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Original Article

Relationship of plasma S100B and MBP with brain damage in preterm infants

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Abstract: To study the relationships of MBP and S100B with PVH-IVH and PVL in preterm infants. 385 cases of preterm infants, whose gestational age was less than 34 weeks, were enrolled in the study. The plasma levels of S100B and MBP were detected within 24 hours and on the 3rd, 7th, 14th day after birth. Cranial ultrasound was preformed 2-3 d, 1 week, 2 weeks, 3 weeks and 4 weeks after birth. They also received Cranial MRI examination before discharge or when the correct gestational age reached 40 weeks. According to the exclusion standard, 73 cases were excluded. The included 312 cases were divided into 3 groups (no brain damage group, PVH-IVH group and PVL group) according to the result of cranial ultrasound and MRI. The differences of plasma levels of S100B and MBP among groups were compared, and the relationships of the plasma levels of S100B and MBP with gestational age in no brain damage group were analyzed. The results of cranial ultrasound and/or MRI showed: 204 cases had no brain damage (enrolled in no brain damage group); 69 cases had PVH-IVH (enrolled in PVH-IVH group); 27 cases had PVL and 12 cases had PVL and PVH-IVH (both enrolled in PVL group). The plasma level of S100B: within 24 h and on the 3rd d after birth, the serum levels of S100B in PVH-IVH group were significantly higher than those in no brain damage group ($P < 0.05$); and the plasma levels of S100B in PVL group were significantly higher than those in no brain damage group and PVH-IVH group (all $P < 0.05$). On 7th d and 14th d after birth, there were no significant differences between PVH-IVH group and no brain damage group ($P > 0.05$); and the plasma levels of S100B in PVL group were still significantly higher than those in no brain damage group and PVH-IVH group (all $P < 0.05$). The plasma levels of MBP: within 24 h and on the 3rd d, 7th d and 14th d after birth, there were no significant differences between PVH-IVH group and no brain damage group (all $P > 0.05$); and the plasma levels of MBP in PVL group were significantly higher than those in no brain damage group and PVH-IVH group (all $P < 0.05$). Correlation analysis of gestational age and S100B, MBP: the plasma level of S100B in no brain damage group had a negative correlation with gestational age ($r = -0.483$, $P = 0.006$), and that of MBP had no correlation with gestational age ($r = -0.295$, $P = 0.105$). The plasma levels of S100B and MBP increased significantly in preterm infants with brain damage within 24 h after birth, and the plasma levels of S100B and MBP in PVL infants were higher than those in PVH-IVH infants. The increased plasma levels of S100B and MBP in PVL infants lasted longer than in PVH-IVH infants. The increased plasma levels of S100B and MBP in preterm infants would have certain clinical significance for judging whether early brain damage and PVL would happen.

Keywords: Periventricular-intraventricular hemorrhage, periventricular leukomalacia, preterm infant, S100B protein, MBP

Introduction

With the development of obstetric and neonatal intensive care technology, the survival rate of preterm children significantly increased, but the incidence of brain injury in preterm children also increased. Brain injury in preterm children mainly manifests as periventricular-intraventricular hemorrhage (PVH-IVH) and periventricular leukomalacia (PVL) [1].

The brain injury mainly occurs in preterm children with a gestational age of less than 34 weeks (or body weight < 2000 g), while white matter damage (WMD) mostly occurs in preterm children with a gestational age of 24 to 32

weeks. WMD incidence of low birth weight infants is about 7% to 26%, and about 10% of survivors may suffer spastic movement defect, also named cerebral palsy; 25%~50% of survivors presented cognitive and behavioral defects or mild dyskinesia, which is the leading cause of nervous system and mental development disorders in premature infants [2], seriously affecting the quality of life in preterm infants. So looking for relatively simple and effective indicators for early diagnosis and timely intervention of PVH-IVH and PVL is the key to achieve better prognosis, having important significance for improving the quality of life in preterm infants, improving population quality and promoting the development of perinatal medicine.

