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Original Article

Effectiveness of Oral Nystatin Prophylaxis in the Prevention of Candida Colonization in Very Low Birth Weight Preterm Neonates; a Randomized Controlled Trial

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ABSTRACT

Background: The present study aimed to assess the effectiveness of oral nystatin as antifungal prophylaxis in the prevention of fungal colonization in premature neonates admitted to neonatal intensive care units (NICUs).

Methods: This randomized controlled trial was conducted on 106 neonates admitted to NICUs in the first 72 h of their life with gestational age, gestational age of fewer than 32 weeks, and birth weight of less than 1500 g. The neonates were randomly assigned to two groups: the case group (received nystatin, n=53) and the control group (did not receive nystatin, n=53). After one week, swabs were prepared from the oral and rectal mucosa of the subjects for smear and fungal culture.

Results: The results obtained from the culture revealed that nystatin prophylaxis significantly decreased the colonization of Candida in premature neonates (P=0.03). Moreover, nystatin prophylaxis was significantly associated with Candida colonization in preterm infants with the following characteristics: a gestational age of 28-32 weeks (0 (0.0%) vs. 5 (10.9%)) (P= 0.05), very low birth weight (VLBW) infants (0 (0.0%) vs. 8 (16.0%)) (P=0.007), neonates born via normal vaginal delivery (NVD) (1 (11.1%) vs. 8 (72.7%)) (P=0.01), infants born after preterm rupture of the membrane (PROM) (1 (10.0%) vs. 6 (75.0%)) (P=0.01), and neonates taking broad-spectrum antibiotics (1 (3.7%) vs. 7 (26.9%)) (P=0.02).

Conclusion: The present study demonstrated that nystatin prophylaxis might be considered an effective drug in the prevention of Candida colonization and might lower the risk of SFIs; nonetheless, it had no significant effect on extremely low birth weight (ELBW) neonates. Since nystatin is safe, well-tolerated, affordable, and effective, further studies are required to confirm it as a therapeutic option for ELBW newborns with Candida infections.

Keywords: Candida colonization, Neonatal intensive care unit, Nystatin prophylaxis, Premature infants

Introduction

Nowadays, great advances in medical sciences have increased the survival rate of premature neonates. Systemic fungal infections (SFIs) are a major cause of mortality and morbidity, and susceptibility to these types of infections is found to be high among preterm infants admitted to neonatal intensive care units (NICUs); therefore, they have become a major health problem (1). Most cases of fungal colonization occur during the first 2-3 weeks and can be vertical or nosocomial (2).

Recent reports have indicated that the incidence of neonatal candidiasis accounts for approximately 0.15% of hospitalized neonates; nonetheless, this rate ranges from 2.0%-9.0% in neonates weighing less than 1000 g. However, some other centers have reported figures up to 10 times higher (1). Moreover, about 10% of full-term neonates undergo colonization in the gastrointestinal tract and respiratory tract in the first five days of life, which reaches 30% in very low birth weight (VLBW) neonates, weighing less than 1500 g (3,4). In a similar vein, nearly 26% of infants with Candida infection and extremely low birth weight (ELBW) die, and the remaining 60%

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develop neurodevelopmental disorders (2,5).

Neonatal candidiasis can be fatal in premature neonates; therefore, an unawareness of its risk factors can have adverse consequences. Some of these factors include delayed onset of intestinal feeding, long-term intravenous feeding, long-term intubation and ventilation, long-term use of central venous catheters, and the use of H2 blockers, such as cimetidine and famotidine. Some parameters, such as broad-spectrum antibiotics, steroids, normal vaginal delivery (NVD), abdominal surgery, birth weight, and gestational age, also increase susceptibility to SFIs. Birth weight and gestational age are among the most important risk factors (1). Gestational age is associated with the underdevelopment of the immune system, skin, gastrointestinal tract, and airways of these infants (6).

Antifungal prophylaxis is of utmost importance since it contributes to the reduction of both colonization and SFIs in preterm neonates (7). Oral nystatin is the first drug administered as antifungal prophylaxis. Research has demonstrated that oral nystatin significantly decreases the risk of SFIs in premature infants. As suggested by the evidence, the incidence of fungal infections in VLBW infants decreased from 12% to 1.8% after the introduction of oral nystatin prophylaxis. As a result, this treatment seems to be more effective, safer, cheaper, and more tolerable, as compared to treatment with fluconazole (3).

