Original Article

Effect of Prophylactic Oral Nystatin on Fungaemia Prevention among VLBW Neonates in NICU

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ABSTRACT:

Introduction: *Recent global estimates suggest that > 1 in 10 or an estimated 15 million babies born in 2010 were preterm, of which >1 million died as a result of prematurity and its complications. Bangladesh ranked the 7th on the top-10 country list for high preterm births in 2010. The risk of invasive fungal infections is high in very low birth weight (VLBW) infants (<1500 g) and highest for infants born at the youngest gestational ages who survive past the immediate postnatal period. Overall mortality attributable to invasive candidiasis was 19.3%. Starting empiric antifungal therapy may decrease the high mortality rate of invasive fungaemia in VLBW infants, especially those born at <28 weeks' gestation.* ***Objectives-*** *To evaluate the efficacy of prophylactic oral Nystatin on fungaemia among VLBW neonates.* ***Methodology-*** *It was a RCT; conducted in NICU of the Department of Neonatology, BSMMU, Dhaka; from Dec.15 to Sept. 16. A total of 25 cases (Group-A) and 25 controls (Group-B) were included in this study purposively and grouped by lottery. Group-A got prophylactic Nystatin orally [1 ml (100000units/ml) every 6 hour started, 24 hours after initiation of feeding until discharged] and group B did not get any prophylactic anti-fungal medication. All the babies received supportive treatment as required.* ***Results-*** *Both groups showed similar pattern of distribution; regarding sex, gestational age, anthropometric measurements and vital parameters. For both groups, Jaundice was present in most neonates (>90.0%). Chest indrawing, Apnoea, Hyperglycaemia, Hypoglycaemia, Grunting and Cyanosis were most notable presentations. All the presentations showed higher 'positive' counts in 'Group-B' and there was some positive association for most of the factors (except Hypoglycaemia, Grunting, Cyanosis and Shock). Majority in Group-B needed Phototherapy, Ionotrop and respiratory support (either CPAP or ventilator) than Group-A. Feeding intolerance developed more in Group–B, though not significant (p-0.5558). None in Group–A but 24.0% neonates in Group–B had invasive fungaemia (p-0.009). Neonates of Group-A (16.96 ± 6.931days) had to stay at hospital for shorter duration than Group-B (26.84± 16.062days) for treatment (p-0.009). Death rate (due to any cause) was lower in Group-A group (12.0%) than Group-B group (28.0%). (p-0.3057).* ***Conclusion-****. The study concludes, prophylactic oral nystatin in neonates reduces the frequency of developing fungaemia and also reduces duration of hospital stay.*

Key words: *Oral Nystatin, Fungaemia, VLBW, NICU, Prophylactic.*

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INTRODUCTION:

Recent global estimates suggest that more than 1 in 10 or an estimated 15 million babies born in 2010 were preterm, of which more than 1 million died as a result of preterm birth and related complications.[1] Although neonatal mortality rates have fallen globally between 1990 and 2009,[2] the absolute numbers and rates of preterm birth have increased during this period.[3] Preterm birth complications account for 35% of the estimated 3.1 million global neonatal deaths,[3] and are the second leading cause of child death after pneumonia[4]. The vast majority (85%) of global preterm births occur in Asia and Africa[5] where health systems are weak and access to and utilization of health services are limited, contributing to the higher risks of death and disabilities in preterm babies.[6,7]

Bangladesh ranked 7th on the top-10 country list for high preterm birth rates in 2010.[1] A study in a rural population of Bangladesh showed, among live-born babies, more than one-fifth was preterm (22.3%) and the majority of preterm births (55.1%) were late preterm.[8] The estimate is consistent with data from similar regional community-based research sites in southern Nepal-19% and north-western Bangladesh–23%.[9,10]

