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Original Research Article

Effects of Probiotic Lactobacillus Rhammnosus GG Supplementation on Outcome of Sepsis in Low Birth Weight Neonates

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Abstract:

Background: Probiotics have been used to modulate gut microbiota which have effect on several immunological and metabolic disorders. Probiotics can be utilised to decrease incidence of diseases and promote health. Lactic acid bacteria and Bifidobacteria have been widely studied. Lactobacillus Rhammnosus GG is easily available, low cost medication, has ease of administration and storage compared to other strategies of infection control with favourable cost benefit ratio in resource poor settings.

Methods: This was a prospective, interventional, single blind, case control study conducted at tertiary care center. Lactobacillus Rhammnosus GG was administered to LBW neonates from birth to discharge / death amongst cases while age, socio-economic status matched controls were not given any probiotic. Study period was from March 2020 to June 2021.

Results: Amongst 30 cases and 30 controls enrolled in study there was no statistically significant difference in parameters between two groups with respect to gender, age, antenatal risk factors (leaking PV >18 hours, PROM, PIH), mode of delivery, birth weight, APGAR score, saturations and laboratory findings on first day of life. LBW neonates who received Lactobacillus R GG required significantly less doses of antibiotics and lesser need for change of antibiotics which was statistically significant (p=0.02). Statistically significant (p=0.01) difference was found in association with duration of hospital stay. No significant difference was seen between two groups with respect to mortality (p=0.78).

Conclusions: LBW neonates who were given Lactobacillus RGG had lesser requirement for antibiotics, lesser need for change / up gradation of antibiotics and shorter duration of hospital stay. Probiotics confer better cost-benefit ratio in management of LBWs with sepsis in resource poor settings. Larger population size is needed for further studies.

Keywords: Probiotics, Lactobacillus Rhammnosus GG, Low Birth Weight neonates, antibiotics, hospital stay.

Introduction

Enteric microbiota is a complex organism established at birth and changes dynamically there after till adult like microbiota is established by 2 to 3 years of age. Manipulation of microbiota in neonatal period as a strategy to reduce the severity and incidence of infection in this period.[1]

Mainly Lactic acid bacteria and Bifido bacteria have been investigated in clinical trials. There is a vast difference in gut microbiota colonisation between term versus preterm infants. Preterm infant gut predominantly harbours Firmicutes Proteo bacteria and Enterobacteria along with pathogens, Klebsiella or Serratia. Further colonisation of preterm infant gut with Bifidobacterium and Bacteroides species is delayed and different from classical healthy pattern. Neonatal period is suitable for modulation of composition of gut microbiome to reap optimum health benefits for individual.[1]

Probiotics are used to modulate gut microbiota which have effect on several immunological, metabolic and mental disorders (Gut- Brain axis) [2]. Immense potential of probiotic therapy for the various conditions is evident and can be utilised to decrease incidence of diseases and promote health [2]. Unnecessary antibiotic treatment in neonates disturbs gut microbiota causing gut dysbiosis, possible colonisation with multidrug resistant bacteria. Gut dysbiosis in preterm is a known risk factor for development of NEC a leading cause of morbidity and mortality. [2] Many meta-analyses have evaluated effects of probiotics on preterms. Neonatal sepsis is single most important cause of neonatal deaths worldwide due to their impaired

innate immunity neonates are relatively immunocompromised which predisposes them to infections. In addition invasive devices, prolonged hospitalisation, use of broad spectrum antibiotics alters gut microbiota and colonisation by pathogens leading to increased risk of infections in neonates.

