Original Article

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Role of *Saccharomyces boulardii* in Reduction of Neonatal Hyperbilirubinemia

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ABSTRACT

Introduction: Probiotics are known to reduce the severity of hyperbilirubinemia.

Aim: This study was done to evaluate the effect of probiotic on neonatal hyperbilirubinemia in term neonates.

Materials and Methods: A total of 181 healthy term neonates after birth were divided into a control group (n=95) and a treatment group (n=86) randomly and treated with placebo and probiotic *(Saccharomyces boulardii*) respectively. A total of two doses were given orally in the first two consecutive days. The serum bilirubin levels were detected on day three of life. Babies were exclusively breastfed, clinical outcome was recorded. Comparison between groups was made by the non-parametric Mann-Whitney test. Analysis of Variance (ANOVA) was used to assess the quantitative variables. A p-value of <0.05 using a two-tailed test was taken as being of significance for all statistical tests.

Results: On day 3, mean total serum bilirubin in control group among patient who has not developed clinical jaundice is 6.5mg% and in the treatment group is 5mg%. In patient with clinical jaundice, it is 13.6mg% in control group and 10.7mg% in the treatment group. The p-value was found to be <0.05 which is statistically significant. No obvious adverse reactions noted in either group.

Conclusion: Probiotics lowered the serum bilirubin level of healthy neonate with jaundice safely and significantly without any adverse reaction.

Keywords: Enterohepatic circulation, Jaundice, Neonate, Phototherapy, Probiotics

INTRODUCTION

Jaundice is the most common clinical sign in neonatal medicine [1]. About 60% of healthy term infants and 80% of premature infants develop clinically visible jaundice in the first week of life. In the majority of cases, jaundice is mild and transient, resulting from an immaturity of the liver's excretory pathway for bilirubin at the time of its heightened production. Neonatal jaundice remains one of the most common reasons for re-admission to hospital in the first week of life [1].

Phototherapy is the most commonly used intervention to treat and prevent severe hyperbilirubinemia. It results in the reduction of total bilirubin concentration so that exchange transfusion can be avoided [2]. Although phototherapy is a benign modality of treatment, it has its own side effects. Phototherapy can result in a change in the infant's thermal environment (hyperthermia), which lead to increased peripheral blood flow and insensible water loss [3,4]. Infants receiving phototherapy have an increased incidence of diarrhea [5] and stool becoming darker and have a greenish tinge [6]. Association of phototherapy with type 1 Diabetes Mellitus and Asthma have been proven by Swedish studies [7]. It has been reported to cause bronze baby syndrome and rare purpuric and bullous eruptions in an infant with severe cholestatic jaundice [8-11]. Phototherapy upsets maternal-infant interaction and therefore, should be used only with adequate thought and explanation [12]. Most of the time term normal babies are discharged within 48 hrs of life unless they are delivered by caesarean section or there are some maternal or newborn complications. A 5%-10% of the babies delivered is admitted in the newborn unit for hyperbilirubinemia and are treated with phototherapy. In cases of early discharge from the hospital, the newborn may be subjected to re-admission for phototherapy treatment because of the high level of unconjugated bilirubin. Such re-admissions, in addition to involving extra expenses for both the family and the institution, are exposing a probably healthy newborn to the hospital environment, thus, also brings emotional problems and risk to breastfeeding, and is one of the causes of early weaning [13].

One of the causes of hyperbilirubinemia in a newborn is the failure of conversion of conjugated bilirubin to stercobilin due to the relative lack of bacteria in the gut during the first week of life. Hyperbilirubinemia is also attributed to over activity of the beta-glucuronidase enzyme in the sterile gut and the mild alkaline pH of the proximal intestine [14,15]. Microbiological preparations like probiotics are able to decrease the pH of the intestine. Probiotics can decrease the enterohepatic circulation by changing the intestinal flora and suppressing the activity of the beta-glucuronidase enzyme [16].

Very few studies are available in the literature about therapeutic effects of probiotics on neonatal jaundice. The current study was undertaken in an attempt to evaluate the effect of probiotic *Saccharomyces boulardii* on neonatal hyperbilirubinemia in term newborn in terms of incidence, severity and need for phototherapy.