In developing countries hospital-born babies are at increased risk of neonatal infections because of poor intrapartum and postnatal infection-control practices. Reported rates of neonatal infections were 3–20 times higher than those reported for hospital-born babies in industrialized countries.[11] In Bangladesh according to World Bank at 2012, neonatal mortality rate is 24.40. Sepsis is a common cause of neonatal death and about 21% of VLBW babies have one or more episodes of culture proven sepsis.[12] Fungal sepsis is not also uncommon. Colonization with Candida species is common among babies in the NICU.[13] In China among the cohort of infants with invasive candidiasis, 214 (96.0%) infants had positive blood culture for fungus, 5 infants had positive urine culture, 3 from CSF and 1 from articular effusion in the shoulder joint. Among the cohort of infants with candidemia, 48 (22.4%) infants had fungal meningitis. Candida albicans accounted for 57.4% of total positive cultures. In china, overall mortality attributable to invasive candidiasis was 19.3%.[14]

The risk for invasive fungal infections is high in very low birth weight (VLBW) infants (< 1500 g) and highest for infants born at the youngest gestational ages who survive past the immediate postnatal period.[15,16] These immunocompromised infants usually require invasive therapies, such as central vascular catheters and endotracheal tubes, and are exposed to broad-spectrum antibiotics and parenteral nutrition. In addition, they occasionally receive postnatal steroids and gastric acid inhibitors. All of these factors place them at high risk for fungal infection. The incidence had been increasing in infants <1000 g with the resuscitation and survival of more and more infants prior to the studies of and broad institution of antifungal prophylaxis in high-

risk preterm infants.[17,18] In a recent review article, Chapman reports that invasive candidal infection has a crude mortality of 30%[19], and is associated with overall poorer neurodevelopmental outcomes and higher rates of ROP, compared to matched VLBW control infants. Prevention of fungaemia may reduce this mortality and morbidity.

C albicans remains the most frequently isolated yeast species in infected neonates, followed by C parapsilosis infections in centers not using antifungal prophylaxis.[20,21] Candida albicans and parapsilosis account for 80-90% of infections. Candida can invade the bloodstream and disseminate in these infants because of their immature immune systems. For these reasons, fungal infections are often difficult to eradicate in the preterm infant.[22]

Clinical presentation of fungaemia, depending on patients and risk factors, may span from the absence of specific symptoms to severe sepsis or septic shock. Blood cultures remain the mainstay for the diagnosis of fungaemia, but their sensitivity is limited as they are positive in approximately 50% of cases of fungaemia.[23,24] Furthermore, at least 48 h are required for species identification and susceptibility testing.[25] Non-invasive biomarkers have been extensively investigated and they include serological markers (mannan, antimannan and (1,3)-β-d-glucan) and molecular methods identifying fungal DNA.[26-28]

Preterm birth affects not only infants but also their families who may have to spend substantial time and financial resources to ensure care for their preterm infants; thus, preterm birth has increasing cost implications for families and health services.[29]

In the VLBW infant, an evaluation for signs and symptoms of late-onset sepsis is typically accompanied by antibacterial treatment for at least 48 hours. Some studies have reported on the use of empiric antifungals pending culture results. Some authors propose that starting empiric antifungal therapy while culture results are pending may decrease the high mortality rate associated with fungaemia in VLBW infants, especially those born at <28 weeks' gestation.[30,31] In other studies, empiric therapy has improved outcomes in VLBW infants.[30,32] Evidence also showed that the efficacy of oral nystatin to prevent this problem, however, evidence in Bangladesh in this context is scanty. So the objective of the study was to assess the efficacy of prophylactic oral Nystatin on prevention of invasive fungaemia among VLBW neonates in NICU.

