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Original article

The impact of probiotics (*Lactobacillus reuteri Protectis*) on the treatment, course and outcome of premature infants in the Intensive Care Unit in Mostar

Marjana Jerković Raguž, Jerko Brzica, Sanja Rozić, Darinka Šumanović Glamuzina, Antonija Mustapić, Marija Novaković Bošnjak, Tomica Božić

Department of Neonatology and Intensive Care Unit, Clinic for Children's Diseases, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

Abstract

Aim: The aim of this study was to analyse the treatment, course and outcome of premature infants treated with probiotics (*Lactobacillus reuteri Protectis*) in the Intensive Care Unit (ICU).

Study design: This retrospective cohort study included 100 preterm infants of gestational age up to 30-34$^{+6/7}$ weeks. The first group of infants who were given probiotics in their dairy meal in the course of their medical treatment during hospitalization in the year 2014 were compared to a second group of infants who did not receive probiotics in the year 2013.

Results: A statistically significant difference in the number of days of treatment in the ICU ($p < 0.05$), administration of ranitidine ($p < 0.05$) and feeding intolerance ($p < 0.05$) was found between the two groups of preterm infants. No statistically significant differences were found in the other variables under study.

Conclusion: Probiotics probably have a positive effect on the course and outcome of treatment of premature infants in the ICU. Our newborns who received probiotics spent shorter time in intensive care, they began full peroral intake of milk sooner and received antiulcer medicine for shorter time, which is an important step towards the improvement of treatment outcome in premature infants.

Keywords

Probiotics, neonatal intensive care, preterm, necrotizing enterocolitis.

Corresponding author

Marjana Jerković Raguž, Department of Neonatology and Intensive Care Unit. Clinic for Children's Diseases. University Clinical Hospital Mostar; tel.: +387 63 699 089; e-mail: marjanajerkovic@yahoo.co.uk.

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Introduction

Probiotics are live microbiotic supplements which colonize the intestine, ensuring benefits for the host [1]. The colonization pattern of the gastrointestinal system is significantly different in premature infants than in healthy full-term newborn, and is dependent on the feeding method. During the early neonatal period the relation between intestinal microflora and nutrition is the most important factor for normal intestinal and immune system development, particularly in the premature infant. Early colonization of the infant gastrointestinal tract with non-pathogenic intestinal microbiota is crucial for the overall health of the infant, and may prevent the development of intestinal tract inflammations [2]. Experts agree that probiotics reduce the risk of necrotizing enterocolitis (NEC), the evidence for their claims being of the same order as the evidence for already substantiated interventions, such as, for example, antenatal corticosteroids, surfactant and hypothermia. *Lactobacillus reuteri* (*L. reuteri*) prevents antibiotic diarrhea, as well as the development of intestinal infection, and has even proven effective during the administration of antibiotics to children [3]. A systematic examination of studies published to date on the prophylactic administration of probiotics to preterm infants leads to the conclusion that probiotic supplementation reduces the risk and mortality rate of NEC in preterm infants. However, the optimal strain, dosage and intervals of dosage for the administration of probiotics need further investigation, as concluded by a study published in 2016 [4]. Therefore, indecisiveness still exists regarding the introduction of routine probiotic prophylaxis in preterm infants during hospitalization. Improvement of food tolerance is a significant benefit of probiotics, bearing in mind that nutrition is a priority for prematurely born children, including those children diagnosed with intrauterine growth retardation, who run an even higher risk of developing infections [5]. Even though the mechanism of actions of probiotics has not been elucidated completely, there is evidence to suggest that they act on the level of the immune system through apoptotic mechanisms, stimulating cell survival, cell adhesion and angiogenesis [6]. However, several studies have indicated that the probiotic mechanism of action can be translocated, thereby increasing the risk of developing subsequent sepsis and bacteraemia. It is therefore essential to determine the optimal dosage for the best effect without side-effects, as suggested by a study conducted in Italy in 2016 [7]. A serious condition that affects premature infants is sepsis, where probiotics have shown their positive effect. Since one of the functions of probiotics is the stimulation of the immune system to respond on time, they could be beneficial in the treatment of infection without any side effects. Studies have also shown significant benefits in administering probiotics in the prevention of infantile colic [8]. Another study published in 2016 suggests that the use of probiotics may have its downsides. Strains of lactobacilli producing bacteriocins and other antimicrobial substances may, in the short term, have an 'antibiotic-like' effect and eradicate bacteria similar to themselves, and thus upset the normal gut microbiota, giving rise to other health problems [9]. Bearing in mind the results of current research, resistance to the introduction of probiotics in routine clinical practice is partly justified. However, the benefits of its implementation are great, considering its proven effect on the occurrence and severity of infections in premature infants.

