· Original Article in English ·

Erythropoietin levels in serum and cerebrospinal fluid of neonates with hypoxic-ischemic encephalopathy

Ning CHEN, Jian MAO, Yue DU

Department of Pediatrics, Second Affiliated Hospital, China Medical University, Shenyang 110004, *China*

Abstract: Objective To observe the changes of erythropoietinerythrogenin (Epo) in serum and cerebrospinal fluid (CSF) in neonates with hypoxic-ischemic encephalopathy (HIE), and to study the relationship between Epo levels and brain injury. **Methods** Serum Epo levels were measured by radioimmunoassay in 26 neonates with HIE (HIE group, 8 mild, 10 moderate and 8 severe) and 8 normal neonates (Control group) at 0-24 hrs, 48-72 hrs and 7-10 days of their lives. CSF Epo levels were measured at 48-72 hrs of their lives and brain MRI scans were taken 7-10 days after birth in the HIE group. **Results** In the Control group, serum Epo levels decreased significantly within days after birth ($P<0.05$); However, in the HIE group serum Epo levels increased during 1-3 days then decreased thereafter; Significantly decreased levels were observed only in mild HIE neonates ($P<0.05$). In every time period, the serum Epo concentration in severe HIE neonates was significantly higher than in mild and moderate HIE neonates. It was also observed that CSF Epo levels in severe HIE neonates were significantly higher than those of mild and moderate HIE neonates ($P<0.01$). There was a significant linear and positive correlation between serum and CSF Epo levels at 48-72 hrs in severe HIE neonates ($r = 0.76$, $P < 0.05$), but not in mild and moderate HIE neonates. CSF Epo levels in neonates with severe cranial MRI abnormalities were significantly higher than those of neonates with mild and moderate cranial MRI abnormalities ($P < 0.01$). **Conclusions** The maintained and increased serum Epo levels may be a marker of severe hypoxic-ischemia in neonates with HIE. CSF Epo levels can reflect the severity of cerebral hypoxia-ischemia; Impaired blood-brain barrier might account for the increased CSF Epo levels. [**Chin J Contemp Pediatr, 2005, 7(2): 107-111**]

Key words: Hypoxia-ischemia, brain; Erythropoietin; Infant, newborn

新生儿缺氧缺血性脑病血清及脑脊液中促红细胞生成素的变化

陈宁,毛健,杜悦 中国医科大学附属第二医院儿科,辽宁 沈阳 110004

[**摘 要**] **目的** 探讨缺氧缺血性脑病(HIE)患儿血清和脑脊液(CSF)中促红细胞生成素(Epo)的变化,观察 Epo 与脑损伤的关系。**方法** 对 26 例 HIE 患儿(轻度 8 例,中度 10 例,重度 8 例)和 8 例正常对照组进行研究,在生后 0 ~ 24 h、48 ~ 72h 及 7 ~ 10 d 抽取静脉血,HIE 组在生后 48 ~ 72 h 腰穿取 CSF,放射免疫法测定血清和 CSF Epo 含量,HIE 组生后 7 ~ 10d 做头部 MRI 检查。**结果** 对照组血清 Epo 随日龄增加呈下降趋势,有统计学差异($P<0.05$);HIE 各组血清 Epo 水平呈先上升而后下降趋势,其中仅轻度组血清 Epo 变化显著($P<0.05$);各时间段重度组较轻度组、对照组血清 Epo 明显升高,有统计学差异。重度 HIE 组较轻、中度组 CSF Epo 明显升高($F = 6.86$, $P<0.01$)。重度 HIE 组 48 ~ 72 hr 血清与 CSF Epo 水平有较强直线相关关系($r = 0.76$, $P < 0.05$);而轻、中度 HIE 组血清与 CSF Epo 无直线相关关系($r = 0.12$, $r = 0.09$, $P > 0.05$)。头部 MRI 为重度改变的 HIE 患儿 CSF 中 Epo 水平均较头部 MRI 为轻、中度改变的 HIE 患儿显著升高($F = 8.56$, $P < 0.01$)。**结论** HIE 患儿血清 Epo 显著升高,持续不降是病情危重的标志。CSF 中 Epo 显著升高提示 HIE 患儿脑损伤严重,预后不良。重度 HIE 患儿可能存在着血脑屏障的破坏,Epo 可能透过血脑屏障。 [**中国当代儿科杂志,2005, 7(2): 107- 111**]