METHODS AND MATERIALS:

This was a randomized controlled clinical trial (RCT), conducted at Neonatal Intensive Care Unit (NICU) of the Department of Neonatology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahabag, Dhaka, from December 2015 to September 2016, over a period of 09 (nine) months. All inborn very low birth weight (<1500 gram),

who were admitted in the NICU of BSMMU, Dhaka, during the study period, were the study population. Calculated sample size was 50, comprising 25 in each group, a total of 64 babies had to be enrolled in this study by convenient sampling, as 14 died even before establishment of oral feeding. Inclusion criteria was inborn patients, very low weight babies (< 1500 gm), survival of the neonate till initiation of oral feeding and neonates; whose parents gave written consent. Exclusion criteria was out born patients**,** gestational age <27 weeks**,** incredible low weight babies (<750 gm), patients already suffering from any fungal infection, babies of mother who are known case of AIDS, and neonates; whose parents didn't give written consent. Enrolled babies were randomized by lottery in two groups. Group A got Nystatin and group B did not get any prophylactic anti-fungal medication. At the end of the study group A were taken as a case & group B were as control.

After enrolment each patient detailed history of gestational age (assessed by New Ballard Score), socio-economic information and risk factors along with physical findings were recorded in a predesigned questionnaire. In group A 1.00 ml of nystatin suspension (100 000 units/ml) were given every 6 hour started after 24 hours after initiation of feeding until discharged from intensive care.[33] The oral cavity was coated with 0.5 ml nystatin and the rest was given through an orogastric tube.[33] The doses were ensured by trained NICU nurses. It was withheld if there was evidence of peritonitis or concern about a diagnosis of necrotizing enterocolitis. Drug of a same pharmaceutical company was prescribed (Drop Candex, Square pharmaceuticals, batch-509001). Clinical follow-up along with blood for bacterial & fungal culture (in Brain-heart infusion media[34]) were sent. Culture was done in Department of Microbiology of BSMMU. All the babies received supportive treatment i.e. thermal care, parental nutrition, oxygen therapy with respiratory support and ionotrops (if required).

Finally, all the data were documented, tabulated and entered into computer for final analysis by using computer software SPSS (Statistical Package for Social Sciences) version 21. Comparison between the two groups were evaluated statistically, and expressed in mean, and SD. Chi-square test, student's t-test, RR (relative risk) and OR (odds ratio) with 95% CI was done for statistical analysis. The level of significance was set at $p<0.05$. This study was approved by the Institutional Review Board (IRB) of BSMMU.

Operational definitions: Neonate: a period of human life, measured from birth to 28 days. **Fungaemia:** the presence of fungi in the blood isolated in blood culture. **VLBW**: Neonate of less than 1500 grams. **ELBW:** Neonate of less than 1000 grams. **Neonatal sepsis:** Neonatal sepsis is defined as a clinical syndrome of bacteraemia with systemic signs & symptoms of infection in first 28 days of life. **Clinical Sepsis:** neonates having clinical signs of sepsis such as hypo/ hyperthermia, poor feeding, fast breathing, convulsion ETC. **Prophylactic:** It is a preventive measure or

medication or treatment designed and used to prevent a disease from occurring

RESULTS:

This study was undertaken with the objective to evaluate the role of prophylactic oral nystatin for very low birth weight babies to prevent fungaemia in NICU. Expected sample size was n-25 (approx.) for each group, that is a total of 25x2=50 babies were to be included in the study. However, during the study period a total of 73 neonates were admitted into the Neonatal Intensive Care Unit. Among them 67 could be included in the study according to the inclusion and exclusion criteria, out of them 64 had to be enrolled in this study to get 50 participants, as 14 died even before establishment of oral feeding. Among the remaining 50, 25 were treated with oral Nystatin and 25 did not receive any prophylactic anti-fungal medication, the division was decided through lottery.

Most of the participants in both Group; Group-A [16 (64.0%)] and Group-B [15 (60.0%)] were Males. Male: Female ratio was about 1:0.56 and 1:0.67 in Group-A and Group-B respectively which was not significant (p-0.7706).

Majority of neonates in Group-A 14(56%) and Group-B 13(52%) were between 27 to <30week gestational age and 11(44%) in Group-A and 12 (48%) in Group-B were between 31-34weeks of gestational age, which was not significant (p-0.7759). Mean gestational age was 31.32±1.952 weeks in Group-A and 31.12±1.810 weeks in Group-B, which was also not significant (p-0.745). Majority of Group-A 21(84%) and Group-B 23(92%) neonates were between birth weight of 1000-1499 grams, while only 4(16%) in Group-A and 2(8%) in Group-B were between 750 - 999 gm (0.384).

