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Original Article

Intestinal Colonization Rate of *Candida albicans* among Low Birth Weight Neonates after Using Oral Synbiotic Supplementation: A Randomized Placebo-controlled Trial

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ABSTRACT

Background: The present study aimed to evaluate the effect of synbiotics on the intestinal colonization rate of *Candida albicans* in low birth weight neonates (i.e., under 2,500 g), which is one of the most important events for necrotizing enterocolitis (NEC).

Methods: During one year, 106 preterm neonates with a birth weight of less than 2,500 g, admitted to the Neonatal Intensive Care Unit (NICU) of Imam Reza Hospital, affiliated to Kermanshah University of Medical Sciences, Kermanshah, Iran, were randomly selected and investigated in two groups of case and control. In the case group, 5 drops of synbiotics (under the trade name of Pedilact in which 5 drops are equivalent to 2.5×10^8 CFU), containing three probiotics of *Bifidobacterium infantis*, *Lactobacillus rhamnosus,* and *Lactobacillus reuteri,* as well as the prebiotic of fructooligosaccharide, were administered. On the other hand, 5 drops of distilled water were used for the control group. In the present single-blind study, the subjects were divided into two groups using a random number table. The stool cultures were obtained on the 1st and 10th days of admission. Then, the two groups were compared in terms of the amount of positive stool culture for *Candida albicans*, time of feeding initiation and full nutrition, duration of hospitalization, and time of discharge.

Results: The incidence rate of positive stool culture for *Candida albicans* was 6.6%. A significant relationship was observed between gestational age and positive culture (P=0.009). However, there was no significant difference between the two groups in terms of the duration of hospitalization, time of feeding initiation and full feeding, good physical examination results, and wellbeing. In addition, the relationship between positive culture and birth weight was statistically significant (P=0.045) since the rates of positive culture were 57.1% and 42.9% in cases with the birth weight of ≤ 1,500 and > 1,500 g, respectively.

Conclusion: Based on the results, synbiotic use showed no significant relationship with enteral positive cultures for *Candida albicans*, time of enteral feeding initiation and full feeding, and hospitalization duration.

Keywords: *Candida albicans*, Enteral colonization, Neonates, Synbiotic

Introduction

Nowadays, the complications of long-time hospitalization and drug administration or invasive therapeutic interventions have increased by enhancing the survival possibility of preterm and/or low birth weight neonates (1). *Candida albicans* is one of the infective agents causing some diseases, such as severe blood infection with multiorgan involvement, necrotizing enterocolitis (NEC), and late-onset bloodstream infections, with up to 54% mortality (2, 3). Obviously, exact prevention and candidiasis treatment play a significant role in the health of premature or low

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birth weight neonates.

Oral prebiotic or probiotic supplements are regarded as the evolving suggested therapeutic interventions (4). Probiotics are live micro-organisms related to the genus of *Lactobacillus,* which normally coexists in the gastrointestinal tract and decreases the possibility of the proliferation of infectious microorganisms, such as *Candida albicans,* by consuming foodstuffs and fermenting simple sugars, such as fructoo-ligosaccharides (prebiotics). They also prevent the pathogen penetrance by increasing the intestinal mucosa resistance through decreasing the intestinal acidity (4). Synbiotics, such as Pedilact, refers to the presence of prebiotics and probiotics in a nutrient material simultaneously. The incidence rates of candidemia in the neonates who are admitted to neonatal intensive care units are reportedly 1.6-9% and up to 15% in the neonates weighing < 1,500 and < 1,000 g, respectively (6).

Manzoni et al. indicated that the use of *Lactobacillus casei* in the neonates with a birth weight of < 1,500 g decreased the enteral colonization of *Candida* species significantly (6). In a prospective study, Romeo et al. evaluated the effect of *Lactobacillus reuteri* and *Lactobacillus rhamnosus* among 249 neonates with a birth weight of < 2,500 g over three years. They reported that *Lactobacillus reuteri* induced a more preventive effect, compared to other species (7). In another study, Roy et al. observed the same results for *Bifidobacterium infantis*, *Lactobacillus acidophilus,* and *Lactobacillus lactis* (4). The main reason for performing the present study was that the rate of the enteral colonization of *Candida* species in Imam Reza Hospital, located in Kermanshah, Iran, is ambiguous, and no standard protocol is available for utilizing synbiotics in preventing and treating candidiasis and its related disorders in any of the therapeutic centers of Iran.

Methods

In order to conduct the study, 106 preterm neonates with a birth weight of less than 2,500 g who were admitted to the Neonatal Intensive Care Unit (NICU) of Imam Reza Hospital, affiliated to Kermanshah University of Medical Sciences (KUMS), Kermanshah, Iran, were randomly selected based on the inclusion and exclusion criteria (Table 1). The subjects were divided into two groups by using a random number table. The project was confirmed by the Iranian Registry of Clinical Trials (No. IRCT201708614333N79). The Ethics Committee of KUMS also approved the study with the number of kums.rec.1396.32.