Although nystatin has been presented as the first drug to prevent fungal colonization, concerns over increasing resistance to this drug have restricted its use. Compared to alternative drugs, such as intravenous fluconazole, this medicine is easily accessible and affordable in Iran, has no known side effects, and has not been administered in premature neonates born in Iran. In light of the aforementioned issues, the present study aimed to evaluate the effectiveness of oral nystatin as antifungal prophylaxis in the prevention of fungal colonization in preterm newborns admitted to NICUs.

Methods

Trial design and population

This randomized controlled trial was performed to evaluate the effect of oral nystatin prophylaxis on the prevention of Candida colonization in preterm neonates, weighing less than 1500 g, who were admitted to NICUs. All the infants born in Ayatollah Mousavi Hospital in Zanjan, Iran, or admitted to other hospitals in Zanjan province, who were admitted to NICUs in the first 72 hours of life and had a gestational age of fewer than 32 weeks or a birth weight of less than 1500 g were examined. The study was conducted in accordance with the Declaration of Helsinki.

Inclusion and exclusion criteria

The inclusion criteria were as follows: gestational age less than 32 weeks and birth weight less than 1500 g. Moreover, participants with severe congenital or chromosomal abnormalities, severe sepsis, disseminated intravascular coagulation (DIC), intraventricular hemorrhage (IVH), congenital heart disease (CHD), oral thrush, congenital malformations requiring surgery, and infants without parental consent were excluded from the study.

Randomization

The neonates were randomly assigned to two groups with a 1:1 aspect ratio to receive either nystatin or placebo through a computer-based program, which follows a random number generator protocol. The case group received nystatin, whereas the control group did not. The participants were classified via an online calculator at www.calculator.net, and each patient was randomly assigned a number based on the output of the calculator. Subjects numbered 1-53 were in The intervention group, while the control group contained numbers 54-106. Further, both groups were the same regarding age, gender, gestational age, and other anthropometric findings.

Trial Procedures

The most effective time to start antifungal prophylaxis treatment is on the first day of life. The initiation of prophylaxis five days after birth is less effective since fungal colonization has already occurred. The neonates in the nystatin group received an oral suspension of nystatin produced by Emad Darman Pars Company (100,000 units per ml) at the dosage of 1 ml (0.5 ml in the oral cavity and 0.5 ml through an orogastric tube) three times a day, while the control group did not receive antifungal prophylaxis. After one week, the swabs were prepared from the oral and rectal mucosa for smear and fungal culture.

More than 90% of Candida albicans isolates can be detected within 2-3 h. In the laboratory, the sample was transferred to a Sabouraud dextrose agar medium. After 24-48 h, it was removed from a pure yeast culture with a sterile inoculation loop, and a suspension of yeast containing 100-1000 cells was prepared in the serum of calves, cattle, rabbits,

or humans with a volume of 0.3-0.5 ml. The prepared suspension was kept at 37°C for 2-3 h; thereafter, a drop of it was placed on a sterile slide using a sterile inoculation loop. After covering the slide with a coverslip, the presence or absence of germ tubes was assessed under a microscope. About 90% of clinical isolates of Candida form germ tubes if kept in the serum at 37°C for 2-3 h.

Sometimes, under similar conditions, the arthroconidia of the geotrichum trichosporon form long tubes that are similar to the germ tubes of Candida albicans. Nonetheless, Candida albicans produces active germ cells, in addition to the germ tubes, and also lacks arthroconidia in the true mycelium; therefore, it can be distinguished from other fungus (8). The neonates with fungal infections proven by blood or urine cultures were excluded from the study and treated with appropriate antifungal drugs, such as amphotericin at the standard therapeutic dose. Data were collected using a predefined checklist.

Trial outcomes

A checklist was prepared containing all the information, such as gestational age, birth weight, type of delivery (normal vaginal delivery (NVD) or cesarean section), premature rupture of the membrane (PROM), use of broad-spectrum antibiotics (third-generation cephalosporins and carbapenems), receive invasive respiratory ventilation, oral culture and smear for fungi, rectal culture and smear for fungi.

Statistical analysis

Descriptive variables are presented in terms of numbers (percentages). Chi-square and Fisher exact tests were used to compare the descriptive variables between the two groups. In order to eliminate the effects of confounding factors, such as weight and the use of non-invasive methods, logistic regression was used. In this method, The status of oral colonization, therapeutic intervention (antibiotic prophylaxis), weight, and the use of non-invasive methods were entered into the model as outcome, independent, and potential confounding variables, respectively. The data ere analyzed in SPSS software (version 16.0). P-values less than 0.05 were considered statistically significant.