[**关 键 词**] 缺氧-缺血,脑;促红细胞生成素;婴儿,新生

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Hypoxic-ischemic encephalopathy (HIE) is a common neonatal disease. Severe HIE may lead to death and sequelae such as cerebral palsy, epilepsy and cognitive disablitiy in some survivors. Currently,

[Received] July 30, 2004; [Revised] September 28, 2004
[Biography] Ning CHEN (1971 -), Female, Lecturer, Specializing in neonatology.
[Correspondence Author] Jian MAO, Department of Pediatrics, Second Affiliated Hospital, China Medical University, Shenyang 110004, China (Email: 7ning@sina.com.cn).

no effective therapeutic methods have been reported.

It had been believed for a long time that erythropoietin (Epo) only exists in blood, stimulating haematogenesis and differentiation processes. But recent studies have indicated that Epo and Epo-receptor expression can be detected in the central nervous system (CNS)[1,2]. Studies have shown that, similar to the changes in blood, hypoxia increases the expression of Epo-mRNA in the CNS[1,3]. It has also been reported that Epo and its neuron specific Epo receptor have neuroprotective effects[4-7]. In this study, serum and CSF Epo levels of HIE neonates were measured using radioimmunoassay (RIA) in order to clarify the relationship between Epo and brain injury.

Subjects and methods

Grouping of patients

Twenty-six HIE neonates (20 males, 6 females) hospitalized in the Neonatal Division of the Second Hospital of China Medical University from August 2000 to August 2001 were enrolled in this study (HIE group). The HIE group was subdivided into Mild HIE ($n = 8$), Moderate HIE ($n = 10$) and Severe HIE groups ($n = 10$). The diagnosis and grading of HIE met the criteria established by the 1996 Hangzhou Conference[8]. The gestational age of the HIE group was between 38 and 42 weeks and body weight was between 2 500 and 4 000 g. Subjects with the following conditions were excluded from the study: inherited metabolic disease, hemolytic disease of the newborn, anemia, polycythemia; neonates born from diabetic mothers; neonates born from mothers with severe cardio-pulmonary, and renal diseases. All neonates with HIE were hospitalized within 24 hours after birth and none of them had no hypoxia after admission ($SaO_2 > 96\%$). Regular treatment was performed for them. Cranial MRI scans were taken in 24 cases (7 mild, 10 moderate, and 7 severe) at 7-10th day after birth; scanning results were classified as mild, moderate and severe MRI abnormalities[9]. The grading principle of MRI changes was as follows: Mild: spot or strip like high signals in cortex and subcortex region, with or without supra/sub-tentorium subarachnoid hemorrhage; Moderate: symmetrical spot-like high signals in deep white matter in bilateral frontal lobe and strip-like high signals along walls of lateral cerebral ventricle in addition to manifestations of mild grade with topical cerebral edema; Severe: high signals in basal nuclei, thalamus, along with relatively low signals in the posterior limb of internal capsule, with subcortex cystic-form low signal necrosis region, diffused cerebral edema, intraventricular hemorrhage and enlargement of lateral ventricle of cerebrum in addition to the Mild group findings. Among the 7 cases of severe MRI changes, 6 were in the Severe HIE group and 1 in the Moderate HIE group.

Eight (6 males, 2 females) normal full-term newborns hospitalized within 24 hours after birth were chosen as Control group. None of them had asphyxia, CNS symptoms, anemia or respiratory/circulatory system diseases.

Population characteristics of both groups are shown in Table 1.

Table 1 Population characteristics

	Control group	HIE groups		
		Mild	Moderate	Severe
n	8	8	10	8
Mother age(year)	27 ±4.5	26.8 ±3.7	26.1 ±4.3	25.4 ±7.1
Delivery routes				
Vaginal delivery	5	6	7	6
Cesarean section	3	2	2	2
Gestational age(week)	39.4 ±0.8	39.8 ±0.9	39.5 ±1.08	39.2 ±1.46
Birth weight (kg)	3.24 ±0.13	3.26 ±0.29	3.31 ±0.48	3.33 ±0.47
1- min Apgar score				
4-7	0	5	6	0
<4	0	3	4	8
5- min Apgar score				
>5	0	6	8	0
≤5	0	2	2	8
MRI changes grading				
Not done	8	1	0	1
Normal		3	0	0
Mild		3	4	0
Moderate		1	5	1
Severe		0	1	6

Methods

Collection of samples

A sample of 2 mL venous blood was collected in the morning at 0-24 hours, 48-72 hours and 7-10 days after birth in both groups. Serum was isolated by centrifuging at 2 000 rpm for 15 minutes after full clotting and kept at -70° C. CSF were collected by lumbar puncture in the HIE group at 48-72 hours after birth and were then stored at -70°C until assayed. CSF samples with contaminated blood were excluded.