Human microbiome is variable with substantial interindividual and intraindividual variations at different time points. There are longitudinal differences along GIT and also spatial differences in distribution of microbiome [3,4,5,6]. Bacteroides, Bifidobacterium, Streptococcus, Enterobacteriaceae, Clostridium, Lactobacillus and Ruminococcus are dominant in lumen, while Clostridium, Lactobacillus and Enterococcus are predominant in mucosa and mucus. [4]

The profile of gut microbiota of a full term, vaginally delivered, breast-fed infant is considered as "ideally healthy "[3]. Several factors influence assembly of gut microbiota during infancy. Gestational age, birth mode, antibiotic administration, feeding type and environment of care all have an important influence on acquisition and shaping of gut microbiota. The process of bacterial gut colonisation in preterm infants is more challenging because of several environmental factors influencing, including use of antibiotics, parenteral nutrition and long term hospitalisation. Studies in human and animal models have found both qualitative and quantitative differences in gut microbiota between preterm and term infants. [3,4,5].

Overwhelming research is on-going on probiotics world over. Probiotics are defined by WHO (in 2001) as live microorganisms which when administered in adequate amount confers health benefit to the host. The word probiotic means "for life" and is used in reference to bacteria associated with beneficial effects on human and animals for disease management, infection control, health improvement.

There are number of different organisms that can be classified as probiotics including Lactobacillus, Bifido bacterium, Streptococcus, Propioni bacterium, Bacillus and Leuconostoc mesenteroides but the most commonly used strains belong to genera Lactobacillus and Bifido bacterium.

Probiotics ingestion modifies homeostasis of gut which is important in infection prevention. Best evidence of specific probiotic strain efficacy is obtained from RCTs and meta- analysis on prevention and treatment of antibiotic induced diarrhoea, gastroenteritis and acute diarrhoea. [7,8]

Wang and colleagues found less disease gut flora in preterm [9, 10] with gut flora dominated by gamma proteobacteria (>90%) in NEC patients [9]. Similar findings were found in Swedish study, many preterm are highly colonised with Proteobacteria without developing NEC [11] hence prophylactic use of probiotics has been tried for reducing rates of NEC. Numerous systematic reviews and meta-analysis have shown a significant reduction in risk of NEC after probiotic supplementation. [13,14]

Considering all evidence in favour of probiotics many would administer all hospitalised preterms with probiotics. Some cases of sepsis attributable to Lactobacillus species have been documented in patients supplemented with probiotics. [15,16]

Therefore objective of this study was to evaluate effect of Lactobacillus R GG on outcome of LBW neonates. And to study duration of antibiotics administration and hospital stay, requirement of change of antibiotics, mortality.

Materials and Methods:

This was prospective, interventional, single blind, case- control study conducted in a tertiary care centre from March 2020 to June 2021. Bang et al have reported incidence of neonatal sepsis in community - 17% accordingly sample size was calculated. Minimum 55 neonates were to be taken for study. To avoid loss of data and to enhance reliability of study total 60 LBWs were recruited to study. Permission from IEC was obtained. Written informed consent in English/ Hindi/ Local language was taken. Confidentiality of patients was maintained. Inclusion criteria were newborns with birth weight between 1500 to 2500gm having sepsis and who gave consent for being included in study. Exclusion criteria were VLBWs (<34 weeks), those with congenital malformations and those whose guardians did not give consent.

Presence of >3 signs and symptoms of sepsis were mandatory for clinical diagnosis of sepsis like Refusal of Feeds, irritability, fever/ hypothermia, vomiting, abdominal distension, respiratory distress, lethargy, apnoea, convulsions, need for supplementation of O_2. Total 60 patients were included in study. 30 cases and 30 age and socio-economic status matched 30 controls were recruited to study. Cases were administered Lactobacillus R GG after dissolving each sachet of 6 million units in 6 ml of breast milk then 3ml of this preparation was given twice daily through NGT at 12 hourly interval until discharge/ death/development of NEC. Controls did not receive probiotic.

Start of intervention was on admission and end was at discharge, diagnosis of NEC or death. The study placebo was given in 3ml breast milk through NGT twice daily at 12 hourly interval to controls till discharge/ death/ development of NEC. For both groups freshly expressed mother's breast milk was feed of choice followed by milk from milk bank. None received formula feeds. Probiotic used was

Ecogram GG by Brinton Pharma batch no's-1911303 containing 6 Billion CFU of Lactobacillus R GG per 1 Gm of sachet.