Trial oversight

The present study was registered at (irct.ir) (registration number: IRCT20201222049802N2). Moreover, it was approved by the Institutional Review Board of Zanjan University of Medical Sciences and obtained the approval of the Ethical Committee (IR.ZUMS.REC.1399.330).

Results

A total of 122 VLBW neonates were hospitalized. Out of 16 patients who were excluded, four cases showed major congenital defects, seven patients declined to participate, and five neonates died before 72 hours of life. A total of 106 VLBW neonates were recruited and randomly assigned to the case (nystatin = 53) and control (placebo = 53) groups (Figure 1). Among the studied variables, only the frequency distribution of neonatal weight in the two groups demonstrated a statistically significant difference (Table 1). Table 2 displays the prevalence of baseline variables based on Candida culture results. All positive cases were observed in NDV, and in cesarean delivery, the culture results of all neonates were negative (P<0.001). Gestational age (P=0.02), weight (P=0.002), type of delivery

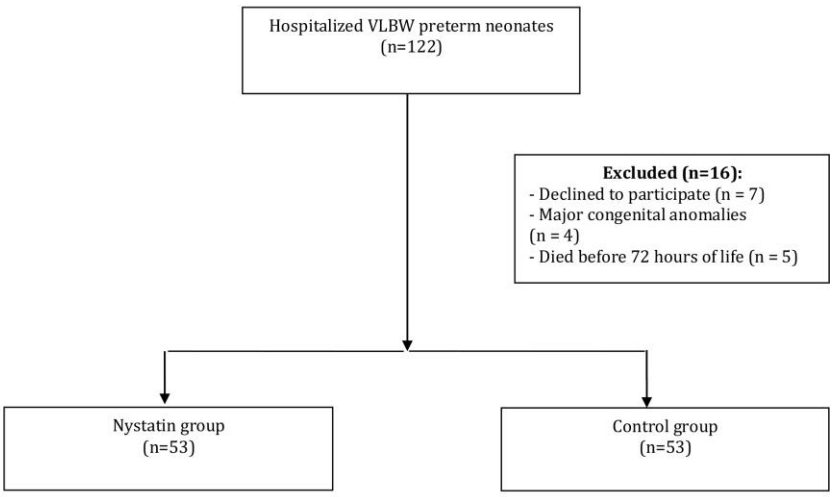

Figure 1. Flowchart of the participants

Table 1. Frequency of baseline variables in the case and control groups

Variables		Groups		P-value
		Control	Case	
Sex	Male	27 (50.9)	26 (49.1)	0.846
	Female	26 (49.1)	27 (50.9)	
Gestational age	<28 months	7 (13.2)	9 (17.0)	0.587
	28-32 months	46 (86.6)	44 (83.0)	
Weight	ELBW	3 (5.7)	12 (22.6)	0.012
	VLBW	50 (94.3)	41 (77.4)	
Type of delivery	NVD	11 (20.8)	9 (17.0)	0.620
	Cesarean	42 (79.2)	44 (83.0)	
PROM	No	45 (84.9)	43 (81.1)	0.605
	Yes	8 (15.1)	10 (18.9)	
Broad-spectrum antibiotics	Yes	27 (50.9)	26 (49.1)	0.846
	No	26 (49.1)	27 (50.9)	
Invasive respiratory ventilation	No	23 (43.4)	15 (28.3)	0.142
	≤3 days	20 (37.7)	30 (56.6)	
	>3 days	10 (18.9)	8 (15.1)	

ELBW: extremely low birth weight, VLBW: very low birth weight, NVD: normal vaginal delivery, PROM: preterm rupture of the membrane

(P<0.001), and PROM (P<0.001) differed significantly between the two groups of positive and negative culture samples.

Table 3 shows a comparison of Candida colonization results between the case and control groups based on such variables as gestational age, weight, type of delivery, PROM, broad-spectrum antibiotics, and invasive respiratory ventilation. The results obtained from the culture revealed that nystatin prophylaxis was significantly associated with candida colonization in premature infants
(P= 0.03). At 28-32 weeks of gestation, nystatin prophylaxis was significantly associated with candida fungal colonization in preterm neonates from 11%-0% (P=0.05).

In addition, it was found that in VLBW neonates (P= 0.007), nystatin prophylaxis had a significant relationship with the colonization of Candida, and the number of positive cases decreased from 16% in the control group to 0% in the case group.

