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Risk factors for neonatal nosocomial enteric infection and the effect of intervention with BIFICO

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Abstract. – OBJECTIVE: This study aims to investigate the risk factors for neonatal nosocomial enteric infection (NNEI) and the effect of intervention with BIFICO.

PATIENTS AND METHODS: Between May 2013 and June 2015, 215 neonates admitted to our institution were randomly divided into the study group and the control group, 47 for each group. Patients in the study group were treated for primary diseases combined with the oral admission of BIFICO, whereas patients in the control group were treated for primary disease alone. Statistical analysis was performed to obtain the occurrence of enteric infection and univariate, as well as multivariate analysis of clinical data, were performed to investigate the underlying risk factors.

RESULTS: Univariate and multivariate analysis of variance showed that gestational age, birth weight, length of hospital stay, invasive procedures and underlying diseases were risk factors affecting NNEI. The occurrence of NNEI in the study group was significantly lower than that in the control group [17.02% (8/47) vs. 29.79% (14/47), $X^2 = 19.394$, $p = 0.004$].

CONCLUSIONS: Preterm infant, low-birth-weight infant, length of hospital stay, invasive procedures and comorbidity are independent risk factors for NNEI. Prophylactic therapy with BIFICO can effectively decrease the occurrence of infections and can be widely used in clinical practice.

Key Words:
Newborn, Enteric infection, Risk factors, BIFICO.

Introduction

Nosocomial infection is a part of the spectrum of pediatric diseases frequently occurred in neonates who are susceptible to various external factors due to compromised immunity and incomplete development of organs. Intestinal infection, as an important type of nosocomial infection, prolongs hospitalization of newborns, affects normal neonatal development and increases the economical burden of patients' families[1,2]. To this end, the discovery of risk factors for neonatal nosocomial infection assists the prevention and treatment of the disease. Currently, antibiotics, while as the mainstay treatment of infectious diseases, result in the imbalance in the intestinal bacteria flora[3]. Human gastrointestinal (GI) tract is a gigantic reservoir of microbiota, where various bacteria cohabit, mutually influence and constrain. Disruption of this balance can lead to a variety of infectious diseases[4]. In recent years, micro-ecology has been emerging as a novel branch of life science. Microbioecological preparation, a group of agents developed based on the theories of micro-ecology, is composed of microbiota and their metabolic products that are beneficial to human body. These agents can improve host physiological status through correcting flora imbalance and adjusting the function of biological barrier[5]. The microbioecological preparations commonly used in pediatric units include probiotics, probiotic and synbiotics, among which probiotics is the most widely used agent. BIFICO, which is used in the present study, contains a mixture of viable bacteria including *Enterococcus faecalis* (EF), *Bifidobacterium longum* (BL) and *Lactobacillus acidophilus* (L)[6,7]. In the present study, BIFICO is used to treat intestinal infection in newborns to investigate the risk factors for the disease, in an effort to provide theoretical evidence for clinical treatment.

Patients and Methods

Patients

Between May 2013 and June 2015, 215 newborns including 94 with an intestinal infection

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and 121 healthy subjects admitted to our institution were included in the present study. Exclusion criteria are listed as follows: (1) Intestinal infection was present before hospitalization. (2) Hospitalization was less than 72h. (3) Patients with congenital digestive tract malformations. (4) Neonatal hemolysis. (5) Patients with incomplete clinical data and those who could not complete follow-up. Newborns with nosocomial enteric infection were randomly divided into the study group and the control group, 47 cases for each group, by using a random number table. The study group consists of 27 males and 29 females with an age range of 1-24 days, mean age of (12.22 ± 2.87) days, weight range of 1531-3605 g, mean weight of (2597.38 ± 80.34) g. These patients were born at 34-38 weeks gestation (mean gestation of (33.87 ± 6.22) weeks. Of these newborns, 21 were vaginally-delivered and 26 caesarean-delivered. The length of hospital stay ranged from 7-24 days, mean of (14.27 ± 4.15) days. Eight of them experienced neonatal asphyxia, 7 pneumonia, 4 jaundice and 5 with other diseases.