MATERIALS AND METHODS

The study was an experimental study conducted at a tertiary care teaching hospital of South India from October 2012 to September 2013. Ethical committee of the Institute approved the study protocol.

All healthy term neonates with birth weight more than 2.5kg in the post-natal ward were included. The exclusion criteria were any newborn becoming sick during the study period, babies admitted in NICU, unable to get consent, ABO incompatibility, Rh incompatibility, at risk of sepsis, clinical evidence of sepsis. Cephalhaematoma, subgaleal bleed, clinical suspicion of hypothyroidism, infant of diabetic mother, babies >4kg birth weight, intra-uterine infections, respiratory distress syndrome, complementary feeding.

The sampling technique was non-probability sampling and purposive sampling. A sample size of 174 was derived, based on a confidence level of 95%, an expected frequency of the factor under study 13% [17] and a margin of error 5%. The sample was further increased by 5% to account for contingencies such as non-response or recording error. Hence, the sample size came to be 182. While compiling the data we lost details of one case, hence, 181 patients has been included in this study.

Written informed consent was taken from the parents of all neonates enrolled in the study. Age sex, birth weight, mode of delivery matched samples were taken from all healthy newborn who fullfill the inclusion and exclusion criteria and were assigned into two groups, the case group, and the control group.

According to the group, babies were given either Probiotics (*Saccharomyces boulardii*) or Placebo. Case group was given *Saccharomyces boulardii* 250mg once a day for two days. *Saccharomyces boulardii* is available as a lyophilized powder for oral suspension each sachet containing 250mg. It was mixed with 10ml of sterile water and administrated orally within 4 hours of birth. A total of 2 doses were given orally in the first two consecutive days. Probiotics were not given on the 3rd day. The control group was given 10ml sterile water. Both groups were followed up for 3 days, for the development of clinical jaundice. The number of stool output per day was also noted in the two groups. Babies were exclusively breastfed during the study period. All newborns in the study were subjected to Total Serum Bilirubin (TSB) estimation on day 3 using semi autoanalyser KIM [7]. In those cases with clinical jaundices or increasing jaundice serum bilirubin was repeated. Total serum bilirubin was plotted on the nomogram for newborn infant 35 or more weeks gestation. Those neonates, whose values were above phototherapy line were admitted and treated with phototherapy as per hospital protocols [18].

Data collection was done on the first day and 3rd day of life. Mother's details like blood group and the history of Gestational Diabetes Mellitus or any such complications (if present) were collected from her medical record. Baby's details like name, hospital number, sex, date of birth, time of birth, birth weight, blood group, serum bilirubin on day 3 and phototherapy if received or not, were all collected from baby's record. All data were filled up in the prescribed proforma.

STATISTICAL ANALYSIS

The data obtained was entered into Microsoft Excel. The data are reported as the mean ± SD or the median, depending on their distribution. The differences in quantitative variables between groups were assessed by means of the unpaired t-test. The chi-square test was used to assess differences in categorical variables between groups. A p-value of <0.05 using a two-tailed test was taken as being of significance for all statistical tests. All the data was analysed with a statistical software package. (SPSS, version 16.0 for windows).

RESULTS

Total of 181 babies were studied. Out of this 86 babies received probiotics and 95 were taken as controls. Out of 95 controls, 51 (54%) were male babies and 44 (46%) were female babies. Out of 86 babies who were given probiotic 46 (53%) were male and 40(47%) were female. On comparing this with Pearson Chi-Square test we get a p-value 0.15, which is not significant. Hence, sex distribution between study and control groups was not statistically significant.

Mean birth weight between 2 groups: Mean birth weight in the control group was found to be 2.9Kg and that of the test group was found to be 3.0Kg. Both the group had a standard deviation of 0.4. This was compared and the p-value was found to be 0.826. There was no statistically significant difference in birth weight between the two groups.

Distribution of mother's blood group among two groups: In the control group mother's blood group A+ 15%, B+ 33%, AB+ 3%, O+ 27% and B- 3%. In the test group A+ 10%, B+40%, AB+ 1%, O+ 21%, A- 3% and B- 2%. On comparing this with Pearson Chi-square test we get a p-value of 0.28 which was not statistically significant.