The present study indicated that in NVD (P=0.01), the number of positive cases in the control group was 72%, while in the case group, nystatin prophylaxis significantly reduced Candida colonization by 11%. In addition, the results showed that nystatin prophylaxis significantly decreased the colonization of Candida from 75% to 10% in preterm neonates born after PROM (P=0.01). Regarding the use of broad-spectrum antibiotics (P=0.02), nystatin prophylaxis led to a significant reduction in the colonization of Candida in preterm infants (P=0.30), while nonuse of broad-spectrum antibiotics had a significant effect on Candida colonization in preterm neonates. Invasive respiratory ventilation also did not contribute to

Table 2. Prevalence of baseline variables based on *Candida* culture results

Variables		Culture results		P-Value
		Positive	Negative	
Total frequency		9 (8.5)	97 (91.5)	------
Gestational age	<28 months	4 (44.4)	12 (12.4)	0.02
	28-32 months	5 (55.6)	85 (87.6)	
Weight	ELBW	6 (66.7)	15 (15.5)	0.002
	VLBW	3 (33.3)	82 (84.5)	
Type of delivery	NVD	9 (100.0)	11 (11.3)	< 0.001
	Cesarean	0 (0.0)	86 (88.7)	
PROM	No	2 (22.2)	86 (88.7)	< 0.001
	Yes	7 (77.8)	11 (11.3)	

ELBW: extremely low birth weight, VLBW: very low birth weight, NVD: normal vaginal delivery, PROM: preterm rupture of the membrane

Table 3. Comparison of *Candida* colonization results between the case and control groups in terms of gestational age, weight, type of delivery, PROM, broad-spectrum antibiotics, and invasive respiratory ventilation

Variables			Culture results		P-Value
			Negative	Positive	
Gestational age	<28 months	Case	8 (88.9)	1 (11.1)	0.26
		Control	4 (57.1)	3 (42.9)	
	28-32 months	Case	44 (100.0)	0 (0.0)	0.05
		Control	41 (89.1)	5 (10.9)	
Weight	ELBW	Case	11 (91.7)	1 (8.3)	>0.99
		Control	3 (100.0)	0 (00.0)	
	VLBW	Case	41 (100.0)	0 (0.0)	0.007
		Control	42 (84.0)	8 (16.0)	
Type of delivery	NVD	Case	8 (88.9)	1 (11.1)	0.01
		Control	3 (27.3)	8 (72.7)	
	Cesarean	Case	44 (100.0)	0 (0.0)	---
		Control	42 (100.0)	0 (0.0)	
PROM	No	Case	43 (100.0)	0 (0.0)	0.16
		Control	43 (95.6)	2 (4.4)	
	Yes	Case	9 (90.0)	1 (10.0)	0.01
		Control	2 (25.0)	6 (75.0)	
Broad-spectrum antibiotics	Negative	Case	26 (100.0)	0 (0.0)	0.30
		Control	26 (96.3)	1 (3.7)	
	Positive	Case	26 (96.3)	1 (3.7)	0.02
		Control	19 (73.1)	7 (26.9)	
Invasive respiratory ventilation	No	Case	15 (100.0)	0 (0.0)	0.24
		Control	21 (91.3)	2 (8.7)	
	≤3 days	Case	29 (96.7)	1 (3.3)	0.28
		Control	17 (85.0)	3 (15.0)	
	>3 days	Case	8 (100.0)	0 (0.0)	0.21
		Control	7 (70.0)	3 (30.0)	
Candida colonization		Case	1 (1.9)	52 (98.1)	0.03
		Control	8 (15.1)	45 (84.9)	

ELBW: extremely low birth weight, VLBW: very low birth weight, NVD: normal vaginal delivery, PROM: preterm rupture of the membrane

Table 4. Multiple logistic regression regarding the outcome of oral and rectal colonization

Type of colonization	Variables	Odds ratio (95% CI)	P-Value
Oral colonization	Weight	0.97 (0.86-11.00)	0.985
	Non-invasive methods	2.03 (0.50-5.00)	0.161
	Groups	10.10 (1.09-92.98)	0.041
Rectal colonization	Weight	0.96 (0.76-12.00)	0.977
	Non-invasive methods	2.84 (1.59-9.00)	0.080
	Groups	7.40 (0.73-74.36)	0.089

the reduction of Candida colonization in preterm neonates by nystatin prophylaxis (P>0.05 for all comparisons) (Table 3).

The results revealed that despite adjusting the regression model for potential confounding variables, the therapeutic intervention had a significant effect on the mitigation of oral colonization (P=0.041). However, the results of rectal colonization outcome did not show a statistically significant relationship between rectal colonization and the therapeutic intervention (P > 0.05 for all comparisons) (Table 4).