In recent years, myelin basic protein (MBP) and S-100B (S100 calcium-binding protein B) [3-6] attract great attentions, as the sensitive and specific markers of brain damage. MBP is a major component of the central nervous myelin membrane and the changes of MBP levels in blood or cerebrospinal fluid may reflect the extent of WMD in oligodendrocyte glial cell myelin [3, 4]; S-100B mainly exists in astrocytes, and their blood or cerebrospinal fluid levels may reflect the extent of WMD in astrocytes [5]. Which of these biomarkers increased more obvious in a short time after brain injury in preterm infants; which is more specifically; which can reflect the severity of brain damage better; and their relationships with disease outcomes and so on; all of these are not entirely clear, pending further clarification.

Materials and methods

Subjects

Inclusion criteria

Preterm infants in 24 h after birth, whose gestational age was less than 34 weeks, admitted in the NICUs of Guangzhou Women and Children's Medical Center, Guangzhou Huadu District Maternal and Child Health Hospital and Dongguan Hospital Affiliated to Jinan University from Jan. 2010 to Jun. 2013, were enrolled in the study.

Exclusion criteria

Subjects who rejected Cranial MRI during hospitalization, or dead, dropped out of treatment and transferred; Subjects, with serious complications of other systems, include multiple malformations, cyanotic congenital heart disease, neonatal necrotizing enterocolitis, neonatal sepsis and bilirubin encephalopathy.

Sample collection

A total of 385 cases of preterm children met the inclusion criteria; excluding the subjects who dead, self checkout, transferred during hospitalization, and had incomplete medical records, a total of 312 preterm children were included in this study. Plasma samples of every subject were collected at the time points of 24 h, 3 d, 7 d and 14 d after birth. 2 ml whole bloods of fasting preterm infants were drawn from the femoral vein with 5ml sterile syringes and anticoagulated with EDTA. Then the samples were centrifuged in a low temperature centrifuge (4°C, 3000 r/min, 3 min); the upper plasma was separated and injected into cryotubes, which was placed in -80°C refrigerator for batch testing.

Imaging examination and group division

For included subjects, cranial ultrasound was preformed 2-3 d, 1 week, 2 weeks, 3 weeks and 4 weeks after birth. They also received Cranial MRI examination before discharge or when the correct gestational age reached 40 weeks.

Cranial ultrasound and diagnostic criteria: France KONTRON SIGMA210 ultrasonic diagnostic apparatus was used and the sector mechanical probe frequency was 5 Hz; coronal and sagittal plane examinations were performed using anterior fontanel as acoustic window. PVH-IVH showed hyperechoic reflections of subependymal and (or) intraventricular in ultrasound. According to Papile indexing, PVH-IVH was divided into four degrees [7]. Early partial PVL (edema period) in the coronal plane ultrasound mainly performed as bilateral symmetry inverted triangle hyperechoic areas above the lateral external; hyperechoic areas in the sagittal plane mainly distributed in the outer top of the lateral ventricles, and the echo intensity was the same as that of choroid plexus (normal periventricular echo was weaker than that of choroid plexus). In about two weeks, the original echo enhancement area transformed into hypoechoic multiple small cysts changes (advanced partial PVL). The dis-

Table 1. General information of no brain damage group, PVH-IVH group, PVL group

Group	The number of cases	Male	Female	Gestational age (W)	Birth Weight (g)
No brain damage group	204	117	87	30.7±1.6	1528±359
PVH-IVH group	69	38	31	31.5±2.1	1460±275
PVL group	39	22	17	29.2±3.7	1347±312

PVH-IVH: periventricular-intraventricular hemorrhage; PVL: periventricular 1 eucumalacia.

ease outcome of local PVL usually presents four degrees in ultrasound: echo enhancement period (edema period); relatively normal period (preliminary formation of cysts); cyst formation stage; cysts disappearance period. Disease severity of local PVL in ultrasound was divided into four degrees, according to de Vries grading [8]. No abnormalities in each cranial ultrasound represented normal.