Objectives

The objective of this study was to compare the treatment, course and outcome and the duration of hospitalization in the Intensive Care Unit (ICU) of the Department of Neonatology and Intense Treatment of Newborn and Premature Infants of the Children's Diseases Clinic, University Clinical Hospital Mostar, as well as the frequency of complications in the first group of premature infants who were given probiotics (*L. reuteri Protectis*) and the second group which did not receive probiotics in their dairy meal in the course of their medical treatment during hospitalization.

Study population and methods

The study was conducted at the ICU of the Department of Neonatology and Intense Treatment of the Newborn and Premature Infants of the Children's Diseases Clinic at the University Clinical Hospital Mostar in the period from January 1 to December 31 2013 and from January 1 to December 31 2014. The data were collected from medical documents, medical history and discharge letters of the premature infants.

The retrospective cohort study consisted of 100 newborns, who were born preterm at the Clinic for Gynecology and Obstetrics of the University Clinical Hospital Mostar and treated at the ICU. During 2014, the study population consisted of 50 newborn who were administered probiotics along with all other necessary therapies. In 2013, the study population consisted of 50 newborns who were given antibiotic supportive therapy without probiotics during hospitalization. Treatment protocols remained unchanged in the course of the two years.

The newborns were of a gestational age from 30 to $34^{+6/7}$ weeks in both groups. They weighed over 1,000 grams at birth. All the newborn who had gastrointestinal anomalies or were of a gestational age under 30 and above 35 weeks were excluded from the study.

Parameters recorded for children: gender, gestational age, weight at birth, Apgar score, laboratory parameters (values of blood count, C-reactive protein [CRP]), duration of hospitalization in the ICU, type of therapy, duration of ranitidine treatment and commencement of peroral feeding or duration of feeding intolerance.

Feeding intolerance is a well-known phenomenon in the neonatal ICU and is linked to morbidity and mortality in the premature infant. However, premature infants receive enteral nutrition in the form of breast milk or formula. Feeding intolerance in the premature infant is the inability to digest enteral feedings presenting as more than 50% prefeeding gastric residual volume, abdominal distention or emesis or both, and the disruption of the patient's feeding plan. Some researchers defined feeding intolerance in premature infants using outcome measurements, including the failure to reach enteral feedings on a specific timeline or the number of interruptions or delays in the process of reaching enteral feedings [10].

Neonatal sepsis is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first month of life. Early-onset occurs in the first 5-7 days of life. Late-onset disease occurs after the first week of life.

NEC is a multifactorial disease, representing the common end-point of multiple predisposing conditions. Frequently associated risk factors that have been postulated in the pathogenesis of NEC include inappropriate colonization of the neonatal intestinal tract, an excessive inflammatory response by the immature intestinal epithelial cells, anemia and transfusion-related gut injury, prolonged exposure to antibiotics, patent ductus arteriosus, aggressive advancement of enteral feedings, absence of enteral feedings, non-human milk feedings, reduced gastric acid production, and reduced gut motility. The clinical syndrome has been classified into stages by Walsh and Kliegman (1986) to include systemic, intestinal and radiographic findings. Stage I: suspected NEC. Stage IIA: mild NEC. Stage IIB: moderate NEC. Stage IIIA and IIIB: advanced NEC.

Perinatal infection is a term which is used in our neonatal ICU for diagnosis purposes if the infant's hemocultures are negative and if the symptoms (fever, feeding intolerance) and laboratory signs (elevated finding of CRP) of infection occurred immediately following birth [11].

Peroral feeding of small amounts of formula for preterm babies (10 ml/kg per day, distributed in 8 to 12 feeds) was introduced to the premature infants from the 1st to the 3rd day of life. Every morning meal included 5 drops of probiotics BioGaia® which contained live active bacteria *L. reuteri Protectis* dispersed in oil. The BioGaia drops were kept in a refrigerator at a temperature of 2-8ºC at the Department. The children's parents gave their consent for the adminstration of probiotics, as they purchased it individually for their child and brought it to the Department. The manufacturer and representative for the sales of probiotics for Bosnia and Herzegovina, Ewopharma Ltd., were not familiar with the course of the study nor did they financially support the development of the study. The children, along with daily therapy (antibiotics), supportive therapy (plasma, blood products transfusion) and symptomatic therapy (H2 blocker – ranitidine), also received probiotics immediately upon the commencement of peroral feeding. Ranitidine reduces the amount of acid in the stomach, which reduces the symptoms of acid reflux and helps intestinal motility.

Ranitidine treatment protocol, neonatal dosing, venous injection: loading dose 1.5 mg/kg as a single dose; maintenance dose: 12 hours after

loading dose give 1.5 mg/kg/day divided every 12 hours. Administration: infuse over 15-30 minutes at a usual concentration of 0.5 mg/ml. Ranitidine is included in the treatment protocol of newborns to establish the unhindered oral intake of milk [11].