Discussion

The results of the present study revealed that nystatin prophylaxis might be effective in the prevention of colonization and might lower the risk of SFI; however, it did not demonstrate much effect in ELBW neonates. Premature neonates are at a higher risk of invasive fungal infections, as compared to term neonates. The high rates of colonization (between 22% and 87%) were observed in preterm infants who did not receive any fungal prophylaxis (9,10). A similar result was observed in the study by Rundjan et al. (11) who

reported the colonization rates of 6.3% and 29.8% in the control and nystatin groups, respectively.

The present study pointed out that oral nystatin significantly reduced the rate of Candida colonization in preterm neonates. Consistent with the results of the current research, previous studies have suggested that nystatin can prevent colonization and SFIs (10,12). A randomized control trial study by Aydemir et al. revealed that fluconazole or nystatin prophylaxis prevents SFI in VLBW neonates (10). The findings of the stated study suggested that no significant differences were found between the nystatin and fluconazole groups in terms of invasive fungal infection and fungal colonization. Moreover, in agreement with the results of the present study, they proposed nystatin as a prophylactic option for the treatment of VLBW neonates with fungal colonization and invasive fungal infection.

In the same context, Rundjan et al. pointed to a significant reduction in fungal colonization in the nystatin group, as compared to that in the control group. In their study, all cases diagnosed with SFI were in the case group, whereas the nystatin group comprised no such cases. This result confirmed the decreasing trend of SFI risk and the potential preventive effect of nystatin prophylaxis against SFI (11). The findings of the current research were in accordance with those of the study by Howell et al. on the use of oral nystatin prophylaxis. They reported a significant association between the use of oral nystatin prophylaxis and a reduction in fungal infections (3).

Randomized clinical trials by Ozturk et al. (9) also showed that nystatin prophylaxis significantly reduced SFI; however, no significant differences were detected between the studied groups in terms of mortality rates. A study by Ganesan et al. reported reduced systemic fungal infections in neonates with a gestational age of fewer than 33 weeks due to the use of oral nystatin prophylaxis (12). Therefore, it can be stated that nystatin prophylaxis is effective in the prevention of Candida colonization and may potentially diminish the risk of fungal infections.

In the study by Islam et al., such variables as infant weight, type of delivery, and duration of pregnancy were demonstrated not to be associated with a significant reduction in candida colonization due to nystatin prophylaxis (13). Nevertheless, the present study revealed that nystatin prophylaxis significantly reduced Candida colonization in preterm neonates with 28-32 weeks of gestation, VLBW neonates, and infants born with NVD and PROM. It should be noted that nystatin prophylaxis had no significant effect on infants with a gestational age of fewer than 28 weeks and ELBW neonates. Ozturk et al. also reported that gestational age, NVD, and antibiotics were statistically associated with invasive candidiasis in preterm infants (9).

Previous studies have demonstrated that the density and number of reported colonization foci are positively associated with the risk of subsequent SFI (14), and accordingly, an increased risk of fungal transmission and spread (14,15). Other studies have reported that high fungal densities and multiple foci of fungal colonization are associated with an increased risk of SFI. Kaufman et al. (15) indicated that the risk of SFI increases with each of the additional colonization foci.

Among the notable limitations of the present study, we can refer to the lack of blinding. Moreover, although acceptable sample sizes were examined, the follow-up time was short. Since most cases of fungal colonization occur during the first two to three weeks, it is recommended that each neonate be sampled more than once-that is to say, a sterile swab sample should be taken orally and rectally until weekly discharge. Given the lack of colonization with fungi in most infants at birth, as well as the diminutive colonization in the study, it was not required to measure colonization before the intervention. Furthermore, this study only examined the effect of nystatin on the Candida fungus. It is recommended that future studies assess the effects of other prophylactic antifungal drugs, in parallel with nystatin, on other species that lead to widespread fungal infections.

Conclusion

As evidenced by the results of the present study, nystatin prophylaxis is effective in the prevention of colonization and is likely to lower the risk of SFI, even though it does not show a significant effect on ELBW neonates. Accordingly, it can be concluded that nystatin might be considered a suitable prophylactic drug in this group of high-risk neonates to prevent Candida infection since it is an effective, safe, easy to use, inexpensive, and tolerable drug. It is recommended that similar studies be performed on larger sample sizes in the future, and other SFI risk factors, such as corticosteroid use, intravenous nutrition, use of the gastric tube, and central venous catheters, be investigated.

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None.

Conflicts of interest

The authors declare that they have no conflict of interest.

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