Mean weight in Group-A was 1229.80±212.370gms and in Group-B was 1296.40±163.907gms, mean length in Group-A was 39.400±3.8837cm and in Group-B was 38.740±1.9805cm. Mean OFC in Group-A was 28.120±1.7042cm and in Group-B was 28.008±1.6065cm. All were not significant (p-0.262, p-0.514, p-0.850 respectively).

Table-I: Distribution of the neonates by their vital parameters

Parameters	Group - A (n=25) (Mean ± SD)	Group – B (n=25) (Mean ± SD)	T-test	p-value
Temperature (°C)	36.708 ± 0.4725	36.808 ± 0.3013	0.937	0.358
Heart rate (bpm)	139.08 ± 13.687	139.28 ± 11.820	0.055	0.957
Respiratory rate (rpm)	63.80 ± 12.315	61.20 ± 12.503	0.663	0.514

There was no statistically significant difference in vital parameters among groups.

Table-II: Distribution of the children by their clinical presentation

Signs	Group - A (n=25) Percent	Group - B (n=25) Percent	RR and OR (95%CI)
Nasal flaring	0.0	8.0	OR=∞; 95% CI: NA
Grunting	28.0	28.0	OR=1.0; 95% CI: 0.5394-1.854
Chest indrawing	72.0	78.0	OR=1.3788; 95% CI: 0.7243-2.6247
Cyanosis	24.0	24.0	OR=1.0; 95% CI: 0.5226-19137
Pallor	12.0	16.0	OR=1.3968; 95% CI: 0.6239-3.1274
Jaundice	92.0	96.0	OR=2.087; 95% CI: 0.6077-7.1675
Convulsion	4.0	8.0	OR=2.087; 95% CI: 0.6077-7.1675
Oedema	0.0	4.0	OR=∞; 95% CI: NA
Apnoea	56.0	60.0	OR=1.1786; 95% CI: 0.6718-2.0677
Hypoglycaemia	36.0	36.0	OR=1.0; 95% CI: 0.6718-2.0677
Hyperglycaemia	40.0	44.0	OR=1.1786; 95% CI: 0.7751-2.399
Shock	12.0	12.0	OR=1.0; 95% CI: 0.4261-2.3466
Total	100.0	100.0	

For both Group-A and Group–B, Jaundice was present in most neonates (>90.0% in both groups). Chest indrawing (72.0% & 78.0%), Apnoea (56.0% & 60.0%), Hyperglycaemia (40.0% & 44.0%), Hypoglycaemia (36.0%), Grunting (28.0%) and Cyanosis (24.0%) were most notable presentations found among the participants. All the presentations showed higher 'positive' counts in 'Group-B' and there was some positive association for most of the factors (except Hypoglycaemia 36.0%, Grunting 28.0%, Cyanosis 24.0% and Shock 12.0%).

Table-III Distribution of the neonates by their General managements

General management	Group - A (n=25) Percent	Group - B (n=25) Percent	Statistical calculations
Phototherapy	88.0	92.0	OR=1.5682; 95% CI: 0.6119-4.0191
Oxygen	88.0	88.0	OR=1.0; 95% CI: 0.4261-2.3466
Ionotrop	12.0	12.0	OR=1.0; 95% CI: 0.4261-2.3466
Respiratory support	36.0	48.0	OR=1.461; 95% CI: 0.9315-2.8909
Ventilator	20.0	32.0	OR=1.188; 95% CI: 0.9871-3.5895
Total	100.0	100.0	

More neonates in Group - B needed Phototherapy, Ionotrop and respiratory support (either CPAP or ventilator) than those in Group -A group. Among the neonates of both groups, who required respiratory support, 20.0% of Group –A and 32.0% of Group – B needed ventilator.