In the case group, five drops of synbiotics, including three probiotics of *Bifidobacterium infantis*, *Lactobacillus rhamnosus,* and *Lactobacillus reuteri,* at a dosage of 2.5×10^8 CFU, as well as the prebiotic of fructooligosaccharide, were administered. On the other hand, the control group received five drops of distilled water. The dosage was recommended by the Research Department of Zistkhavar Co., and five drops of the Pedilact drop were chosen for the safety of the premature neonates and administered per day. Then, the stool cultures were obtained on the 1st and 10th days of admission. In the next step, the selected groups were compared in terms of the time of enteral feeding initiation and completion, rate of positive stool culture for *Candida albicans,* and discharge time.

All specimens were inoculated onto Sabouraud dextrose agar, and the plates were incubated at 25-30°C in ambient air. The germ tube test plays a significant role in identifying the isolated yeast. The traditional germ tube test involves the inoculation of a tube of human serum. The tube was incubated at 35°C for up to 3 h. The germ tubes which are elongated fingers, like extensions from a yeast cell, represent the beginning of a true hypha. No constriction was seen at the junction of the yeast cell and germ tube. The wall of the germ tube was parallel. In addition, the germ tube, in combination with the result of morphology on CM

Table 1. Inclusion and exclusion criteria

Inclusion Criteria
1. Birth weight ≤2500 g
2. The age of admision≤72 h
3. The mother has not applied oral or local antifungal therapy at the last month of pregnancy
Exclusion Criteria
1. Contraindications of oral feeding due to oropharyngeal or gastrointestinal anomalies
2. Justified NEC
3. Sepsis
4. Administration of oral or intravenous antifungal therapies
5. Consumption of milk powders containing of pro or prebiotics
6. Impaired liver function tests, history of gastroschisis, omphalocele before reconstruction surgery

agar, can confirm the identification of *C. albicans*.

The results were analyzed in SPSS software, version 22. The Kolmogorov-Smirnov test was used to check the normality of the continuous variables. Intergroup comparison was performed by applying an independent sample test. In this regard, t-test was used for the normally distributed data, and Mann-Whitney U test was applied for non-normally distributed data. Chi-square test or Fisher's exact test was used to compare the qualitative variables between the two groups. The study was ethically acceptable as synbiotics normalize the microflora of the gastrointestinal tract. In other words, the prevention of a fetal neonatal disease is regarded as an intervention.

Results

According to the results, just two participants in the case group had positive cultures for *Candida albicans*, which is one of the most important causes of NEC in preterm neonates. However, in the control group, five cases had a positive culture. The results are important in terms of clinical aspect although there was no significant relationship in this regard. As shown in tables 2-5, a significant relationship was observed between positive culture and gestational age (P=0.009). In addition, the incidence rate of positive fecal culture was 6.6%.

However, no significant relationship was reported between synbiotic use and enteral culture. Regarding the control group, a positive culture was observed in 5 (9.43%) samples, while it was found in 2 (3.77%) samples in the case group. In other words, the protective effect of synbiotic against *Candida albicans* colonization in the gastrointestinal tract was confirmed.

Table 2. The different variables between case and control groups

Variables	Synbiotic	N	Mean	Std. Deviation	P value
Gestational Age (week)	yes	53	32.32	2.471	0.059
	no	53	33.08	2.046	
Birth Weight (g)	yes	53	1789.62	441.196	0.391
	no	53	1862.26	426.148	
Start of treatment (day)	yes	53	1.83	.580	0.462
	no	53	2.13	.556	
Start of nutrition (day)	yes	53	2.98	1.232	0.462
	no	53	3.13	1.210	
The time of full nutrition (day)	yes	53	9.70	2.750	0.126
	no	53	10.38	2.719	
The time of hospitalization	yes	53	13.55	6.927	0.105
	no	53	14.30	7.043	

Table 3. The frequency of variables between case and control

Variables		control groups n(%)	case groups n(%)	P value
Gestational Age (week)	≤32	17(32.1%)	27(50.9%)	0.059
	>32	36(67.9%)	26(49.1%)	
Sex	male	24(45.3%)	33(62.3%)	0.08
	female	29(54.7%)	20(37.7%)	
Type of delivery	C/S	25(47.2%)	27(51%)	0.698
	NVD	28(52.8%)	26(49%)	
Birth Weight (g)	1000≥	3(5.7%)	6(11.3%)	0.766
	1001-1500	8(15.1%)	7(13.2%)	
	1501-2000	26(49.1%)	24(45.3%)	
	>2000	16(30.2%)	16(30.2%)	
Time of hospitalization (day)	1-14	37(69.8%)	38(71.7%)	0.21
	15-28	11(20.8%)	14(26.4%)	
	>28	5(9.4%)	1(1.9%)	
Full nutrition (day)	1-7	8(15.1%)	11(20.8%)	0.479
	8-14	39(73.6%)	39(73.6%)	
	≥15	6(11.3%)	3(5.7%)	