For quality control and safety study batch and placebo was tested for presence of pathogens using standard microbiological techniques. Nutrition varied from patient to patient. Enteral nutrition administration was started with 1ml human milk given every 2 or 3 hourly and amount was increased by 1ml over 3-4 hours as tolerated. Human milk was supplemented with parenteral glucose for day 1 of life and with amino acids and lipid administration from day 2 of life as per institutional protocol. Milk given was progressively increased if tolerated and parenteral nutrition was progressively decreased and stopped. Infants were weighed daily and examined minimum twice daily for symptoms or clinical signs of sepsis.

Ultrasonography (Abdominal, Pelvic, Trans - Fontanellar), chest X- rays was done whenever required. Patients in both groups were studied in terms of symptoms of sepsis at time of admission and later during hospital stay, severity of sepsis, presence or absence of complications, duration of hospital stay, and requirement of NICU admission.

Outcome in terms of death/discharge and requirement of change of antibiotics due to development of antibiotic resistance was evaluated. Data was collected on demographics, antenatal, intranatal history and details of antibiotics, shock, respiratory distress and sepsis was recorded. CBC, CRP, blood culture and sensitivity, USGs, chest X-ray, urine routine microscopic examination was done. Outcomes in form of duration of hospital stay, death/ discharge were evaluated. Data was compiled on Microsoft office excel sheet analyser using appropriate statistical tests.

Results & Discussion:

18 males and 12 females were in cases while 16 males and 14 females were in controls. date, age , gender distribution, birth weight, comparison of sepsis in severity of sepsis, complications and duration of hospital stay and outcome in both groups. Chi square comparison between these 2 groups was found to be insignificant (p=0.54) Infants were weighed daily and examined by doctors twice daily for symptoms and clinical signs of infection were monitored. Antenatal risk factors for sepsis were studied. Leaking per vaginum for more than 18 hours was seen in 9 out of 30 mothers (30%) in cases group and in 10 mothers (33.3%) in control group.

Premature rupture of membrane was seen in 10 mothers (33.33%) in each group. pregnancy induced hypertension was noted in 2 mothers (6.7%) in cases and in 3 (10 %) mothers in control group. History of maternal fever, urinary tract infections, major systemic illnesses, laboratory evidence of focus of fever (eg. Dengue, Malaria, Leptospirosis, etc.), meconium stained liquor and foul smelling liquor were not present in any of the mothers of cases and controls.

Table 1: Distribution of mode of delivery and postnatal parameters

Parameters	Cases (n=30)(mean)	Percentage (%)/SD	Control (n=30)(Mean)	Percentage (%)/SD	P Value
Normal Vaginal Delivery	11	36.7	12	40	
Birth Asphyxia	1	3.33	1	3.33	
Birth Weight	1.82	0.4	1.84	0.3	
APGAR score	8.5	1.1	8.4	1.3	
SPO2	94.9	4.6	94.2	4.9	

Table 2: Distribution of laboratory data

Parameters	Cases (n=30)(mean)	Cases SD	Control (n=30)(mean)	Control SD	P value
Haemoglobin	15.7	2.3	14.9	1.9	0.18
WBC	15237.7	7161.9	15015	8139.4	0.91
Platelets	253596.7	150256.6	275020	191485	0.63
ANC	7163	3631.8	6966.5	3861.9	0.84

Independent sample 't' test was used. P-value <0.05 statistically significant.