Feeding intolerance developed more in Group-B [10(40.0%)] than Group–A [8(32.0%)], which was not statistically significant (p-0.5558).

'Invasive fungaemia' of neonates on blood culture was present 6(24.0%) Group–B and none (0.0%) in Group–A There was a statistically significant difference in development of invasive fungaemia between the groups [χ^2-6.818, df-1; p-0.009].

Table-IV: Distribution of the patients by their duration of hospital stay

Hospital stay	Group -A (n=25) (Percent)	Group - B (n=25) (Percent)
< 10 d	3 (12.0)	3 (12.0)
10-20 d	11 (36.0)	9 (44.0)
≥ 20 d	11 (52.0)	13 (44.0)
Total	25 (100.0)	25 (100.0)

On an average neonate of Group-A (16.96 ± 6.931days) had to stay at hospital for shorter duration than those of Group-B (26.84 ± 16.062 days) for their treatment; which was statistically significant (p-0.009).

Four (16%) patients in group A died and 21(84%) were discharged, while 7(28%) patient in Group-B died and 18(72%) patient discharged. Death rate (due to any cause) was lower in Group-A group than Group-B group, however no statistically

significant difference in mortality was observed between the groups (χ^2-1.049, df-1; p- 0.3057).

DISCUSSION:

This study was aimed to evaluate the role of prophylactic oral nystatin for pre-term babies on invasive fungaemia in NICU. Administering prophylactic nystatin to neonates born before 34 completed weeks of gestation has been associated with a significant decrease in the incidence of invasive fungaemia in these neonates. There were no significant differences in the baseline characteristics of the neonates cared for on our unit between the two groups.

Most of the participants in both Group-A group [16(64.0%)] and Group-B group [15(60.0%)] were Males. Male: Female ratio was about 1**:**0.56 and 1**:**0.67. As both groups showed similar distribution, there was no statistically significant difference in male-female distribution between the groups (p-0.7706). In both groups more than half of the neonates were in 27-31 weeks of gestational age category (56.0% and 52.0%). Mean gestational age was 31 weeks for both groups (p-0.7759, Chi-square and 0.745, t-test); which explains no statistical significance between the groups. Ganesan et. al found that, more than half of the babies were male (55.1% in nystatin prophylaxis group and 56.9% in pre-prophylaxis group, p-0.45); for both groups mean of gestational age was 29 weeks (p-0.65).[33]

Both groups had similar anthropometric measurements. Most of the neonates in both groups weighted '1000-1499 grams', 84.0% in Group-A and 92.0% in Group-B. Both groups showed similar Mean ± SD for weight (1229.80±212.370grams) and (1296.40±163.907grams), (p- 0.262). Mean length and OFC were also not significant (p=0.514) (p=0.850). Ganesan et. al found that, average birth weight was 1226 gm (in nystatin prophylaxis group) and 1164 gm (in pre-prophylaxis group); (p-0.16).[33]

For vital parameters, there was no statistically significant difference between the groups, temperature (p=0.358), heart rate (p=0.957) and respiratory rate (p=0.514). Considering clinical presentations, for both Group-A and Group-B, Jaundice was present in most neonates (>90.0% in both groups). Chest indrawing (72.0% & 78.0%), Apnoea (56.0% & 60.0%), Hyperglycaemia (40.0% & 44.0%), Hypoglycaemia (36.0%), Grunting (28.0%) and Cyanosis (24.0%) were most notable presentations found among the participants. All the presentations showed higher 'positive' counts in 'Group-B' and there was some positive association for most of the factors (except Hypoglycaemia 36.0%, Grunting 28.0%, Cyanosis 24.0% and Shock 12.0%). Ozturk et al. stated, the reasons for admission of premature babies were respiratory distress syndrome, perinatal asphyxia, apnoea, hyperbilirubinaemia and septicaemia.[35] Similarly Sims et.al. found colonized infants were dependent on the respirator (p<0.001), had indwelling catheters (p<0.01), and received antibiotics (p<0.05) for a longer period than infants free from fungi.[36]

For their management, more neonates in Group-B needed Phototherapy, Ionotrop and respiratory support (either CPAP or ventilator) than those in Group-A. Among the neonates of both groups, who required respiratory support, 20.0% of Group–A and 32.0% of Group–B needed ventilator.