Cranial MRI examination: Holland Philips Achieva 3T superconducting magnetic resonance imaging system was used for routine horizontal, sagittal and coronal scan, using spin-echo (SE) sequences, with a thickness of 5 mm. T1-weighted images and T2-weighted images were selected. Cranial MRI showed abnormal signals in white matter regions; focal abnormal signals prompted white matter malacia; It also showed diffuse high T2 signal or low T1 signal; T2-weighted images displayed the reduced capacity of white matter, lateral expansion, irregular edges and poor myelination, suggesting the presence of white matter damage [8].

Grouping: According to the results of imaging examination, all cases were grouped. No brain damage group: Cranial ultrasound and cranial MRI findings showed no obvious abnormalities; there was also no significant damage in the nervous system, which can be considered as no brain damage; PVH-IVH group: cranial ultrasound showed hyperechoic reflection of subependymal and (or) intraventricular, suggesting PVH-IVH; no significant abnormal signal was found in white matter regions by cranial ultrasound and MRI; PVL group: cranial ultrasound found localized or widespread hyperechoic reflection in double periventricular at early stage; a few weeks later (earliest in two weeks after birth), it transformed into the local small cysts changes or extensive cysts changes in periventricular; or cranial MRI showed diffuse white matter signal abnormalities or encephalomalacia. Subjects complicated with PVH-IVH were also included in PVL group.

Plasma MBP and S100B detection

Human S100B ELISA kits and human MBP ELISA kits were purchased from Guangzhou Dahui biological Company. Referring to the kit instructions, operation was conducted. OD values of each well were read on a microplate reader at the wavelength of 450 nm; with OD values as the vertical and the concentrations of the standard as the horizontal, graphs were drawn; find the corresponding concentration on the graph based on sample OD range. The corresponding concentration range was determinate according to the sample OD values in the graph.

Statistical methods

Statistical software package SPSS 13.0 was used for statistical description and analysis; data were expressed as mean ± standard deviation (x±s). S100B and MBP in different groups were compared using one-Way ANOVA; differences between the two groups were compared using LSD test; Pearson correlation analysis of S100B, MBP and gestational age was performed. $P < 0.05$ was considered statistically significant.

Results

General information of included cases

A total of 385 cases of premature infants were enrolled and 73 cases were excluded; finally 312 cases were included in the study, including 175 males and 137 females. Gestational age ranged from 27 to 34 weeks, with a mean age of 31±4.35 weeks; weigh ranged from 870 to 1890 g, with an average of 1485±553 g. 204 cases (65.4%) of 312 cases of preterm children had normal cranial ultrasound and MRI results; there are 108 cases (34.6%) with brain injury, including 69 cases (22.1%) of PVH-IVH, 27 cases of PVL and 12 cases of PVL with PVH-IVH (totally 12.5%). Gestational age and birth weight of each group were shown in **Table 1**.

Imaging results

All 312 subjects received Cranial MRI examination before discharge or when the correct ges-

Table 2. S100B & MBP level in different point in time of no brain damage group, PVH-IVH group, PVL group

Group	The number of cases	MBP (μg/L)				S100B (μg/L)			
		24 h	3 d	7 d	14 d	24 h	3 d	7 d	14 d
No brain damage group	204	3.37±0.71	3.25±0.65	3.40±0.62	3.28±0.92	5.24±1.89	5.48±0.97	5.57±0.59	4.81±0.78
PVH-IVH group	69	4.17±1.52	4.32±1.75	4.78±1.90	3.55±0.98	8.93±2.47[a]	7.66±1.85[a]	6.25±0.71	5.13±1.43
PVL group	39	8.59±3.36[a,b]	9.02±2.68[a,b]	9.71±3.93[a,b]	9.17±3.52[a,b]	12.52±4.60[a,b]	11.64±3.59[a,b]	12.73±4.82[a,b]	10.91±4.32[a,b]

PVH-IVH: periventricular-intraventricular hemorrhage; PVL: periventricular leucumalacia; compared with no brain damage group, [a]$P < 0.05$; compared with PVH-IVH group, [b]$P < 0.05$.

