = urine 8-iso-PGF2 level, and X = umbilical blood 8-iso-PGF2 level). The correlation coefficient was 0.6513 and the coefficient of determination (R^2) was 0.8070.

Umbilical cord blood and urine 8-iso-PGF2 α levels in pre-term babies

The blood and urine 8-iso-PGF2α levels of the premature babies with gestational ages of

Table 6. Blood and urine 8-iso-PGF2α levels in babies with gestational ages of 28-32 weeks

	Numbers	8-iso-PGF2α (pg/ml)	95% CI
umbilical cord blood	50	188.42 ± 59.34	171.56~205.29
Urine	50	32.14 ± 7.32	30.06~34.22
t		18.4887	
p		0.0000	

Values are means ± standard deviations or ranges.

Table 7. Blood and urine 8-iso-PGF2α levels in babies with gestational ages of 33-36 weeks

	Numbers	8-iso-PGF2α (pg/ml)	95% CI
Umbilical cord blood	62	189.37 ± 68.46	171.99~206.76
Urine	62	30.46 ± 8.83	28.21~32.70
t		18.1271	
p		0.0000	

Values are means ± standard deviations or ranges.

Table 8. Umbilical cord blood and urine 8-iso-PGF2α levels in babies with gestational ages of 42-43 weeks

	Numbers	8-iso-PGF2α (pg/ml)	95% CI
umbilical cord blood	25	252.01 ± 46.41	232.85~271.17
urine	25	44.00 ± 8.50	40.49~47.50
t		22.0434	
p		0.0000	

Values are means ± standard deviations or ranges.

28-32 weeks (Group A) and 33-36 weeks (Group B) are shown in **Tables 6** and **7**, respectively. **Table 6** shows that the umbilical cord blood levels of 8-iso-PGF2α of the premature babies in group A (gestational ages of 28-32 weeks) when these babies were born (188.42 ± 59.34 pg/ml) and the urine levels of 8-iso-PGF2α at 6 hours after birth (32.14 ± 7.72 pg/ml) were dramatically different ($P < 0.01$). The 95% CI's were 171.56-205.29 pg/ml and 30.06-34.22 pg/ml, respectively.

Table 7 shows that the umbilical cord blood levels of 8-iso-PGF2α in the premature babies in Group B (gestational ages of 33-36 weeks) when these babies were born (189.37 ± 68.46 pg/ml) and the urine levels of 8-iso-PGF2α at 6 hours after birth (30.46 ± 8.83 pg/ml) were also dramatically different ($P < 0.01$). The 95% CI's were 171.99-206.76 pg/ml and 28.21-32.70 pg/ml, respectively.

Because there was no significant difference in the umbilical cord blood and urine 8-iso-PGF2α levels of the preterm babies in Groups A and B, these groups were combined into one group. The umbilical cord blood and urine 8-iso-PGF2α levels of the 112 pre-term babies (gestational ages of 28-36 weeks) in this combined group were 188.90 ± 63.90 pg/ml and 31.30 ± 8.08 pg/ml, respectively, and these levels were dramatically different compared with the levels of the 85 pre-term babies with gestational ages of 37-41 weeks (both $P < 0.01$).

Perinatal umbilical cord blood and urine 8-iso-PGF2α levels in post-term babies

Table 8 shows the blood and urine 8-iso-PGF2α levels in the post-term babies (Group D) with gestational ages of 42-43 weeks. This shows that the umbilical cord blood levels of 8-iso-PGF2α (252.01 ± 46.41 pg/ml) of the 25 post-mature babies when they were born and the urine levels of 8-iso-PGF2α at 6 hours after birth (44 ± 8.50 pg/ml) were also dramatically different ($P < 0.010$). The 95% CI's were 232.85-271.17 pg/ml and 40.49-47.50 pg/ml, respectively.

Comparisons of umbilical cord blood and urine 8-iso-PGF2α levels in babies of different gestational ages

Table 9 and **Figure 2** show the umbilical cord blood and urine 8-iso-PGF2α levels in the babies of different gestational ages. The umbilical cord blood 8-iso-PGF2α levels when they were born were all obviously higher than those of urine 8-iso-PGF2α regardless of the gestational ages and these differences were statistically significant. The umbilical cord blood and urine 8-iso-PGF2α levels in the post-term babies (gestational ages of 42-43 weeks) were the highest, the umbilical cord blood and urine 8-iso-PGF2α levels in the premature babies (gestational ages of 28-32 weeks and 33-36 weeks) were next highest, and the umbilical

cord blood and urine 8-iso-PGF2α levels in the full term babies (gestational ages of 37-41 weeks) were the lowest.

