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The effect of intestinal flora on the neural development of severe hyperbilirubinemia neonates

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Abstract. – OBJECTIVE: To investigate the effect of intestinal flora on the neural development of severe hyperbilirubinemia neonates.

PATIENTS AND METHODS: The clinical data of 108 severe hyperbilirubinemia neonates admitted to the Dezhou People's Hospital from January 2015 through January 2018 were analyzed, and all newborns had a serum total bilirubin level > 342 μmol/L. Based on whether they suffered from neural development abnormalities, the neonatal patients were divided into the neural abnormality group (n=52) and the non-neural abnormality group (n=56). The unconjugated bilirubin levels in serum and cerebrospinal fluid (CSF) and the composition of intestinal flora were compared.

RESULTS: Among 108 neonates, there were 55 cases with developmental abnormalities, in which 52 (48.13%) cases had neural developmental abnormalities, mainly epileptic patients. The serum and CSF unconjugated bilirubin levels of the neonatal patients in the neural abnormality group were (466.25±97.64) μmol/L and (9.64±2.98) μmol/L, respectively, which were higher than those in neonatal patients of the non-neural abnormality group [(357.89±72.53) μmol/L and (6.73±3.11) μmol/L], with statistically significant differences ($p<0.05$). The abundance of intestinal flora genus in the neonates in the neural abnormality group was lower than that in the non-neural abnormality group, and the comparisons of *Fusobacterium, Catabacter, Succinivibrio, Clostridium* and *Bacteroides* between the two groups showed statistically significant differences ($p<0.05$).

DISCUSSION: The intestinal micro-ecological environment of newborns was vulnerable and easily affected by many factors such as methods of delivery, feeding ways and eating habits of their mothers. This study investigated the effects of intestinal flora on the neural development of neonates with severe hyperbilirubinemia. The results showed that, due to decreased intestinal flora diversity, the serum and cerebrospinal fluid bilirubin levels were elevated, and the abnormal rate of neural development was increased.

CONCLUSIONS: Severe hyperbilirubinemia neonates with neural abnormalities have decreased diversity of intestinal flora genus and relatively high serum and CSF bilirubin levels, probably because the decrease in the diversity of intestinal flora genus leads to the change of the blood-CSF barrier permeability, leading to raised levels of bilirubin in serum and CSF, thus affecting the neural development of neonatal patients.

Key Words
Neonates, Severe hyperbilirubinemia, Intestinal flora, Neural development.

Introduction

Hyperbilirubinemia is a common neonatal disease, which occurs in about over 50% of neonates. In clinical practice, hyperbilirubinemia is diagnosed when a full-term neonate has a serum bilirubin concentration >225.6 μmol/L, or the serum bilirubin concentration of a premature infant >256 μmol/L, and severe hyperbilirubinemia is defined when the serum total bilirubin level >342 μmol/L[1]. The pathogenic factors of hyperbilirubinemia are complicated, including heredity, infection, perinatal period and breastfeeding. If no treatment is carried out timely, the disease will continue to progress, and leads to intelligence, hearing and nervous system development abnormalities, causing permanent sequelae[2]. Sachdeva et al[3] have pointed out that bilirubin enters the central nervous system and may induce the occurrence of neural development abnormality and

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encephalopathy. Because neonates have relatively high blood-cerebrospinal fluid barrier (BCSFB) permeability, and their nervous systems are highly sensitive to toxic substances, the high serum conjugated bilirubin level in neonates is much likely to affect the normal neural development. The intestine is an important digestive organ in the human body, and the intestinal micro-ecological system that consists of epithelial cells, resident floras and the local mucosal immune system is a key player in the immunity, metabolism and maturation of the human body[4]. Intestinal floras and the host restrict and depend on each other, maintaining the dynamic balance of organism[5]. However, the intestinal micro-ecological environment of neonates tends to be affected by multiple factors, such as the method of the delivery, feeding way and eating habits of their mothers, and is highly vulnerable to destruction[6]. Therefore, this work investigated the effect of intestinal flora on the neural development of severe hyperbilirubinemia neonates.