Measurement of Epo levels

Epo levels were assayed by the RIA. All measure-

ments were performed by one person at one time. The banlance method was applied in the experiment. The inner batch CV was 5% and the inter one was 10%.

Statistical analysis

Values in the text were given as $\bar{x} \pm s$. The one-way ANOVA were used to analyze differences between groups. A two-two comparison among multiple samples was done by the q test using SPSS 11.5 software. The linear regression between paired samples was used to determine the correlation between CSF and serum Epo levels.

Results

Serum and CSF Epo levels changes

In the Control group, serum Epo levels decreased significantly within days after birth ($F = 4.04$, $P < 0.05$). However, in the HIE groups serum Epo levels increased during 1-3 days and then decreased, but significant differences were observed only in the Mild HIE group ($F = 4.88$, $P < 0.05$); serum Epo concentration on the 3rd day was significantly higher than that on the 7th day ($q = 4.5$, $P < 0.05$). In every time period, serum Epo concentration in the Severe HIE group was significantly higher than that of the Mild and Moderate HIE groups; no remarkable ascending trend was noticed when comparing the Mild and Moderate HIE groups with the Control group. CSF Epo levels in the Severe HIE group were significantly higher than in the Mild and Moderate HIE groups ($F = 6.86$, $P < 0.01$). See Table 2.

Table 2 CSF and serum Epo levels ($\bar{x} \pm s$, ng/mL)

Group	n	Blood			CSF
		0-24 hrs	48-72 hrs	7-10 days	48-72 hrs
Control	8	4.61 ± 0.95	4.39 ± 1.23	3.22 ± 0.96[e]	
HIE					
Mild	8	4.71 ± 1.00	5.44 ± 0.80[f]	4.00 ± 0.96	2.34 ± 0.44
Moderate	10	5.02 ± 1.39	5.44 ± 1.30	4.09 ± 0.89	2.65 ± 0.67[e]
Severe	8	6.69 ± 2.16[a,c]	7.54 ± 2.05[b,d]	5.93 ± 1.87[b,d]	3.40 ± 0.68[d]
F		3.58	7.02	7.11	6.86
P		<0.05	<0.01	<0.01	<0.01

a vs the Control group $P < 0.05$; b vs the Control group $P < 0.01$; c vs the Mild group $P < 0.05$; d vs the Mild group $P < 0.01$; e vs the paired serum group (0-24 hrs) $P < 0.05$; f vs the paired serum group (7-10 days) $P < 0.05$

Relationship between serum and CSF Epo levels

A linear correlation was observed between serum and CSF Epo levels at 48-72 hours in the Severe HIE group ($r = 0.76$, $P < 0.05$). However, no linear relationship was noticed between them in the Mild and Moderate HIE groups ($r = 0.12$, $r = 0.09$, $P > 0.05$).

Relationship between cranial MRI changes and CSF Epo levels

CSF Epo levels in neonates with severe cranial MRI changes were significantly higher than those of neonates with mild and moderate cranial MRI changes ($F = 8.56$, $P < 0.01$). See Table 3.

Table 3 Relationship between brain MRI changes and CSF Epo levels ($\bar{x} \pm s$, ng/mL)

MRI changes	n	Epo levels
Mild	7	2.24 ± 0.51[a]
Moderate	7	2.76 ± 0.50[a]
Severe	7	3.38 ± 0.57
F		8.56
P		<0.01