Table 3. The correlation between S100B and MBP

24 h	R = 0.535	P < 0.01
3 d	R = 0.598	P < 0.01
7 d	R = 0.617	P < 0.01
14 d	r = 0.509	P<0.01

tational age reached 40 weeks. The examination age was (50.4±22.3) d. 65.4% (204 cases) of 312 cases of preterm infants had normal cranial ultrasound and MRI results, without brain parenchymal echo enhancement, intraventricular and intracerebral hemorrhage, ventricular dilatation and abnormal signal of cranial MRI in white matter area; with normal morphology of ventricular system. Cranial ultrasound suggested that 69 cases (22.1%) had PVH-IVH, mainly presenting as hyperechoic reflection of subependymal and (or) intraventricular, including 28 cases of grade I and 41 cases of grade II~III; Later cranial MRI examination revealed no abnormal signals in white matter of the other parts. 39 cases (12.5%) had periventricular hyperechoic on the 3rd d in cranial ultrasound; 28 cases had sustained hyperechoic on the 7th d; eight cases were detected malacia on the 3rd week; cranial MRI showed that 28 cases of preterm infants had varying degrees of white matter damage, including 16 cases with diffuse abnormal signal in white matter area and 12 cases with focal malacia; cranial ultrasound showed that there were 12 of the 39 cases also with grade I~II PVH-IVH between 7 and 21 d after birth.

Plasma S100B and MBP levels

The plasma level of S100B: within 24 h and on the 3rd d after birth, the plasma levels of S100B in PVH-IVH group were significantly higher than those in no brain damage group (P < 0.05); and the plasma levels of S100B in PVL group were significantly higher than those in no brain damage group and PVH-IVH group (all P < 0.05). On 7th d and 14th d after birth, there were no significant differences between PVH-IVH group and no brain damage group (P > 0.05); and the plasma levels of S100B in PVL group were still significantly higher than those in no brain damage group and PVH-IVH group (all P < 0.05). The results were shown in **Table 2**.

The plasma levels of MBP: within 24 h and on the 3rd d, 7th d and 14th d after birth, there were no significant differences between PVH-IVH group and no brain damage group (all P > 0.05); and the plasma levels of MBP in PVL group were significantly higher than those in no brain damage group and PVH-IVH group (all P < 0.05). The results were shown in **Table 2**.

Correlation analysis of gestational age and S100B, MBP: the plasma level of S100B in no brain damage group had a negative correlation with gestational age (r = -0.483, P = 0.006), and that of MBP had no correlation with gestational age (r = -0.295, P = 0.105). The correlation between S100B and MBP was shown in **Table 3**. There were significant correlations between S100B and MBP at 24 h, 3 d, 7 d, and 14 d, respectively.

Discussion

Preterm children with brain injury lack of specific clinical manifestations in the early days. Early diagnosis of preterm infants with brain injury depend on imagine inspection [9]. Currently, cranial B-ultrasound was the first choice for screening preterm children with brain injury. It showed a higher rate in germinal layer matrix, intraventricular hemorrhage, posthemorrhagic hydrocephalus as well as the focal cystic PVL. However, it has some limitations in early diagnosis of focal PVL without cystic and diffused PVL [10]. It is reported that in the preterm infants weighing less than 1500 g, the incidence of focal PVL was only 3% to 5% while that was 20% to 50% in diffused PVL [11]. When the diameter of cysts was greater than 5 mm, cranial B-ultrasound showed high sensitivity in focal PVL. While the diameter of cysts was less than 5 mm, it showed low diagnosis sensitivity. In addition, the diagnosis results of ultrasound were largely depending on the technical of the operator. Compared with cranial B-ultrasound, MRI has a higher sensitivity in focal and diffused PVL [12]. In particular, diffusion weighted image (DWI) [13] has a higher sensitivity for early diagnosis of PVL. But for preterm infants with very low birth weight infants, their condition change frequently and moving is very difficult for them. As the check time of MRI is long, there are certain difficulties and risks. Diffused PVL may be more common than local PVL, while B-ultrasound is insensi-