Patients and Methods

General Data

Retrospective analysis on the clinic data was performed for 108 cases of severe hyperbilirubinemia neonates who were admitted to the Dezhou People's Hospital from January 2015 to January 2018. There were 67 males and 41 females with the age of 3-38 d, the birth weight of (2560.83 ± 352.18) g, and the gestational age of (38.57 ± 4.28) w. They were aged (5.47 ± 2.51) d at admission. In terms of delivery method, 12 cases were born by cesarean section and 96 were born vaginally. All 108 neonates consisted of 52 mixed-feeding cases and 56 breastfeeding cases.

Inclusion criteria: 1) Neonates clinically diagnosed with severe hyperbilirubinemia, 2) those with the gestational age ≥ 37 w, 3) those with the weight at birth > 2500 g, 4) those who didn't receive the antibiotic therapy, probiotic bacteria therapy, or phototherapy, and 5) neonatal patients whose guardians signed the informed consent.

Exclusion criteria: 1) Neonates with neural development abnormalities induced by sepsis, bacterial meningitis, anoxic-ischemic encephalopathy, genetic metabolic disorders, epilepsy, intracranial hemorrhage or hypoglycemia, etc., 2) those with the congenital neural development abnormalities, 3) those accompanied with enteritis, gastrointestinal dysplasia, intestinal dysfunction or other disease affecting intestinal floras, or 4) neonatal patients whose mothers had histories of major diseases in the duration of pregnancy.

This study was approved by the Ethics Committee of the Dezhou People's Hospital.

Methods

The developmental abnormality and clinical data of 108 severe hyperbilirubinemia neonates were retrospectively analyzed, and the neonatal patients were divided into the neural abnormality group (n=52) and the non-neural abnormality group (n=56) based on whether neural development abnormalities occurred. The comparisons of the unconjugated bilirubin levels in serum and CSF and the composition of intestinal flora were performed between the two groups.

Bilirubin level

Within 3 h after neonates were admitted to the Dezhou People's Hospital, 2 mL of venous blood was extracted and 2 mL CSF was sucked by percutaneous lumbar puncture. The automatic biochemical analyzer was used to detect the serum bilirubin level. All procedures were conducted according to the instructions of the reagent kit.

Intestinal flora

After the neonatal patients were hospitalized, their fresh stool samples were obtained using the sterile stool collector (Huaneng Pharmaceutical Co., Ltd., Taizhou, Jiangsu, China) immediately and stored in a refrigerator at -20°C for testing. The deoxyribonucleic acid (DNA) of bacteria in the stool samples were extracted using the enzymatic method, and the amplification was carried out for regions V3-V4 of the bacterium 16S ribosomal DNA (rDNA). Illumina library preparation kit (Shanghai Yanjin Biological Co., Ltd., Shanghai, China) was applied to construct the sample library. Then, Illumina Miseq platform (Shanghai South Gene Technology Co., Ltd., Shanghai, China) was employed for the sequencing of the Polymerase Chain Reaction (PCR) products. Effective data were obtained by stitching and filtering the original data, and afterward, the analyses on operational taxonomic units (OTUs) clustering and species classification were conducted. With the OTU and species annotation combined, the basic analysis results on the OTUs and classification pedigree of each sample were obtained. Finally, the flora structures at each classification level were further statistically analyzed.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 software was used to conduct the statistical analysis. The measurement data were expressed as mean ± standard deviation ($\bar{x}\pm s$), and the count data were represented as frequency. *t*-test was used for the comparisons between independent samples in the two groups. $p<0.05$ suggested that differences were statistically significant.

Results

Abnormalities Development in Neonates

Of all the 108 neonates, there were 55 cases of developmental abnormalities, in which 52 (48.13%) cases had neural development abnormality, mainly neonatal patients with epilepsy (Table I).

Comparisons of Serum and CSF Unconjugated Bilirubin Levels

The serum and CSF unconjugated bilirubin levels of the neonatal patients in the neural abnormality group were (466.25 ± 97.64) μmol/L and (9.64 ± 2.98) μmol/L, respectively, which were higher than those of the neonatal patients in the non-neural abnormality group [(357.89 ± 72.53) μmol/L and (6.73 ± 3.11) μmol/L], with statistically significant differences ($p<0.05$) (Table II).