Feeding intolerance developed more in Group-B [10(40.0%)] than Group–A [8(32.0%)], but was not statistically significant (p-0.5558). On blood culture, out of 25 participants in each groups none (0.0%) was found to be positive in Group – A, while in Group–B 24.0% neonates were found to have invasive fungaemia, which was statistically significant (p- 0.009). Similarly Ozturk et al. found that, there were 215 (14.2%) patients positive for invasive candidiasis during hospitalization in group A1 and in group B, only 36 (1.8%) patients were positive for invasive candidiasis(p-0.004). The numbers of positive blood culture specimens were lower in group B (n-22, 61.1%) than in groups A1 (n-180, 83.7%, p<0.001).[35] Ganesan et. al found that, a step reduction in the rate of systemic fungaemia occurred when the nystatin prophylaxis policy was introduced. The rate of blood culture positive infection fell from 4.1% in group B to 1.8% in group A (p-0.008). There was only one case of blood culture negative fungal meningitis in group B, and none in group A, and there were no blood culture negative renal tract fungal infections.[33] Sims et. al. reported infants treated with nystatin had a statistically significant reduction in the incidence of systemic fungal infection with four (12%) of the 33 nystatin-treated infants had positive cultures, two (6%) developed systemic infection. The control group consisted of 34 infants, 15 (44%) had positive fungal cultures and 11 (32%) developed systemic infection [RR 0.19 (0.04, 0.78); RD -0.26 (-0.44,-0.09)].[36]

On an average, neonates of Group-A (16.96 ± 6.931 days) had to stay at hospital for shorter duration than those of Group-B (26.84 ± 16.062 days), which was statistically significant (p- 0.009).

Death rate (due to any cause) was lower in Group-A [4 (12.0%)] than Group-B [7 (28.0%)]. However, there was no statistically significant difference in mortality between the groups (p-0.3057). Ozturk et al. found that, there were 101 deaths (6.6%) in group A1 and 127 deaths (6.4%) in group B (p-0.95). The mortality rates were not significantly different among group A1 (7.5% and 7.5%, respectively) and group B (7.2% and 7.7%) for the infant's birth weight of <1500 g (p-0.96) and <1000 g (p-0.98).[35] Ganesan et. al found that, mortality among infected babies was 46.7% in pre-prophylaxis group and 46.1% in nystatin prophylaxis group, suggesting that, although nystatin prophylaxis is associated with a reduction in the incidence of invasive fungal infection, there was no reduction in mortality for babies who developed these infections.[33] Sims et. al. also found mortality of colonized infants was significantly higher (p-<0.05).[36]

Nystatin is a polyene antifungal with a good safety profile in neonates. Unlike the azole group of antifungal agents, nystatin prophylaxis does not require monitoring of

liver function tests, as it is not absorbed from the gastrointestinal tract. No adverse effect related to nystatin was noted in neonates in this study and also by Ganeshan.[33]

CONCLUSION:

Prophylactic oral nystatin in neonates reduces the frequency of developing invasive fungaemia and also reduces the duration of hospital stay in preterm new born. For generalization of this findings, a large scale, multi-centre study over long duration will be required.

This was a single centre study conducted on a small sample size in a brief period mostly among VLBW babies, very few ELBW babies were also included in this study. Fungal culture was not performed at the time of discharge. This study was conducted in a tertiary care hospital in Dhaka. So the findings may not reflect the exact scenario of the country regarding invasive fungaemia. There was scarcity of study of invasive fungaemia in Bangladesh, in perspective of objective of the study; so, difficulty was faced to compare the study findings with others.

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