Distribution of baby's blood group among two groups: In the control group baby's blood group as mentioned in [Table/Fig-1] and then on comparing this with Pearson Chi-square test we get a p-value of 0.28 which is not statistically significant. Blood group distribution between the two groups was not statistically significant.

Mean serum bilirubin among two groups: Mean serum bilirubin value was 12.3mg/dl in the control group and was 9.4mg/dl in the test group. Minimum bilirubin value noted was 4.5mg/dl and the maximum value was 22.9mg/dl in the control group. Minimum bilirubin in the test group was 2.4mg/dl and the maximum was 18.6mg/dl in the test group. After comparing these data using unpaired t-test, the p-value was found to be <0.01 and thus is statistically significant.

A total of 81% of babies under control group developed jaundice, whereas only 77% from test group developed jaundice. This was compared using Chi-Square test and P-value was found to be less than 0.05, 15% of babies under control group required phototherapy, whereas only 3% from test group required phototherapy. This was compared using Pearson Chi-Square test and p-value was found to be 0.047. Hence, there is a statistically significant difference between the two groups.

Mean total serum bilirubin in control group among patient who has not developed jaundice is 6.5mg/dL and among patient with jaundice is l3.6mg/dL. Mean Serum Bilirubin in the test group who has not developed jaundice is 5mg/dL and among patient with jaundice is 10.7mg/dL. After comparing this data using t-test and p-value was found to be <0.01 which is statistically significant. Hence, a significant reduction in mean bilirubin value is noted after giving the probiotic.

	Control (n=95)	Test (n=86)	Significance
Gender			
Male	44%	41%	p > 0.05
Female	37%	36%	
Total	81%	77%	
Birth weight			
<=3.0	51%	48%	p > 0.05
3,1 - 3.5	20%	20%	
>3.5	10%	9%	
Total	81%	77%	
Mother Blood Group			
A+	15%	10%	p > 0.05
B+	33%	40%	
B-	3%	2%	
AB+	3%	1%	
O+	27%	21%	
A-	0%	3%	
Total	81%	77%	
Baby Blood Group			
A+	19%	9%	p > 0.05
B+	24%	27%	
B-	1%	1%	
AB+	1%	1%	
0+	35%	36%	
A-	1%	2%	
0-	0%	1%	
Total	81%	77%	

[Table/Fig-1]: Incidence of clinical jaundice in various subgroups.

[Table/Fig-1] revealed that the difference in terms of incidence of neonatal jaundice in subgroups like gender, different birth weight, mother and baby blood group in test group and control group were not statistically significant.

Mean stools per day among two groups: Mean stools per day among test group was 5.03 and control group was 5.01 and was compared using student's t-test. p-value was found to be 0.795 thus, is not statistically significant.

DISCUSSION

Neonatal hyperbilirubinemia is a common problem in neonates requiring treatment, and can often reach dangerous toxic levels leading to kernicterus if unchecked. Despite its potential toxicity, bilirubin may have an important and positive physiological role [19,20]. It plays an important role in the prevention of oxidative membrane damage *in vitro* [21].

There are lots of studies available for the use and safety of probiotics in newborns [22,23]. Rautava S et al., reported that probiotics during pregnancy and breastfeeding confer immunomodulatory protection in the infant [21]. Bin-Nun A et al., showed that pre-term human infants randomly assigned to receive a daily feeding supplement of a probiotic mixture had a relative risk reduction in Necrotizing Enterocolitis (NEC) and death and also appeared to have decreased late onset sepsis [24]. Ling-ling W et al., observed that the probiotic Mami Ai lowered breast milk jaundice by constructing intestinal microflora [25]. Gayathri Athalya et al., did a systematic review of 25 Randomised Controlled Study (RCTS) (n=5895) to assess the benefits of probiotics on enteral nutrition in preterm or low birth weight neonates [26]. They found that time to full enteral feeds was shorter in the probiotic group.

In our study total of 181 babies were studied. Out of this 86 babies received probiotics and 95 were taken as controls. There was no statistically significant difference in the distribution of gender, mean birth weight mother's blood group and baby's blood group among control and probiotic groups.