Comparisons of Intestinal Floras

The abundance of intestinal flora genus in neonates in the neural abnormality group was lower than that of neonates in the non-neural abnormality group, and the differences of *Fusobacterium, Catabacter, Succinivibrio, Clostridium* and *Bacteroides* in neonatal patients of the two groups were statistically significant ($p<0.05$) (Table III).

Table I. Development abnormalities in neonates.

Development abnormality	Case (No.)	Ratio (%)
Physical development abnormality	3	2.77
Intelligent abnormality by Denver Developmental Screening Test	7	6.48
Cretinism	9	8.33
Abnormality by Picture Vocabulary Test	10	9.25
Epilepsy knockdown group	26	24.07

Table II. Comparisons of serum and CSF unconjugated bilirubin levels (μmol/L).

Group	Case (No.)	Serum	CSF
Neural abnormality group	52	466.25±97.64	9.64±2.98
Non-neural abnormality group	56	357.89±72.53	6.73±3.11
t		2.569	2.396
p		0.012	0.031

Discussion

The unconjugated bilirubin in severe hyperbilirubinemia neonates causes the poisoning pathological changes of nerve cells through the blood-brain barrier (BBB), which is a major danger. Clinically, newborn infants with severe hyperbilirubinemia tend to experience long-term nervous system and mental sequelae[7]. There are over 100 trillion bacteria in more than 100 kinds in human intestines, including pathogenic bacteria and normal bacteria. The balance state is maintained among floras. Of them, B*ifidobacterium* and *Streptococcus faecalis* are closely correlated with human health[8]. The *Bifidobacterium* and *Streptococcus faecalis* in the neonatal intestines can convert the bilirubin to stercobilinogen, reducing the bilirubin readsorption capacity of organisms[9]. Neonatal intestines lack bacteria and normal floras, so bilirubin cannot be reduced into urobilinogen and excreted in feces. Furthermore, neonatal BBB development is less mature and highly permeable, and bilirubin traverses BBB easily and enters cerebral tissues, damaging central nervous systems, delaying neural conduction and triggering bilirubinemia[10]. To improve the quality of life for neonates, the present work explored the effect of intestinal flora on the neural development of severe hyperbilirubinemia neonates.

Among all the 108 study subjects, 55 cases exhibited development abnormalities, in which 52 (48.13%) cases were diagnosed with neural development abnormalities, mainly epileptic patients. Epilepsy is a chronic recurrent transient cerebral dysfunction syndrome and a common nervous system disease, characterized by recurring epileptic due to the abnormal discharge of brain neurons. Then, neonatal patients in the neural abnormality group had the serum unconjugated bilirubin level of (466.25 ± 97.64) μmol/L and the CSF conjuga-

Table III. Comparison of intestinal flora composition.

Genus	Non-neural abnormality group (No.=56)	Neural abnormality group (No.=52)	*q*	*p*
Fusobacterium	0.0435±0.0000	0.0006±0.0002	0.0000	0.0000
Akkermancia mucinophilia	0.0138±0.0000	0.0139±0.0025	0.0000	0.0000
Barnesiella intestinihominis	0.0006±0.0000	0.0007±0.0000	0.0000	0.0000
Negativicoccus	0.0006±0.0000	0.0006±0.0000	0.0000	0.0000
Butyricicoccus	0.0004±0.0000	0.0005±0.0000	0.0000	0.0000
Desulfovibrio	0.0002±0.0000	0.0003±0.0000	0.0000	0.0000
Butyricum degradative bacteria	0.0002±0.0000	0.0003±0.0000	0.0000	0.0000
Alistipes	0.0001±0.0000	0.0001±0.0000	0.0000	0.0000
Catabacter	0.0001±0.0000	0.0001±0.0000	0.0000	0.0000
Faecalicoccus	0.0001±0.0000	0.0001±0.0000	0.0000	0.0000
Gemella	0.0000±0.0033	0.0001±0.0000	0.0023	0.0002
Succinivibrio	0.0001±0.0000	0.0001±0.0000	0.0000	0.0000
Clostridium difficile	0.0000±0.0029	0.0001±0.0000	0.0012	0.0001
Anaerobic cocci	0.0000±0.0000	0.0001±0.0000	0.0000	0.0000
Gardnerella	0.0000±0.0000	0.0000±0.0000	0.0000	0.0000
Clostridium	0.0003±0.0000	0.0000±0.0000	0.0000	0.0000
Camyplobacter genus	0.0000±0.0000	0.0000±0.0000	0.0000	0.0000
Bacteroides	0.0002±0.0000	0.0000±0.0000	0.0000	0.0000