tive for early diagnosis of diffused PVL. Therefore we advocate to do routine MRI before pre-discharge or at corrected gestational age of 40 weeks in order to discovery the genetic change of misdiagnosed locally PVL and diffused PVL at early stage [14].

Early diagnosis can be carried out by cranial B-ultrasound screening for preterm infants with intracranial hemorrhage (eg PVH-IVH). In recent years, because of prevention and early diagnosis of PVH-IVH, the incidence of PVH-IVH showed gradually downward trend [15]. While white matter damage (WMD) especially PVL has become the main type of brain injury in preterm infants. The etiology of WMD is complex, and there is little knowledge about its pathogenesis and neurobiology. Therefore the occurrence of WMD is also difficult to avoid.

In this study, bedside cranial B-ultrasound combined MRI was used for the diagnosis. The patients were divided into three groups, no brain damage group, PVH-IVH group and PVL groups. What is worth mentioned is that, in the early injury of white matter, because of edema reasons, the main manifestations of ultrasound was echo enhancement, including a transient and persistent echogenic. Some studies showed that, a transient periventricular echogenicity generally recovered about 7 d, while there will be about 20% of cysts in the persistent echogenic [16]. In this study, 39 cases were found echogenic by cranial B-ultrasound in the 2~3 d. 28 cases were found echogenic in the 7 d. Cyst was found in 8 cases in the last examination of MRI. Therefore, regular cranial B-ultrasound inspection played an important role in the judgment and prognosis for preterm infants with brain injury. Given the inclusion and exclusion criteria of the study, discontinuous observation in this study and many patients in Guangzhou Women and Children Medical Center NICU were transferred from other hospitals, it is inappropriate to do the incidence analysis of preterm infants with brain injury.

Early diagnosis and intervention of preterm infants with brain injury was very important for its prognosis. Currently, imaging was dependent for diagnosis. 3 to 4 weeks after brain injury was the best time for diagnosis of PVL by B-ultrasound. In most cases, 1 to 6 month after both was the best diagnosis time by MRI. In fact, before radiographic changes occurred, acute injury of nerve cells had already occurred. Therefore, in recent years, myelin basic protein (MBP), S-100B etc. were served as sensitivity and specificity biomarkers of brain injury, and these studies aroused much attention [17, 18].

MBP is a myelin-rich protein, which accounted for 30% of total myelin protein. Only mature oligodendrocytes could synthesize MBP to complete the myelination of nerve fibers. MBP covalently bound to the serosal surface of myelin in the oligodendrocytes and closely integrated with myelin lipid, maintaining the structure and function stability of myelin; it is the marker of oligodendrocytes; excluding the nerve system, MBP levels are very low in other tissues; when myelin is destructed, MBP can be released into the cerebrospinal fluid and blood, so MBP is a specific biochemical indicator for white matter damage, especially for myelin damage [4]. Studies on infants with hypoxic-ischemic encephalopathy (HIE) found that MBP levels were positively correlated with the degree of brain damage [19]; in mild and moderate HIE, MBP increase was not obvious, while in severe HIE, MBP significantly increased [20].