Table 4. The frequency of the results of enteral culture between case and control

			Synbiotic			P value
			No(Control)	Yes(case)	Total	
culture	Neg	Count	48	51	99	0.437
		%	90.6%	96.2%	93.4%	
	Pos	Count	5	2	7	
		%	9.4%	3.8%	6.6%	
Total		Count	53	53	106	

Table 5. The comparison of the results of enteral culture with Variable

Variables		Culture		P value
		Neg n(%)	Pos n(%)	
Gestational Age (week)	≤32	40(40.4%)	4(57.1%)	0.314
	>32	59(59.6%)	3(42.9%)	
Sex	male	54(54.6%)	3(42.9%)	0.701
	female	45(45.4%)	4(57.1%)	
Type of delivery	C/S	49(49.5)	3(42.9%)	1
	NVD	50(50.5)	4(57.1%)	
Birth Weight (g)	1500 ≥	20(20.2%)	4(57.1%)	0.045
	>1500	79(79.8%)	3(42.9%)	

Discussion

Candidiasis is regarded as the third cause of mortality due to blood infections, as well as the major reason for fungal infections among infants (8, 9). Based on the reports, candidiasis accounts for 10-20% of nosocomial blood infections in intensive care units (10, 11). In addition, the incidence rates of *Candida* infections in very low and extremely low birth weight neonates have been reported as 1.6-9% and up to 15%, respectively (5). Furthermore, the mortality of the infantile candidiasis has been reported as 20-30% (6, 8). Normal intestinal microbiota are complex and considered a balance of microorganisms, which are normally present in a healthy gastrointestinal tract (1-3). Candida is considered a normal microflora of a human alimentary tract (1, 2).

The risk factors for neonates with invasive candidiasis who are admitted to NICU include prematurity, central venous catheterization, abdominal surgery, NEC, systemic steroids, broad-spectrum antibacterial treatment, antacids, and intratracheal intubation (12). Additionally, the infants with lower birth weight are more frequently affected by invasive candidiasis (12). Because of the different kinds of fungal infections and colonization, up to 25% of infants have very low birth weight (6, 7). The incidence of the enteral colonization of candidiasis in the center under study was 6.6%, which seemed to be acceptable.

A large number of studies have shown that the application of probiotics, including *Bifidobacterium* and *Lactobacillus,* can lead to a significant decrease in NEC occurrence among the neonates with very low birth weight (6, 7, 10, 11). However, no significant effect was observed in some studies (13). In addition, some other studies indicated that probiotics can result in the reduction of oral feeding intolerance and hospitalization duration (5, 10).

Based on the results, a significant relationship was reported between positive enteral culture for candidiasis and lower gestational age. However, there was no significant relationship between synbiotic administration and *Candida* colonization. The results are inconsistent with those obtained by Oncel et al. (13). In addition, the relationship between lower birth weight and positive enteral culture was significant. However, the results indicated no relationship between the use of synbiotics and time of initiating oral feeding. Therefore, synbiotics induced no effect on oral feeding tolerance, which is inconsistent with the results obtained by Oncel et al. (13).

In another study, Pammi and Abrams reported that lactoferrin with *Lactobacillus rhamnosus* significantly decreased the incidence of NEC among the neonates with a birth weight of < 1,000 g (14), which is consistent with the results reported by Vongbhavit et al. (15). However, all of these reports have been focused on NEC (16-18),

using synbiotics added to mother' milk. Nandhini et al. evaluated 220 preterm neonates with a birth weight of < 1,500 g. They investigated the effects of synbiotics, including the probiotics of *Lactobacillus acidophilus*, *Bifidobacterium longum*, *Lactobacillus rhamnosus*, *Lactobacillus plantaris*, *Lactobacillus casei*, *Lactobacillus bulgaricus*, *Bifidobacterium infantis*, and *Bifidobacterium breve*, as well as 100 mg fructooligosaccharide as a prebiotic (17). Based on the results, synbiotics could not play any role in decreasing NEC severity, sepsis, or mortality.