The study group included 26 males and 21 females with an age range of 2-25 days, mean age of (12.67 ± 4.12) days, weight range of 1500-3618 g, mean weight of (2619.26 ± 92.15) g. These neonates were born at 34-38 weeks gestation, mean gestation of (36.14 ± 5.34) weeks. Of these newborns, 20 were vaginally-delivered and 27 were caesarean-delivered. Length of hospital stay ranged from -28 days, mean stay of (17.31 ± 5.02) days. Of these newborns, ten presented with neonatal asphyxia, six with pneumonia, six with jaundice and seven with other diseases.

The probiotic group included 16 males, and 14 females, with an age range of 2-20 days, mean age of (12.67 ± 3.17) days, weight range of 1482-3673 g, mean weight of (2637.13 ± 87.15) g. These neonates were born at 33-39 weeks gestation, mean gestation of (36.59 ± 3.44) weeks. Of these newborns, 10 were vaginally-delivered and 20 were caesarean-delivered. Length of hospital stay ranged from 6-29 days, mean stay of (17.64 ± 5.33) days. Of these newborns, nine presented with neonatal asphyxia, five with pneumonia, four with jaundice and six with other diseases.

The BIFICO group included 11 males, and 21 females, with an age range of 2-25 days, mean age of (12.67 ± 4.12) days, weight range of 1500-3618 g, mean weight of (2619.26 ± 92.15) g. These neonates were born at 34-38 weeks gestation, mean gestation of (36.14 ± 5.34) weeks. Of these newborns, 18 were vaginally-delivered and 14 were caesarean-delivered. The length of hospital stay ranged from 7-28 days, mean stay of (17.31 ± 5.02) days. Of these newborns, ten presented with neonatal asphyxia, six with pneumonia, six with jaundice and seven with other diseases.

No significant differences were observed between three groups in gender, age and other clinical data ($p < 0.05$). The study protocol was approved by the Ethics Committee of our institution and written informed consent was obtained from all parents of these patients.

Diagnosis Criteria[8]

NNEI was diagnosed according to the Diagnostic Criteria of Nosocomial Infection (trial implementation) published in 2001 by Chinese Ministry of Health. Patients with mild conditions presented common GI symptoms, such as diarrhea up to 10 times daily accompanied with low-grade fever, milk spit up, listlessness and mild dehydration. Patients with severe conditions displayed diarrhea of above 10 times daily, significant symptoms of fever, refusal to milk, vomiting, prolonged sleep time and cold extremities.

Methods

Treatment

Patients in the study group were treated for primary diseases as well as received oral administration of BIFICO (Shanghai Sinepharm, China, A0172, ½ capsule, twice daily) for preventive intervention. Each BIFICO capsule contains > 1.0× 107CFU of *Streptococcus faecalis, Lactobacillus acidophilus and Bifidobacterium*. Each half capsule of powder was dissolved in warm water and administered through nasogastric feeding in premature infants until discharge. Patients in the probiotics group were treated for primary diseases as well as received oral administration of prebiotic mixture (Shaanxi Song Ling Pharmaceutical Co., Ltd. 150323, twice daily, 1 bag) for preventive intervention. Each dosage was dissolved in warm water or milk (< 37°C) and administered through nasogastric feeding in premature infants until discharge.

In the control group, patients were treated for primary diseases alone. Statistical analysis and comparison of the two groups of patients were obtained with the occurrence of nosocomial intestinal infection.

Data Collection

At least two professional and part-time staffs performed statistical analysis on patient data, including gender, age, gestational age, mode of delivery, birth weight, length of hospital stay, invasive procedures, incubator placement, antibiotic prophylaxis, application of glucocorticoids and underlying diseases. After at least 6 months follow-up through telephone or home visits, the recurrence of infection in children was with statistics.