a vs neonates with severe cranial MRI changes $P < 0.01$

Discussion

Up until the present the erythroid lineage has been thought to be a sole physiological target of Epo. In the fetal stage, Epo is produced in the liver where erythropoiesis takes place. In adults, Epo produced by the kidneys travels in the circulation to reach erythropoietic tissues, bone marrow, and spleen (in rat, mouse, etc.). Synthesis of Epo is regulated by oxygen, and hypoxia may up-regulate the Epo gene expression. This study indicated that serum Epo levels in each HIE group and each time period were higher than in normal controls. Significant differences were observed between the Severe HIE group and Mild and Moderate HIE groups as well as the Control group, while no remarkable ascending trend was noticed when comparing the Mild and Moderate HIE groups with the Control group. These results indicated that serum Epo levels might reflect the severity of hypoxic-ischemia, which is consistent with Rolf's report[10]: Increased serum Epo levels were correlated with pH and base excess (BE). Results of this study also indicated that: in the Control group serum Epo levels decreased significantly within days after birth; in the HIE group serum Epo levels increased during 1-3 days then decreased thereafter; Significantly decreased levels were observed in the Mild HIE group, the 3rd day's serum Epo level was significantly different from that of 7th day; however, the decreased level in the Moderate and Severe HIE groups was not significant. It is speculated that the

maintained increased levels of serum Epo in the Severe HIE group might be related to the secondary kidney hypoxic-ischemic injury induced by severe asphyxia.

Juul[11] reported that Epo could be detected in CSF of both full-term and premature infants. Serum and CSF Epo levels increase in neonates with asphyxia[12]. As no CSF data of normal controls were available in this study, changes of CSF Epo concentrations in the HIE group compared with the Control group were unknown. But it was found that CSF Epo levels were significantly increased in the Severe HIE group compared with other HIE groups. It was also noticed that CSF Epo levels were considerately increased in HIE neonates with severe MRI changes. These findings indicated that the CSF Epo concentration could reflect the severity of cerebral hypoxia-ischemia; Significantly increased the CSF Epo levels indicate severe brain injury and poor prognosis. It is speculated that the compensatory increase of Epo synthesis may occur after asphyxia in brain tissues in order to protect neurons against injury by excitatory amino acids and reduce neural cell apoptosis[5,7]; but in severe hypoxia status, neural cells injury induced by hypoxia is beyond of the protective effect of increased endogenous Epo, therefore decompensatory changes occur and manifest clinically as severe HIE symptoms as well as cranial MRI changes.

Juul[11,12] reported that Epo could be detected in the CSF of premature and full-term neonates, and serum/CSF Epo concentrations increased significantly during asphyxia. They also reported that no differences could be observed between CSF Epo levels in normal premature neonates treated with rEpo or not, and CSF Epo was not linearly correlated with serum Epo; This confirmed that Epo could not pass the integral blood-brain barrier (BBB). It was speculated that the close correlation between serum and CSF Epo levels during 0 - 48 hours after birth in neonates with asphyxia was caused by the increased permeability or increased peripheral and CNS Epo synthesis induced by hypoxia[12]. In this study, it was found that the linear correlation between serum and CSF Epo levels only existed in severe HIE neonates, while not in mild and moderate HIE neonates. These different results from Juul′ reports may be caused by: (1) Different methods for choosing study subjects: neonates who 1-min Apgar score indicated asphyxia and had clinical neural system symptoms were eligible to this study, while in Juul′s study subjects were chosen as ①neonates with intrauterine distress, fetal heart abnormality and placental abruption; ②1-min Apgar score ≤ 2, 5-min ≤ 5; ③ convulsion within 12 hours of life associated with fetal distress as noted above; and ④postnatal cord blood lactic acid concentration > 8mmol/L. (2) Different Epo measurement methods: Juul used ELISA method, while RIA for this study. (3) Different time of CSF samples collection: 48 - 72 hours after birth vs 0 - 48 hours after birth. This study indicated that hypoxia and ischemia may increase the CSF Epo level; the increased CSF Epo level in HIE neonates may be related to the brain origin Epo synthesis induced by topical cerebral hypoxia. Intracranial simultaneous acidosis caused by severe asphyxia may exist in severe HIE neonates, which increases the permeability of cerebral vessels and damages BBB. Thereafter peripheral Epo passes through the BBB and increases the CSF Epo concentration. Whether the increased CSF Epo level observed after asphyxia is related to the injury of BBB needs further clarification. Studies are also needed to answer whether peripheral rEpo treatment could increase CSF Epo level and enhance its neural protective effect in HIE neonates.