Table 3: Frequency distribution of requirement /change in antibiotics

Requirement/change In antibiotics	Cases (n=30)	Percentage (%)	Control (n=30)	Percentage (%)	P value
Yes	9	30	15	50	
No	21	70	15	50	0.02*
Total	30	100	30	100	

Table 4 Frequency distribution of hospital stay (days)

Hospital Stay (days)	Cases (n=30)	Percentage (%)	Control (n=30)	Percentage (%)
<7	2	6.7	1	3.3
7-14	24	80	19	63.4
>14	4	13.3	10	33.3
Total	30	100	30	100
Mean(+-)	10.3(+-)4.3		13.2(+-)6.4	

No significant association was found between antenatal risk factors of two groups. 'p' values obtained by comparison between two groups for leaking PV, PROM and PIH were insignificant (0.4, 0.56 and 0.3, respectively). No statistically significant difference was seen between two groups in association with mode of delivery (p= 0.4), birth asphyxia (p=0.6), birth weight (p=0.87), APGAR score (p=0.75) and SpO2 levels (p=0.57) (Table 2). Meta- analysis done through searches and in "The Cochrane Library" by Al Faleh et al found Lactobacillus or Bifidobacterium containing probiotics to be effective in controlling neonatal sepsis. Al Faleh et al found probiotics effective in controlling neonatal sepsis similar to our study. They also found an overall preventive effect of probiotic supplementation on over all 'all cause' mortality in preterm infants [12].

Swah SC, Deshpande et al in their meta-analysais found no difference in incidence of culture proven sepsis between probiotic and control groups in 31 trials (having 8707 patients). [13].

Al Faleh K et al found an overall preventive effect on 'all cause' mortality in probiotic supplementation preterm infants group. Swah SC, Deshpande et al found reduced incidence of 'all cause' mortality in 29 trials having 9507 patients receiving probiotics. Swah SC, Deshpande et al found no beneficial effect on reduction in overall mortality when probiotics were started after 48 hours of life. However they found preventive effect on NEC in infants started on probiotics before or after 48 hours of life. Our study preterm infants too didn't develop NEC. Why initiation of probiotics in preterm infants before 48 hours of life reduced mortality while no such beneficial effect on mortality was seen in studies when probiotics were initiated after 48 hours of life couldn't be explained by Swah et al. Incidence of mortality was reduced significantly in trials where probiotics were started right from first enteral feeds. Probiotic species sepsis was not found in any of the trials studied by Al Faleh et al and Swah et al which is similar to our study. In our study association of laboratory parameters was found insignificant for haemoglobin (p=0.18), white blood cell count (p=0.91) platelets count (p=0.63) and for absolute neutrophil count (p=0.84) (Table 3). Nine (30%) cases and 15 (50%) controls required change in antibiotic treatment and up gradation to higher antibiotics. Comparatively less neonates in cases required antibiotics than controls (p=0.02) which was significant difference (Table 3) Amongst cases 2 (6.7%), 24 (80%) and 4 (13.3%) neonates were hospitalised for less than 7 days, 7 to 14 days and more than 14 days respectively while amongst controls 1 (3.3%), 19 (63.4%) and 10 (33.3%) neonates were respectively hospitalised for above duration. Statistical comparison between two groups was significant (p=0.01). Minimum to maximum duration of hospital stay in cases was 6 to 27 days and in controls was 6 to 37 days, minimum 6 days of stay was seen in both groups as that much is duration required to get blood culture reports which if negative is also criteria for discharge from hospital in our set up (Table 4, Figure 3) None in both groups developed NEC while one case and two controls died during treatment. No significant difference was seen between two groups with respect to mortality (p=0.78). Swah et al found statistically significant effects on hospital stay with shorter duration of stay in patients receiving probiotics which was similar to our study They also found increased weight gain per day and reduced time to reach full enteral feeds all in favour of using probiotics.[13]

Conclusion:

Administration of Lactobacillus RGG is beneficial for LBW neonates leading to fewer requirements of antibiotic doses, lesser need for change and/ or up gradation of antibiotics to higher generations. Also duration of stay in hospital was reduced while no side effects were seen due to probiotic administration considering oral route of administration, ease of availability and less cost of probiotics the cost – benefit ratio would tilt towards more benefit which is of immense value in resourse poor settings. Studies on larger groups are needed to look for any lesser known complications of probiotic use in neonates.

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