Probiotics were administered every day of treatment at the Clinic until discharge from the hospital. Upon discharge, parents gave their children probiotics until they reached a body weight of > 2,500 grams or until the age of 1 month.

During the 2013 study, newborns were not given probiotics along with antibiotic, supportive or symptomatic therapy during treatment at the same Department.

All therapy administered during the years encompassed by the study was recorded in detail by doctors and nurses into treatment protocols and therapy procedure protocols. Medical documentation was stored in medical history, from which records were taken about the duration of administration of the histamine H2 blocker (ranitidine), which was introduced upon admission of the premature infants, the type of antibiotic therapy and number of days of treatment at the Department.

Statistic methods: for presentation of nominal variables, frequency and percentage were used, and for presentation of continuous variables, mean value and standard deviation were applied. To analyze nominal variables, Fisher's exact test was used, while Student t-test was used for testing differences between continuous variables. The possibility of error was accepted at $\alpha < 0.05$ and differences between the groups were accepted as statistically significant for $p < 0.05$. Values of p which could not have been rounded to a maximum of three decimal places were presented as $p < 0.001$.

Results

During 2013, 1,700 live-born children were delivered at the Clinic for Gynecology and Obstetrics at the University Clinical Hospital in Mostar, of which 231 were hospitalized at the ICU for Treatment of the Newborn and Premature Infants. The number of premature infants born from 26 to $36^{+6/7}$ weeks of gestation was 131.

In the course of 2014, there were 1,873 live-born children at the Clinic for Gynecology and Obstetrics. Of these, 178 children were treated at the ICU, and 100 children were born from 26 to $36^{+6/7}$ weeks of gestation, i.e. premature infants.

Comparison of values of blood count, CRP in the serum and gestational age, birth weight, Apgar score of the infants are presented in **Tab. 1**.

The preterm infants of the first group had significantly lower values of platelets than the preterm infants of the second group ($p < 0.001$). Statistically significant differences between the two study groups were not found in other parameters (values of blood count, CRP) nor in gestational age, birth weight, Apgar score.

Comparison of the different tested parameters, diagnosis and type of treatment of the preterm infants according to the year of the study group is presented in **Tab. 2**.

Symptomatic and supportive therapies were prescribed more often in 2014, whereas support and antibiotic therapy was added more frequently during 2013. Fisher's exact test did not show a significant difference in therapy and diagnosis approach between the two study groups of preterm infants.

Distribution of infants in the whole sample and by group, according to the number of days of treatment, administration of ranitidine and feeding intolerance is presented in **Fig. 1**.

Table 1. Comparison of values of blood count, C-reactive protein (CRP) in the serum and gestational age, birth weight, Apgar score of the infants of the studied group.

	Groups				t	p
	2014		2013			
	$\bar{x}$	SD	$\bar{x}$	SD		
Gestational age	31.86	3.08	31.48	3.14	0.610	0.543
Birth weight	1,832.80	610.52	1,662.40	557.85	1.457	0.148
Apgar score 1st min	7.26	3.15	8.00	2.03	1.397	0.166
Apgar score 5th min	8.66	2.44	8.84	1.46	0.448	0.656
CRP mg/dl	31.08	49.87	27.34	51.03	0.371	0.712
Leukocyte x10^9/L	21.81	35.12	13.27	16.73	1.553	0.125
Platelets x10^9/L	145.36	97.93	187.20	82.51	2.310	**0.023**

A statistically significant difference in the number of days of treatment in the ICU ($p < 0.05$), administration of ranitidine ($p < 0.05$) and feeding intolerance ($p < 0.05$) was found between the two study groups of preterm infants. A shorter period of hospitalization in the ICU, fewer days of administration of ranitidine and a shorter period of feeding intolerance were found in preterm infants

Table 2. Comparison of the different tested parameters diagnosis and type of treatment in individual years of studied groups of premature infants.

	Groups				χ^2	p
	2014		2013			
	n	%	n	%		
Diagnosis						
Perinatal infection (negative hemocultures)	38	76.0	40	80.0	2.888	0.781[a]
Late neonatal sepsis	3	6.0	3	6.0		
Early neonatal sepsis	7	14.0	3	6.0		
NEC (stage II B)	2	4.0	4	8.0		
Therapy						
Antibiotics	37	74.0	39	78.0	2.284	0.546[a]
Supportive and symptomatic therapy	10	20.0	5	10.0		
Supportive therapy and antibiotics	3	6.0	6	12.0		

[a] Fisher's exact test.
NEC: necrotizing enterocolitis.