The results of the present study cannot be compared with those in other studies since no study, to the best of our knowledge, has been conducted to evaluate the role of a synbiotic supplement containing three probiotics, namely *Bifidobacterium infantis*, *Lactobacillus reuteri*, and *Lactobacillus rhamnosus,* and the prebiotic of fructooligosaccharide in preventing *Candidia albicans* colonization. The results indicated that the use of synbiotics in the newborns with a birth weight of < 1,500 g had no effect on *Candida* colonization, hospitalization duration, and time of enteral feeding initiation and completion due to matching the groups in terms of some basic variables, such as gestational age, birth weight, type of delivery, and gender.

However, the results cannot be generalized due to the presence of contradictory results with respect to the use of synbiotics. Finally, Nieuwboer et al. reported that the use of probiotics and synbiotics among the children under 2 years of age should be limited, and the clinical application of these products depends on standardization (18).

Conclusion

Based on the results, the use of synbiotics showed no significant relationship with enteral positive cultures for *Candida albicans,* as one of the most important events of NEC in low birth weight neonates, hospitalization duration, feeding initiation time, and time of full enteral feeding. However, no significant relationship was reported between synbiotic use and enteral culture. In conclusion, the protective effect of synbiotics against the colonization of *Candida albicans* in the gastrointestinal tract was supported.

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Conflicts of interest

The authors reported no conflict of interest.

References

1. Polin R, Randis TM. Perinatal infections and chorioamnionitis. Fanaroff and Martin's neonatal-perinatal medicine. 11th ed. Philadelphia, PA: Elsevier; 2020. P. 809-14.
2. Remington JS, Klein JO. Remington & Klein infection disease of the fetus & newborn infant. 8th ed. Philadelphia, PA: Elsevier; 2015. P. 1058-75.
3. MacDonald MG, Seshia MM. Avery's neonatology: pathophysiology and management of the newborn. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2015. P. 983.
4. Roy A, Chandhari J, Sankar D, Ghosh P, Chakraborty S. Role of enteric supplementation of probiotics on late-onset sepsis by *Candida* species in preterm low birth weight neonates: a randomized, double blind, placebo-controlled trial. N Am J Med Sci. 2014; 6(1):50-7.
5. AlFalah K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Evid Based Child Health. 2014; 9(3):584-671
6. Benjamin DK, Stoll BJ, Gantz MG, Walsh MC, Sánchez PJ, Das A, et al. Neonatal candidiasis: epidemiology, risk factors, and clinical judgment. Pediatrics. 2010; 126(4):e865-73.
7. Romeo MG, Romeo DG, Trovato L, Oliveri S, Palermo F, Cota F, et al. Role of probiotics in the presentation of the enteric colonization by candida in preterm newborn: incidence of late-onset sepsis and neurological outcome. J Perinatol. 2011; 31(1):63-9.
8. Steinbach WJ. Epidemiology of invasive fungal infections in neonates and children. Clin Microbiol Infect. 2010; 16(9):1321-7.
9. Zaoutis T. Candidemia in children. Curr Med Res Opin. 2010; 26(7):1761-8.
10. Agarwal RR, Agarwal RL, Chen X, Lua JL, Ang JY. Epidemiology of invasive fungal infections at two tertiary care neonatal intensive care units over a 12-year period (2000-2011). Global Pediatr Health. 2017; 4:2333794X17696684.
11. Aliaga S, Clark RH, Laughon M, Walsh TJ, Hope WW, Benjamin Dk, et al. Changes in the incidence of candidiasis in neonatal intensive care units. Pediatrics. 2014; 133(2):236-42.
12. Manzoni P, Jacqz-Aigrain E, Rizzollo S, Franco C, Stronati M, Mostert M, et al. Antifungal prophylaxis in neonates. Early Hum Dev. 2011; 87:S59-60.
13. Oncel MY, Erdeve O, Dilmen U. Probiotics for necrotizing enterocolitis: not always a success story. J Pediatr. 2014; 165(2):417.
14. Pammi M, Abrams SA. Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev. 2015; 2:CD007137.
15. Vongbhavit K, Underwood MA. Prevention of necrotizing enterocolitis through manipulation of

the intestinal microbiota of the premature infant. Clin Ther. 2016; 38(4):716-32.
16. Johnson-Henry KC, Abrahamsson TR, Wu RY, Sherman PM. Probiotics, prebiotics, and synbiotics for the prevention of necrotizing enterocolitis. Adv Nutr. 2016; 7(5):928-37.
17. Nandhini LP, Biswal N, Adhisivam B, Mandal J, Bhat BV, Mathai B. Synbiotics for decreasing incidence of necrotizing enterocolitis among preterm neonates - a randomized controlled trial. J Matern Fetal Neonatal Med. 2016; 29(5):821-5.
18. van den Nieuwboer M, Claassen E, Morelli L, Guarner F, Brummer RJ. Probiotic and synbiotic safety in infants under two years of age. Benef Microbes. 2014; 5(1):45-60.