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Combination of Probiotic and Zinc in preterms significantly reduce mortality when used as therapeutic adjunct in Neonatal sepsis – An Open Label Randomized Controlled Trial

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Abstract

Background and Objectives: Despite advances in neonatal care, the mortality and morbidity from neonatal sepsis still remains high. Zinc is known to play a central role in the immune system. Decreased rates of infection have been observed following zinc supplementation in several population based studies of different diseases, notably diarrhoea, pneumonia and malaria. Probiotics are live microorganisms that when administered in adequate numbers confer health benefits. Probiotics may reduce mortality and necrotizing enterocolitis (NEC) in preterm infants, with unclear effect on sepsis. This study was done to evaluate efficacy of zinc alone or probiotics alone or their combination on mortality in preterm neonates with sepsis.

Methods: This was a prospective, open label, randomized controlled study of preterm neonates with proven sepsis randomly allocated to receive zinc, probiotics, or both or no supplementation. Zinc was given 10 mg/day. Probiotics used were Lactobacillus and Bifidobacteria. Primary outcome was effect on mortality. Secondary outcomes were effect on treatment failure, need for supportive treatment and duration of hospital stay.

Results: We enrolled a total of 124 neonates with 32 in the zinc group, 32 in probiotics group, 29 in zinc and probiotics group and 31 in the control group. Mortality

was significantly lower in the combination of zinc and probiotics group (P = 0.04). Also, the combination decreased treatment failure significantly (P= 0.02). But zinc or probiotics singly failed to show any significant effect on mortality.

Interpretation And Conclusions: Combination of zinc and probiotics (containing Lactobacillus and Bifidobacterium) given orally in neonatal sepsis reduces mortality and treatment failures in preterm neonates.

Keywords: Mortality, sepsis, neonate, probiotics, zinc

Introduction

Neonatal period is the critical period of life because of various problems which a neonate has to face. Systemic bacterial infections during this period have remained a major cause of infant morbidity and mortality. According to State of India's Newborn report 2014 (SOIN 2014), the incidence of neonatal sepsis in India is 30 per thousand live birth in hospital based studies while community-based studies indicate an incidence of 2.7% to 17% of all live births[1]. Despite advances in neonatal care, the mortality and morbidity from neonatal sepsis still remains high. So, there is need of inexpensive and accessible interventions that will improve treatment outcomes and reduce case fatality.

Zinc has a central role in the immune system. Supplementation of Zinc reduces rates of infection, notably diarrhoea, pneumonia and malaria[2]. Role of Zinc supplementation in reducing mortality in small for gestational age (SGA)[3] infants is shown in previous studies, also its role in enhancing growth of Low Birth Weight (LBW)[4,5] and very low birth weight (VLBW)[6] babies.

Probiotics are live microorganisms that when administered in adequate numbers confer health benefits[7]. Their effects are dose, strain and condition specific[8]. Meta-analyses and systematic reviews of randomized controlled trials (RCTs) of different probiotic strains when used in preterm infants found significant reduction in NEC but not sepsis[9-13].

This study was undertaken to evaluate whether therapy with zinc or probiotics or both in neonates with sepsis would reduce mortality, lead to earlier discharge from hospital, less need of higher lines of antibiotics and supportive treatment.

Material and Methods

A) **Type of Investigation**- Intervention

B) Study Question

(P) In intramural preterm neonates from 28 1/7 day to 36 6/7admitted to NICU with proven sepsis during the study period, (I) does oral zinc and/or probiotics (C) compared to standard treatment, (O) decrease mortality (T) from randomisation to discharge from hospital.

Design: Randomised, open label controlled trial in Neonatal Intensive Care Unit of a level III tertiary care hospital

Allocation: All neonates were randomly assigned (1:1) with a computer generated web based randomisation table. The neonates who were enrolled in the study are randomized in one of the four groups.

Blinding: Caregivers could not be masked because of the nature of bottles used. But treating physicians and statistician were blinded.

Follow up period: Until death or discharge from hospital

Setting: Neonatal Intensive Care Unit of a level III tertiary care hospital between December 2017 and May 2018.

Ethics statement: This trial was registered with Ethical clearance was obtained from the Institutional Ethical Committee. The procedures followed were in accordance

with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964, as revised in 2008. Written informed consent was taken from the parents of all neonates enrolled in the study.

Trial registration: The trial protocol was registered under the Clinical Trial Registry, India (**CTRI/2017/08/009544**).

Patients

Inclusion criteria: All intramural preterm neonates from 281/7 day to 366/7 days admitted to NICU with proven sepsis during the study period whose parents gave consent.

Diagnostic criteria for sepsis was

(a) Positive 'sepsis screen' i.e. presence of at least two of the following three parameters, namely, Total leucocyte count <5000/mm^3, Low absolute neutrophil count (as per standard charts), C-reactive protein>1mg/dl,

(b) Radiological evidence of pneumonia

(c) Culture positive sepsis

(d) Meningitis

Exclusion criteria: 1) In case of parental refusal to consent, 2) neonates < 28 weeks GA, 3) severe birth asphyxia, 4) life threatening congenital malformations, 5) critically ill newborns within 24 hours of life, 6) neonates with necrotizing enterocolitis were excluded from the study.