Statistical Analysis

Data analysis was performed using SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA). The data was expressed as the average mean ± standard deviation (± s), comparison among multiple groups was one-way ANOVA, and *t*-test was used in the two groups. Quantitative data were expressed as percentages (%). Differences between groups were analyzed using X^2 test. The X^2 test for a single variance was used to identify potential risk factors, which were then analyzed by using unconditional multivariable logistic regression model. X^2 values, OR values and OR 95% confidence interval (CI) were obtained. $p < 0.05$ was considered statistically significant. In addition, Kaplan-Meier and Log rank test were performed to compare the recurrence rate data, with significant level $\alpha = 0.05$.

Results

Univariate Analysis of Nnei

Anova Analysis of Neonatal GIInfection

ANOVA analysis showed the gender and age of newborns were not associated with neonatal GI infection. However, delivery mode, birth weight, length of hospital stay, invasive procedures, incubator placement, antibiotic prophylaxis, corticosteroid application as well as underlying diseases were closely associated with the occurrence of neonatal TI infection ($p < 0.05$) (Table I).

Multivariate Analysis on NNEI

Nine factors identified by univariate analysis were further analyzed using multivariate logistic regression model and 5 risk factors affecting neonatal GI infection were identified, including gestational age, birth weight, length of hospital stay, invasive procedures and underlying diseases (Table II).

Table I. Univariate analysis of NNEI.

Relevant factors		n	Number of infections	Incidence of infection (%)	χ^2	*p*
Gender	Male	150	64	42.67	0.458	0.084
	Female	65	30	46.15		
Age (days)	1-7	75	34	45.33	0.841	0.063
	8-14	86	37	43.02		
	14-25	54	23	42.59		
Gestational age (weeks)	≤ 36	102	58	56.86	6.483	0.021
	> 36	113	36	31.86		
Delivery mode	Vaginally	98	34	34.69	3.872	0.032
	Caesarean	117	60	51.28		
Birth weight (g)	≤ 2500	94	54	57.45	3.128	0.036
	> 2500	121	40	33.06		
Length of hospital stay (days)	7-14	116	47	40.52	2.965	0.041
	14-28	99	57	57.58		
Invasive procedures	Present	84	45	53.57	4.021	0.029
	Absent	131	49	37.40		
Incubator placement	Present	124	46	37.10	3.593	0.033
	Absent	91	48	52.74		
Antibiotic prophylaxis	Present	97	52	53.61	3.995	0.030
	Absent	118	42	35.59		
Glucocorticoid application	Present	102	58	56.86	4.194	0.027
	Absent	113	36	31.86		
Underlying disease	Present	53	32	60.38	8.596	0.017
	Absent	162	62	38.27		

Table II. Multivariate analysis of NNEI.

Risk factors	β	SE	Wald	*p*	OR	95% CI
Gestational age	0.754	0.252	10.287	0.004	4.529	1.131~5.912
Birth weight	1.139	0.732	9.385	0.003	4.895	1.485~9.495
Length of hospital stay	1.732	0.223	24.394	0.001	6.405	2.193~12.395
Invasive procedures	1.643	0.245	14.495	0.002	5.327	1.387~8.302
Underlying diseases	1.286	0.617	8.293	0.007	3.394	1.321~9.034

β: regression coefficient; SE: standard error of β; Wald: Chi-square value; OR: relative risk; 95% CI: confidence interval.

Experimental Results of Infection Treatment

Comparison of Infection Rate of Three Groups of Children After Preventive Treatment

The incidence rate of neonatal hospital intestinal infection of BIFICO group, prebiotic group and the control group were 12.50% (4/32), 16.67% (5/30) and 28.13% (9/32). The rate of neonatal hospital intestinal infection in BIFICO group and the prebiotic group was significantly lower than that of the control group, and the difference has statistical significance (2 = 13.524, 12.812, $p = 0.008$), and differences between BIFICO group and probiotic were not statistically significant ($c^2 = 0.326$, $p = 0.082$).