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·病例报告·

遗传性出血性毛细血管扩张症伴肺动静脉瘘 1 例

殷勇

(上海第二医科大学附属新华医院上海儿童医学中心 上海 200127)

[中图分类号] R543.7 [文献标识码] E

患儿,男,10 岁,出生后即发现毛细血管增生,呈弥漫性,以颜面、四肢多见,同时皮肤还有较多血管瘤,阴囊部、腹股沟两侧为多。1 ~ 5 岁期间有反复发热、鼻出血病史,2 岁时左侧腹股沟处血管瘤行冷冻治疗,9 岁发现颜面和指趾甲青紫、活动耐力下降来我院明确诊断。

父母无皮肤病变。患儿有一 5 岁妹妹,体健。

体格检查:神清,精神反应好,营养不良貌,T 37.3℃,P 90 次/min,R 30 次/min,BP 100/70 mmHg,SaO_2 80%,体重 22 kg,特殊面容,鼻梁短,鼻孔小,颜面、躯干部可见毛细血管增生,躯干部见蜘蛛痣,口唇青紫,杵状指趾,颈部、颌下、腋窝、腹股沟处可及数枚直径 1 ~ 2.5 cm 淋巴结,质地中,无压痛,双肺呼吸音粗,无啰音,心前区可及Ⅱ/Ⅵ级的收缩期吹风样杂音,腹平软,无压痛,无包块,肝肋下 2 cm,剑突下1 cm,质地硬,神经系统(-)。

辅助检查:WBC 9.9 × 10^9/L,N 0.55,L 0.43,Hb 135 g/L,血小板 137 × 10^9/L。谷丙转氨酶 77 U/L,谷草转氨酶 92 U/L,总胆红素 12 μmol/L,总蛋白 64 g/L,清蛋白 37 g/L,球蛋白 26 g/L。凝血酶原时间及白陶土部分凝血活酶时间延长。胸片:两肺纹理增多,模糊。胸部 CT:胸廓饱满,心影大小尚正常,上纵隔血管影清晰,气管及其分叉通畅,未见明显狭窄或扩张,两肺纹理偏多。腹部 B 超示肝脏大,肝内光点不均匀。心脏超声示心脏连接位置正常,左室稍增大,射血分数 54%,缩短分数 27%。肺灌注显像:轻度右向左分流。心血管造影:左右肺动脉造影,见两肺弥漫性肺血管扭曲、紊乱,以右肺为甚,肺静脉见提早显影。左室及主动脉造影,见室隔完整,左心室、主动脉形态可,无主动脉缩窄,大动脉水平未见分流,见一小侧支血管形成。上腔静脉回流正常,术中探查下腔静脉回流正常。

根据临床表现与心血管造影证实患儿为遗传性出血性毛细血管扩张症。遗传性出血性毛细血管扩张症,又称 Rendu-Osler-Weber 综合征,系常染色体显性遗传病,以皮肤、黏膜以及内脏的多发性毛细血管扩张和病变部位反复出血为特征。皮肤损害为鲜红色,非搏动性点状或线状毛细血管扩张,扁平或高起,一般涉及鼻黏膜、唇和舌,亦可出现在面、耳、结膜及手掌的皮肤。偶致呼吸道、胃肠道和泌尿道黏膜病变,发生再发性不同程度的出血,鼻出血最突出。这种病变一般较微小约 1 ~ 4 mm,略突出,呈鲜红色,实际上是连接微小动脉和微小静脉之间的小瘘管,破裂时发生局部出血,如病变涉及内脏可致咯血、吐血、血尿等症状,患者大多数为年长儿或青年。本病例 9 岁以后出现的临床表现,包括青紫和活动耐力下降是该症诊断的线索,对临床工作提供了诊断思路。肺动静脉分流可引起低氧血症、继发性红细胞增多症,可产生高动力循环状态,并能产生高排量充血性心力衰竭。本病治疗包括①止血,体表出血以压迫为主,局部可用电烙、冷冻疗法,内脏出血者考虑安络血以助小血管收缩,用垂体后叶素降低内脏血管内压力;②输血,仅用于大量失血,但不宜过量,避免血压过高而使出血难止;③补充铁剂,适用于慢性失血性贫血;④动脉栓塞可用于治疗肝或肺动静脉瘘;⑤β-受体阻滞剂可改善高动力循环状态,降低血流量,使分流量减少。对于本病例的治疗以对症处理为主,原先计划对患儿进行弹簧圈填塞肺动静脉瘘改善临床的青紫,但由于两肺弥漫性病变无法手术。目前患儿已随访 1 年,青紫未见明显加剧但仍有安静时的气急。

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[作者简介]殷勇(1972 年-),男,大学,主治医师。主攻方向:呼吸系统疾病。