With regard to comparisons of 8-iso-PGF2α levels between these four groups, there were significant statistical differences in cord blood and urine levels between the combined A + B group and group C (both $P < 0.01$), between the A + B group and group D (both $P < 0.01$), and between group D and group C (both $P < 0.01$).

Discussion

Previous studies have shown that the blood and urine 8-iso-PGF2α levels in patients with cardiovascular diseases, respiratory diseases, neurodegenerative diseases, and diabetes were increased and were positively correlated with disease severity [6]. Clinical studies of hypertensive disorders in pregnancy showed that the obviously increased levels of 8-iso-PGF2α were correlated with the severity of pregnancy induced hypertension. Zhuang [8] found that the plasma 8-iso-PGF2α levels of normal pregnant women during pregnancy were stable, but that the plasma 8-iso-PGF2α levels of pregnant women with mild preeclampsia and hypertensive disorders during the middle and late stages of pregnancy and the umbilical cord blood and urine 8-iso-PGF2α levels of their newborns were higher than those in a normal pregnancy group. There were positive correlations between the 8-iso-PGF2α levels in the plasma of pregnant women and baby cord blood 8-iso-PGF2 levels, which suggested that the levels of 8-iso-PGF2α in maternal plasma, baby cord blood, and urine might be a good indicator of lipid oxidative damage.

Very little is known regarding the role of 8-iso-PGF2α in determining lipid peroxide status of babies. In general, superoxide dismutase (SOD) activity in the brain tissues of babies and glutathione peroxidase activity are relatively insufficient. In addition, more of their cerebral cell membranes are rich in unsaturated fatty acids, which make them vulnerable to attack by oxygen free radicals and subject to lipid peroxidation. Inder et al. [4] and Brault et al. [1] found that the cerebrospinal fluid 8-iso-PGF2α levels in severe brain damaged children, such as cerebral white matter injured babies with extremely low body weights, were significantly increased. These high levels of 8-iso-PGF2α were toxic and could selectively induce neural vascular endothelial cell death.

Chen et al. [2] determined the urinary 8-iso-PGF2α levels of 126 normal newborns and 151 hypoxic-ischemic encephalopathy (HIE) children using an ELISA method, and their results showed that the urine 8-iso-PGF2α levels in HIE babies were apparently higher those of normal babies and were closely related to the severity of their disease. There was also obvious lipid peroxidation in red endotoxemia babies that was indirectly induced by erythrocyte destruction, and the plasma 8-iso-PGF2 levels were significantly increased and positively associated with indirect bilirubin levels. Zhong et al. [9] found that the pathogenesis of retinopathy of prematurity (ROP) was closely related to the plasma 8-iso-PGF2α levels, which might be used to predict the occurrence of ROP in its early stage.

In this study, we measured umbilical cord blood and urine 8-iso-PGF2α levels in babies of different gestational ages (28-43 weeks). Our results showed that lipid peroxidation products in premature babies (28-36 weeks) and perinatal babies (42-43 weeks) were increased, and that their blood and urine 8-iso-PGF2α levels were obviously higher than those in normal full-term babies (37-41 weeks). There are two possible mechanisms for the enhanced levels of lipid peroxidation products in premature infants.

One is the relatively immature superoxide dismutase activity of the anti-oxidative systems of premature infants and insufficient glutathione peroxidase activity. Another is that some mothers of premature infants during pregnancy were complicated by gestational hypertension, and there was enhanced oxidative stress and lipid peroxidation in the placenta, which could have resulted in the lipid peroxides produced to enter the fetus. The enhanced lipid peroxidation in perinatal infants probably primarily resulted from placental dysfunction, and this increased lipid oxidation might affect fetuses and babies.