Comparison of Clinical Indexes of Three Groups of Patients

Compared with the control group, the high fever duration, heart rate and the average hospital stay was significantly decreased of the patients in BIFICO group and prebiotic group, and the oxygen saturation concentration increased significantly, and the difference is statistically significant ($p < 0.05$); the differences of indicators between the BIFICO group and probiotic group in patients had not statistical significance ($p > 0.05$), as shown in Table III.

Comparison of the Follow-up of Patients in Three groups

The results of follow-up were summarized in Table IV. From the data: follow-up at 1 month, the 3rd month and the 6th month, the intestinal in-

Table III. Comparison of clinical indexes of three groups of patients ($\bar{x} \pm s$).

Gourp	n	High fever duration (days)	Average hospital stay (minutes)	Blood oxygen saturation (%)n
A: Control	32	4.13 ± 0.87	16.15 ± 1.51	90.84 ± 6.23
B: Probiotic	30	2.87 ± 0.42[a]	11.15 ± 0.97[a]	94.24 ± 5.79
C: Bifico	32	2.77 ± 0.31[a]	10.82 ± 0.62[a]	95.14 ± 8.25[a]
Analysis	F, P	52.483, 0.000	234.286, 0.000	3.480, 0.035

Note: Analysis is One-way ANOVA; multiple comparison is LSD-t test, Marked mark a, b compare with A and B groups, respectively. $p < 0.05$.

Table IV. Comparison of the follow-up of patients in three groups (Infection rate %).

Group	n	1 month	3rd month	6th month	Logrank test (χ^2, *p*)	
A: Control	32	10 (31.25)	13 (40.63)	14 (43.75)	B *vs*. A	0.496, 0.481
B: Probiotic	30	6 (20.00)	7 (23.33)[a]	9 (30.00)	C *vs*. A	4.875, 0.027
C: Bifico	32	5 (15.63)	6 (18.75)	6 (18.75)	C *vs*. B	0.032, 0.859

fection rate of patients in prebiotic group and BIFICO group was significantly lower than that in the control group. By using the Kaplan-Meier method and the log rank test, it was known that the intestinal infection rate of BIFICO group patients was significantly lower than that of the prebiotic group. The difference between two groups has statistical significance ($p < 0.05$); however, there was no significant difference of the intestinal infection rate of patients in prebiotic group and BIFICO group, as shown in Table IV and Figure 1.

Occurrence of NNEI After Prophylactic Intervention

The analysis revealed that the occurrence of neonatal GI infection in the study group was significantly lower than that of the control group [17.02% (8/47) *vs*. 29.79% (14/47), $X^2 = 19.394$, $p = 0.004$].

Discussion

The nosocomial infection has become one of the significant reasons impeding the development of hospitals and also an important indicator to evaluate the level of health care. The issue of how to efficiently control nosocomial infection has attracted the attention of the medical professionals[9]. Due to the incomplete development of organs, particularly, the immune function, newborns are susceptible to the pathogenic invasion in a highly pathogenic environment of hospitals[10], which leads to life-threatening infections. With an effort to resolve this issue, investigating the risk factors has significant implications for controlling neonatal nosocomial enteric infection (NNEI). A huge number of bacteria flora are present in the human intestinal tract, among which 99% are obligate anaerobes, such as *Lactobacillus* and *Bifidobacterium*[11]. In fact, a fetus is in sterile status under normal conditions; however, newborns will be invaded by a substantial variety of microbiota, which become imbalance and cause enteric infection influenced by various factors[12]. On the other hand, prophylactic treatment by using microbial agents plays an important role in controlling NNEI. Microbial agents, as non-digestible substances, can not only stimulate the growth of probiotic bacteria, but also efficiently prevent the imbalance of harmful bacteria, thereby maintaining the balance of microflora[13].