The study found that in PVL group within 24 h after birth, 3, 7, 14 d, the MBP levels in serum were significantly higher than injury-free brain and PVH-IVH group, which was same as Huang runzhong's [21] previous report. In addition, the study showed that MBP had no significant correlation with gestational age, which was consistent with Li Jianming et al's [22] study. But Li Jianming did not find that there was significant difference in serum MBP level between preterm infants with brain injury and normal preterm infants group. Maybe because the subjects and the groups were different, brain injury group in Li's research included PVH-IVH and PVL and the main brain injury was mild or moderate. However in this study, brain injury group were classified into PVL and PVH-IVH group. We found that in PVH-IVH group (mainly for the simple periventricular, intraventricular hemorrhage), compared with non-injury group, there was no significant increase of plasma MBP levels, indicating that MBP was more suitable in early diagnosis for PVL with brain injury.

S100B was mainly composed by astrocytes synthesis in central nervous system and gathered in astrocytes, schwann cells and neurons, which was considered to be a specific marker of glial cell [23, 24]. In acute brain injury, S100B released from damaged tissues, part of which

through the blood-brain barrier hemodynamic and redistributed into the environment [25, 26]. The application of S100B in the diagnosis and prognosis of central nervous system injury has become a hot pot. In neonatal field, studies have shown that the S100B levels of infants with HIE significantly increased in the blood [27, 28] and urine [29, 30], and reached a peak in 2~6 h after birth [31], continuing 1~2 d [32-34]. Gazzolo et al [35] found that S100B had begun to rise in 48~72 h before clinical symptoms or radiographic changes occurring in IVH preterm infants. Xie Lijuan et al [36] found that at the 3 d and 7 d, serum S100B levels of white matter injury group were higher than those of no brain damage group and PVH-IVH group.

The results suggested that the serum S100B levels of preterm infants with perinatal brain damage increased rapidly within 24 h, among which the levels of PVL was the highest. Preterm infants with acute PVH-IVH were higher than those of patients without brain damage; and the serum S100B levels of preterm infants with PVL were higher than those of preterm infants with pure PVH-IVH, suggesting that serum S100B protein can be used as an early diagnostic indicator of brain damage in preterm infants, and it has some reference value on the distinction of PVL and other brain injury.

S100B protein is primarily eliminated by renal clearance (98%), and the serum half-life is about 2 h. Therefore, for suspected newborns with acute brain injury, serum samples should be collected for S100B protein measurement as soon as possible within 24 h after birth. infants with brain damage had admitted in NICU. After a series of intervention, due to the etiology of brain injury was excluded, the serum S100B levels of the preterm infants with PVH-IVH alone had been reduced to the similar levels to those of preterm infants without brain damage 7 d after birth. While the serum S100B levels of the preterm infants with PVL would show sustained high levels within 14 d after birth.

The study also found that plasma S100B levels of preterm infants without brain damage were negatively correlated with gestational age; with the increasing gestational age, serum S100B levels gradually decreased, which was consistent with the findings of Gazzolo et al [37] and Li Jianming et al [22]. This may be due to that the smaller the gestational age is, the more immature the brain development in preterm infants is; glial cells have not yet fully differentiated, and free S100B level was high in brain tissue; in addition, the blood-brain barrier is not complete developed, so the S100B in brain tissue can more easily pass through the blood-brain barrier into the blood to be measured.

In fact, the smaller gestational age is and the lower the birth weight is, the higher the incidence of brain injury is, which is closely related to the immaturity of anatomy and physiology and neurobiology in the central nervous system of preterm infants. In the present study, there were differences in gestational age among the three groups; PVL group had the minimum average gestational age; whether this will lead to the higher S100B levels than those of the other two groups is difficult to determine, and few previous studies concerned about it. This study suggested that when applying S100B in the early diagnosis of brain injury in preterm infants, we should fully consider the impact of gestational age and other confounding factors; especially when using it as a diagnostic indicator to analyze the sensitivity and specificity, we should pay attention to the different gestational with different critical value.

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Disclosure of conflict of interest

None.

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