In view of this lipid peroxidation in pre-term and post-term births, clinicians might implement antioxidant interventions early on for these newborns by blocking the adverse effects of lipid peroxidation on a newborn. One study showed that large doses of antioxidants, such

Table 9. Umbilical cord blood and urine 8-iso-PGF2α levels in babies with different gestational ages*

	Numbers	Umbilical cord blood (pg/ml)	Urine (pg/ml)	t	p
28~32 weeks (A group)	50	188.42 ± 59.32	32.14 ± 7.32	18.4887	0.0000
33~36 weeks (B group)	62	189.37 ± 68.46	30.46 ± 8.83	18.1271	0.0000
37~41 weeks (C group)	85	130.09 ± 31.73	27.14 ± 6.73	35.0673	0.0000
42~43 weeks (D group)	25	252.01 ± 46.41	44.00 ± 8.50	22.0434	0.0000
F		41.6800	31.0400		
p		0.0000	0.0000		

*Levels in groups A and B were comparable and were combined into one group (A + B). Values are means ± standard deviations.

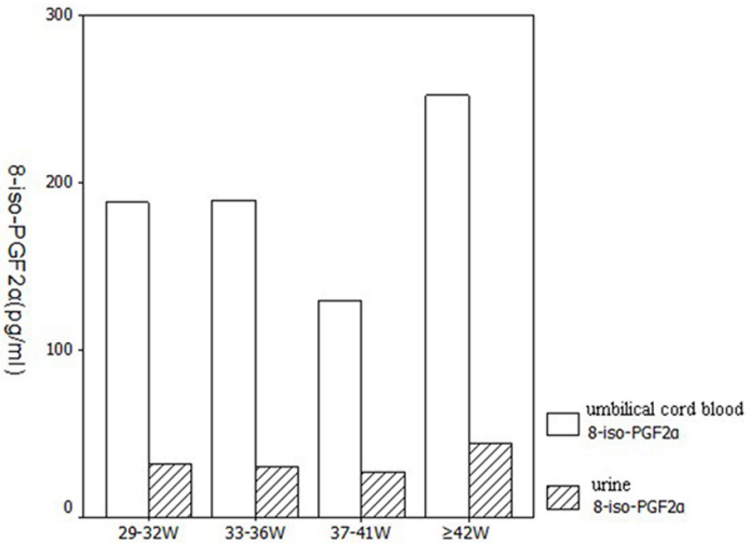

Figure 2. Umbilical cord blood and urine 8-iso-PGF2α levels (pg/ml) in babies of different gestational ages.

as ambroxol hydrochloride and N-acetylcysteine, could significantly reduce the levels of blood and urine 8-iso-PGF2 and alleviate lipid oxidative injury in babies [5].

An ELISA method was recently developed to determine 8-iso-PGF2α levels. This method is simple, does not require expensive instrument, the cost per determination is not high, and it has high sensitivity, specificity and accuracy; thus, this has potential for wide clinical applications. In the present study, 85 full term babies with gestational ages of 37-41 weeks were chosen to establish the normal reference ranges of umbilical cord blood and urine 8-iso-PGF2α levels in normal full-term newborns (within 6 hours after birth), which could eliminate the effects of gestational age on cord blood results. In addition, urine was collected at 6 hours after birth in order to minimize the effects of baby's diseases and clinical intervention on our results.

The normal reference values we obtained for umbilical cord blood and urine 8-iso-PGF2α levels of normal full-term babies were 130.09 ± 31.73 pg/ml and 27.14 ± 6.73 pg/ml, respectively, and the respective 95% confidence intervals were 123.25-136.94 pg/ml and 25.69-28.59 pg/ml. These results should aid in basic clinical research and applications in perinatal medicine.

Because clinical urine collection is simple, convenient and is non-invasive and reproducible, we assessed the correlation between cord blood and urine 8-iso-PGF2 levels in normal full-term babies within the first 6 hours after birth. This showed that urine 8-iso-PGF2 levels were positively correlated with umbilical cord blood 8-iso-PGF2 levels.

In conclusion, we established the umbilical cord blood and urine iso-PGF2α levels of normal full-term babies and their 95% confidence intervals (123.25-136.94 pg/ml and 25.69-28.59 pg/ml, respectively). The urine 8-iso-PGF2α levels might be used to reflect the extent of lipid peroxidation in babies. There was evidence for strong lipid peroxidation in pre-term and post-term babies at their time of birth.

Disclosure of conflict of interest

None.

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