Intervention

The newborns with proven sepsis were enrolled in the study. In addition to antibiotics (as per our NICU protocol) and standard supportive care, newborns were given zinc, probiotics as a therapeutic adjunct.

Neonates were randomized in 4 groups i.e. Zinc group, Probiotics group, Zinc and Probiotics group and Control group.

1. Zinc group was given oral zinc supplementation 10 mg/day irrespective of age of newborn till discharge (Zinc 20 mg elemental zinc per ml)
2. Probiotics group was given as syrup containing L. acidophilus (1.25 billion), B.longum (0.125 billion), B. bifidum (0.125 billion) and B. lactis (1 billion) till discharge.
3. Zinc and Probiotic group was given both zinc 10 mg per day and Probiotic Syrup containing L. acidophilus (1.25 billion), B.longum (0.125 billion), B. bifidum (0.125 billion) and B. lactis (1 billion) till discharge.
4. Control group received only standard treatment.

- Zinc and Probiotics were dissolved in expressed breast milk and given orally or by nasogastric tube in babies kept nil by mouth.
- During the hospital stay, neonates were evaluated twice daily and in case needed supportive treatment i.e. ventilation, inotropic support, blood component transfusion or required second or third line antibiotics, decision is taken by treating consultant who is not part of study.

Outcomes

Primary outcome- Neonatal mortality

Secondary outcomes- treatment failure, need for supportive treatment (i.e ventilation, inotropes and blood component transfusion) and duration of hospital stay.

Safety

All babies receiving zinc and probiotics were monitored for 1) blood culture positive sepsis by bifidobacteria and 2) adverse effects such as abdominal distention, diarrhea and vomiting leading to withdrawal of supplementation.

Statistical Analysis

The data obtained was entered into Microsoft Excel. Pearson Chi-square test and Standard Error between two Means were used to test for statistical significance between the parameters and clinical criteria. P value <0.05 was considered to be statistically significant.

Results

A total of 132 babies were eligible as per study inclusion criteria. 8 parents refused, so total 124 patients were randomised in four groups. Zinc group had 32, probiotics had 32, zinc and probiotics had 29 and control group had 31 cases (Figure 1). None of the cases were discharged AMA or lost to follow up.

In our study, female babies (58%) were affected more than male (42%). Early onset sepsis (EOS) was more common. More than two third were culture positive, most common organism being Klebsiella pneumoniae. Bacterial Meningitis was predominantly seen in Late Onset Sepsis (LOS). Mean gestational age was 31 weeks 6 days and mean birth weight was 1535 gm. Average age of enrollment of babies was 39.6 hours. In this study, we found baseline characteristics were similar in all four groups as shown in table 1 (p>0.05). No adverse effect reported in any intervention group.

Mortality in zinc group was 34.4% (11), Probiotics 37.5% (12), Zinc+Probiotics group 17.3% (5) and Control group 41.9% (13). A total of 6 babies in Zinc+Probiotics group needed stepping up antibiotics for deterioration of disease as compared with Zinc (18), Probiotics (19) and Control (16). Lesser number of babies (08) required supportive treatment in form of inotropes, ventilation or blood product transfusion in Zinc+probiotics group in comparison with other three (Table 2).

A One-way ANOVA between subjects was conducted to compare the effect of Zinc, Probiotics and both on the duration of hospital stay. There was no significant difference between Zinc, Probiotics and both on duration of hospital stay at p<0.05 level for the three conditions [F(3, 120) = 0.56, p = 0.64] (Table 3).

Discussion

We hypothesized that zinc and probiotics alone or in combination reduce mortality in neonatal sepsis in preterm babies.

When we analyzed the data statistically, we found that Zinc and Probiotics in combination rather than independent reduced mortality significantly (p 0.049) with RR 0.41 (95% Cl 0.16-0.99 and NNT 4). Also the combination reduced treatment failure significantly (p value 0.02, RR 0.4, 95% Cl 0.18-0.88, NNT 3).

With combination of Zinc and probiotics, there was reduction in number of babies who needed supportive treatment (RR 0.53, 95% Cl 0.27-1.05, NNT 4) but results were statistically insignificant (p value 0.07).

In our study, there was no significant difference in duration of hospital stay when treated with zinc or probiotics alone or in combination in neonatal sepsis in preterms.

However, Zinc alone reduced number of deaths than control but difference was not statistically significant (p=0.5). Also, zinc or probiotics alone did not reduce need for higher line of antibiotics (p value 0.7 and 0.5 respectively).

Mehta K et al[14] and Bhatnagar S et al[15] in their study on Zinc in neonatal sepsis could not demonstrate either significant reduction in mortality or reduction of treatment failure.

But contrary to our observation, Newton B et al[16] and Sazawal S et al[18] found Zinc supplementation in neonatal

sepsis reduced mortality, improved mental development quotient at 18 months and significantly decreased inflammatory cytokines and serum calprotectin[19].