Note: 1) ***q***: assessed value of false discovery rate for calculating the reliability, 2) ***p***: False positive probability value.

ted bilirubin level of (9.64 ± 2.98) μmol/L, which were higher than those in the neonates of the non-neural abnormality group [(357.89 ± 72.53) μmol/L and (6.73 ± 3.11) μmol/L]. The differences were statistically significant ($p<0.05$), suggesting that the CSF bilirubin level determines the occurrence of bilirubin-induced neural development abnormalities. Besides, the bilirubin level of neonates after birth is elevated, and since neonatal BBB is not fully developed with relatively high permeability, bilirubin passes through BBB and enters cerebral tissues. As a result, it damages the central nervous system and induces bilirubin encephalopathy[11]. Therefore, clinically forming favorable intestinal floras can weaken enterohepatic bilirubin circulation, promote the excretion of conjugated bilirubin and lower the reabsorption of bilirubin, effectively reducing the incidence rate of hyperbilirubinemia in neonates. At the same time, the study results revealed that the neonates of the neural development group have lower abundance of the intestinal flora genus than those in the non-neural development group, and through comparisons, *Fusobacterium, Catabacter, Succinivibrio, Clostridium* and *Bacteroides* of neonates between the two groups are statistically significantly different ($p<0.05$). *Fusobacterium, clostridium, Vibrio succinogenes* and *bacteroides* in colons can produce a series of short-chain fatty acid, such as propionic acid, ethanoic acid, lactic acid and butyric acid by metabolizing carbohydrates, providing the source of the intestinal short-chain fatty acid. The short-chain fatty acid is able to facilitate intestinal peristalsis, adjust appetite and promote the expression of tight junction-associated proteins to strengthen the barrier function of intestinal epithelial cells[12]. Moreover, these acids can be transported into all organs through the circulation system, helping develop BCSFB and improving the BBB function, so intestinal flora can affect the BBB function and structure, lowering the incidence of bilirubin-induced neural development abnormalities[13]. In recent years, with the extensive use of probiotics in the clinic, many clinical trials have investigated the safety and efficacy of probiotics taken by low birth weight infants and premature infants. A research has confirmed probiotics such as *bifidobacteria* can customize the intestinal mucosa, form a biological barrier, produce enzymes that decompose food functions, help eliminate toxins, and produce lactic acid. Lactic acid can inhibit the growth of harmful bacteria, prevent the invasion of various pathogenic bacteria or pathogenic bacteria, promote the phagocytosis of macrophages and enhance the immune function of the in-

testine[14]. Probiotics can also inhibit the activity of nuclear transcription factor JB induced by lipopolysaccharide on the surface of bacteria, attenuate the expression of inflammatory factors such as TNF-A, IL-8, intercellular adhesion molecule 1 and cyclooxygenase-2, reduce apoptosis, as well as inhibits the body's inflammatory response. At the same time, probiotics attached to the intestinal mucosa can produce specific IgA, etc., and improve the abundance of *Clostridium, Catabacter, Vibrio succino, Clostridium* and *Bacteroides,* thereby improving immunity and intestinal infection resistance[15].

Conclusions

We showed that the diversity of intestinal flora genus in severe hyperbilirubinemia neonates with neural abnormalities declines, while the serum and CSF bilirubin levels are relatively high. The possible reason is that the permeability of BCSFB changed due to the decrease in the diversity of intestinal flora genus, so the levels of bilirubin in serum and CSF are raised, thus affecting the neural development of neonatal patients.

Conflict of Interests

The authors declare that they have no conflict of interest.

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