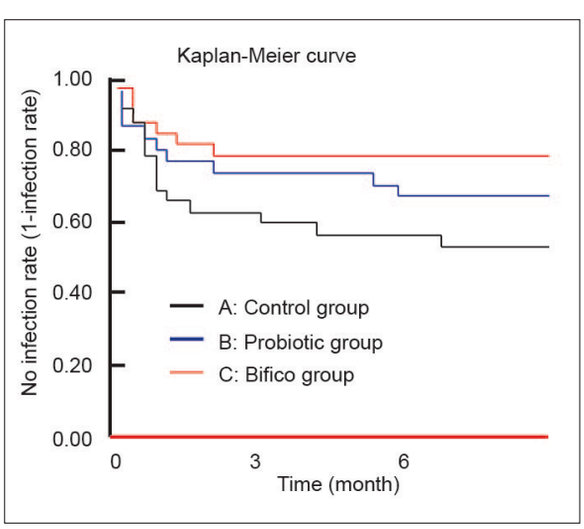

Figure 1. The comparison of the no-infection rate (1-infection rate) of the follow-up of patients in the three groups.

In the present study, the results of univariate and multivariate study showed that preterm neonates and low-birth-weight infants risk factors influencing NNEI, mainly due to the hypomotility of GI tract resulting from its immature structure and function[14]. Additionally, premature rupture of membranes can cause the infection by bacteria migrating from the vagina. Meanwhile, immature immune function impairs infants' resistance to pathogens[15]. Furthermore, prolonged hospital stay rendered neonates susceptible to increased chance of infection and increased the possibility of cross-infection, representing another risk factor for NNEI[16]. Newborns are subject to varying degrees of invasive manipulations during hospitalization. For instance, intubation is likely to cause damage to patient's respiratory tract and mucous membrane. Moreover, pathogens residing on various operating instruments can invade infants and aggravate the infection[17]. Furthermore, immune dysfunction accompanied with underlying diseases decreases the resistance of infants to external pathogens, thereby aggravating infection[18].

Taken together, these five risk factors have implications in the development of neonatal nosocomial enteric infection. Therefore, targeted prevention and treatment should be practiced in an effort to efficiently improve prognostic outcomes in newborns. In addition, this study also found that the occurrence of NNEI in the BIFICO group and prebiotic group were significantly lower than that in the control group [12.50% (4/32), 16.67% (5/30) *vs*. 28.13% (9/32)], indi-

cating that BIFICO plays a role in preventing nosocomial enteric infection in newborns. This probiotic enhances the vitality of beneficial bacteria and indirectly simulates specific as well as non-specific immune system, thereby increasing anti-infection capability. Studies have shown that BIFICO can not only decrease the pH value in intestinal tract but also suppress the growth of harmful microflora, competitively inhibiting pathogens[19-22].

However, the differences between BIFICO group and probiotic group does not have statistical significance ($p > 0.05$), which indicating that the infection effects of these two microbial preparations were very similar.

From the comparison of the hospital intestinal infection in patients, it was found out that compared with the control group, the high fever duration, heart rate and the average hospital stay of patients in the BIFICO group and the probiotic group were significantly decreased, and the oxygen saturation concentration was increased significantly ($p < 0.05$). However, the difference between BIFICO group and probiotic component indicators in patients did not show statistical significance ($p > 0.05$). Therefore, the BIFICO and probiotic can effectively improve the clinical symptoms in patients, but also can be used in clinical practice. The results from a period of 6-month follow-up of patients confirm that follow-up at 1 month, the 3rd month and the 6th month, the intestinal infection rate of patients in the BIFICO group and probiotic group was significantly lower than that in the control group. The prophylactic use of the two types of microorganisms can prevent the relapse of infection in a certain period of time. And the differences between the follow-up results at 1 month and the 3rd month between the BIFICO group and probiotic group of children with intestinal infection rate were not obvious. The intestinal infection rate of patients in BIFICO group at the 6th month was lower than in the prebiotic group, with a great discrepancy. It was noted that compared with the probiotic group, the BIFICO has a stronger long-term curative effect, which should be widely used in clinical trials.

Conclusions

In summary, risk factor directed prevention and therapy should be performed to treat NNEI in an effort to control or decrease the occurrence of infection, providing theoretical evidence for clinical practice. Meantime, prophylactic application of BIFICO can efficiently decrease the occurrence of neonatal enteric infection and can be promoted in clinical practice.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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