Probiotics alone were unable to show statistically significant reduction in mortality (p=0.7) in neonatal sepsis. Similar observations were by Jacobs SE et al[17] and Tewari VV et al[20]. In 2014, Patole S et al[21] used Bifidobacterium breve M16V as probiotic and found that fecal count after supplementation increased significantly but didnt altered all cause mortality or rates of late onset sepsis.

Contrary to our study findings, Baucells BJ et al[22] and found beneficial effect of Probiotics in neonatal sepsis in reducing all-cause mortality. In a meta-analysis in 2017 by Rao SC[23], probiotic supplementation reduces risk of LOS in preterm babies.

We didn't find any study which evaluated combination of Zinc and probiotics, used as therapeutic adjunct in neonatal sepsis.

In our study, we consistently found efficacy of Combination of zinc and probiotics containing lactobacillus and bifidobacterium in reducing neonatal mortality in sepsis and need for higher line of antibiotics. This combination possibly may act by replacing or inhibiting pathogenic bacterial flora in neonates with sepsis and also might have probable **additive** effect with zinc which affects immunity at various levels as explained below. Further larger study is needed to clarify the role of combination of zinc and probiotics in neonatal sepsis.

Zinc affects both, specific and nonspecific immunity at various levels. In nonspecific immunity zinc has role in epithelial integrity, functions of neutrophils, monocytes, natural killer cells and macrophages. Some of the effects of zinc may be mediated by release of glucocorticoids, decrease in thymulin activity and possibly by antioxidant properties.

The WHO committees have recommended 0.7-1.3 mg/day zinc for infants from 0-5 months of age. But little is known about need in preterms and Low birth weight babies as they have limited hepatic reserves and higher postnatal growth. Some studies found increase in postnatal growth rate following zinc supplementation in dose of 2-5 mg/day. Many studies suggest that 10–20 mg Zn/d is able to limit the risk of infections in children.[24-26]

In our study, total zinc intake in the zinc group was 10 mg/d. A dose of 10 mg/d was adopted from studies involving zinc for prevention of neonatal sepsis.[15]

Bifidobacteria and lactobacilli are the species of choice in probiotics, given the evolution of the gut flora in preterm neonates[27-29]. Benefits of probiotics are species and strain specific and results of one strain cannot be extrapolated to other strains. Beneficial effects of probiotics are via different mechanisms.

We used bifidobacteria and lactobacillus because Bifidobacteria are the dominant strains in infancy, and the combination of both is known to promote the growth of indigenous lactic-acid bacteria (bifidogenic effect) by formation of short-chain fatty acids.[28,30,31]

Conclusion

We know that the Zinc supplementation may help in reducing mortality in neonatal sepsis. Probiotics may reduce mortality and necrotizing enterocolitis in preterm neonates.

In our study we observed combination of zinc 10 mg/day and Probiotics containing Lactobacillus and Bifidobacterium may have additive effect in reducing neonatal mortality and risk of treatment failure in preterm babies with sepsis.

Limitations

First, sample size was small. Further studies are needed with large sample size to provide role of zinc and probiotics in neonatal sepsis. Also, we were unable to do blinding because of the different sizes of bottles and colour of formulations.

Strength

Strengths of our study were good randomization with equal allocation in each group, similar baseline characteristics minimizing confounding variables and bias. It is emphasized that this study is the first of its kind reported in literature as four arm trial comparing role of zinc or probiotics alone and in combination with control in neonatal sepsis in preterms.

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Legend Tables

Sn.	Characteristics	Zinc (n=32)	Pre-probiotics (n=32)	Zinc+pre-probiotics (n=29)	Control (n=31)
1	Male	13	11	14	14
2	Female	19	21	15	17
3	Mean Gest Age	31.2±1.7	32.3±1.6	32±1.6	31.2±1.9
4	Mean Birth wt	1500.3±248.2	1619±317.9	1543.6±267.2	1475.9±252
5	EOS	29	28	26	30
6	LOS	03	04	03	01

Table 1: Baseline characteristics in four groups

Sn.	Group	Mortality (%)	Needed higher lines of antibiotics (%)	Needed supportive treatment (%)	Mean Hospital stay (days)±SD
1	Zinc (n=32)	11 (34.4) *p (0.5)*	18 (56.3) *p (0.7)*	17 (53.1) *p (0.9)*	15.8±1.74
2	Probiotics (n=32)	12 (37.5) *p (0.7)*	19 (59.4) *p (0.5)*	16 (50) *p (0.89)*	15.8±1.64
3	Zinc+Probiotics (n=29)	5 (17.3) *p (0.049)*	06 (20.7) *p (0.02)*	08 (27.6) *p (0.07)*	16.1±1.60
4	Control (n=31)	13 (41.9)	16 (51.6)	16 (51.6)	16.2±1.90

Table 2: Comparison between groups based on outcome measures

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	84.901	3	28.300	0.556	0.645
Within Groups	6106.220	120	50.885		
Total	6191.121	123			

Table 3: ANOVA for Hospital stay

Figure 1: Consort flow diagram