In this study, a significant reduction of mean bilirubin value was found in babies given probiotic. When the final outcome in the two groups was compared it was found that 66(77%) neonates in the probiotic group and 77(81%) in the control group developed clinical jaundice. The mean value of TSB in the probiotic group was 9.4 as compared to 12.3 in the placebo group. Thus, a statistically significant difference was noted between the two groups. Mu-Xue Yu et al., enrolled 74 neonates in his study to evaluate the role of probiotic in the prevention of neonatal hyperbilirubinemia [27]. He reported that, incidence of neonatal hyperbilirubinemia in the intervention group was 33.33% and 57.14% in the control group and the difference was found to be statistically significant. Bisceglia M et al., performed a study on 76 newborns who were randomly assigned to receive a formula containing probiotic and placebo [28]. Bilirubin levels were determined by the transcutaneous bilirubin measurement within 2 hours after birth (T1), at 24, 48 and 72 hours and at 5, 7, 10 and 28 days of life. They noticed that, the neonates whose formula was supplemented with probiotics showed a lower transcutaneous bilirubin that was statistically significant from 72 hour of life. In this study, measurement of bilirubin was done using transcutaneous bilirubinometer, but in the present study, serum bilirubin was measured. Armanian AM et al., did a study on 50 pre-term neonates to evaluate the efficacy of prebiotics in the management of hyperbilirubinemia [29]. The control and test group comprised 25 each. They found that the test group given prebiotic oligosaccharides had increased stool frequency improved feeding intolerance and decreased bilirubin level.

A significant reduction in requirement of phototherapy was noted in babies given probiotic when compared to controls, in the test group out of total 86 babies, 20 babies did not develop any clinical jaundice. A total of 66 babies developed clinical jaundice, out of which 3 babies required phototherapy. In the control group out of total 95 babies, 18 babies did not develop any clinical jaundice. A total of 77 babies developed clinical jaundice, out of which 14 babies required phototherapy. This was compared and p-value was found to be <0.05. Hence, a statistically significant difference was noted. Demirel G et al., had enrolled infants of gestational age <32 weeks and birth weight <1500g [17]. A total of 179 infants, 81 in the study group and 98 in the control group were studied. The infants in the study group were given *Saccharomyces boulardii* and the infants in the control group were fed without *Saccharomyces boulardii* supplementation. They had concluded that the *saccharomyces boulardii* supplementation at a dose of 250mg/day is safe for VLBW infants in the short term and effective in reducing the duration of phototherapy in Very Low Body Weight (VLBW) infants. Demirel et al., studied the effect of *Saccharomyces boulardii* on VLBW babies, while in the present study effect of *saccharomyces boulardii* in term well babies has been studied. Liu W et al., studied the therapeutic effects of probiotics on neonatal jaundice, 68 neonates with jaundice were divided equally into a control group and a treatment group (n=34) randomly and treated by blue light phototherapy that in combination with probiotics [30]. In the test group, probiotics were found to lower the serum bilirubin levels significantly. In a randomized controlled trial by Serce O et al., *Sacchromyces boulardii* did not influence the clinical course of hyperbilirubinemia significantly in 35 to 42-week-old neonates (n=119) receiving phototherapy [31].

The factors like sex, birth weight, mother's blood group, baby's blood group did not have a significant association with the development of jaundice in both test and control groups.

Mean stools per day among test group was 5.03 and control group was 5.01 and was found to be statistically insignificant. A study conducted by Bisceglia M et al., has found out that neonates receiving probiotics showed a greater frequency of stools compared to that of those in placebo [28]. But in the present study, there was no statistically significant difference between the two groups.

LIMITATION

Limitations of this study includes, the use of only a single probiotic for the study, the lack of follow-up of the babies beyond day 3 and being a single center study as for larger interpretation, the multi center study is needed.

CONCLUSION

There was a significant difference in the mean value of TSB between probiotic and placebo groups. There is a significant difference in terms of incidence of neonatal jaundice and the need for phototherapy between the probiotic and placebo groups. There was a significant difference in the mean value of serum bilirubin between neonates with jaundice in probiotic group and control group. There was no difference in terms of decrease in incidence neonatal jaundice in a sub group like gender, different birth weight, mother or baby blood group. *Saccharomyces boulardii* is well tolerated in all babies and there was no significant reduction in the number of stools per day when compared to controls.

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