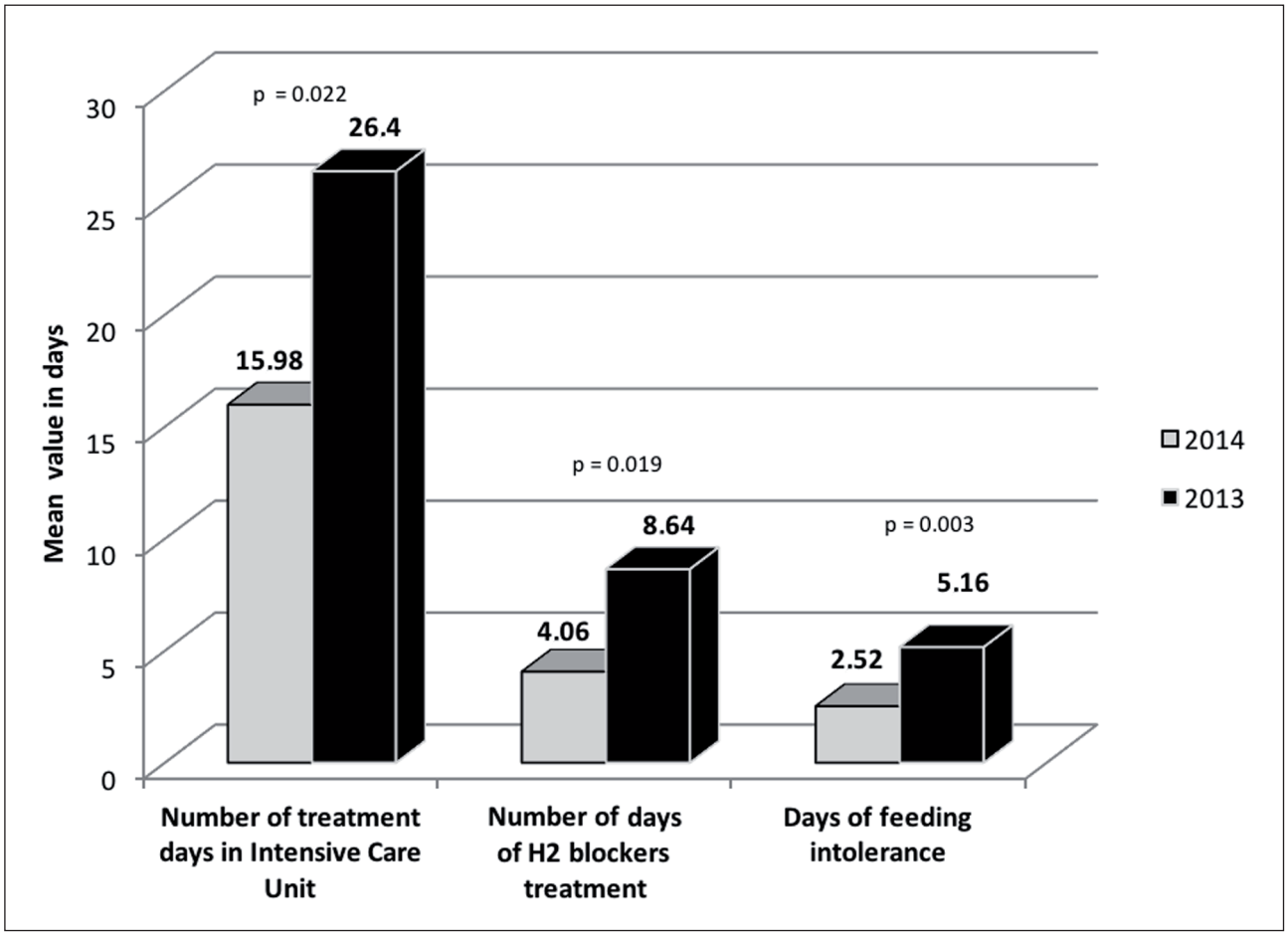

Figure 1. Distribution of preterm infants in the whole sample and by group, according to the number of days of treatment, administration of ranitidine and feeding intolerance of preterm infants.

in the first group, who received probiotics *L. reuteri Protectis*.

Discussion

In recent years, significant progress has been made in the treatment of premature infants. Studies have shown that the introduction of probiotics in ICUs for the treatment of newborn has led to better and more successful treatment [12]. The treatment outcome during the first week of life of the premature infant is closely connected with the adaptation of the organism to food intake, colonization of microorganisms and invasion of antigens from the dairy meal itself [8].

This study was carried out during two one-year periods with the aim of establishing the effect of probiotics on the course, manner and outcome of treatment of premature children. This study included premature infants who were administered probiotics in their dairy meals along with all other antibiotic and supportive therapy during their hospitalization in 2014, and premature infants who were treated in 2013 with the necessary protocol therapy without introducing probiotics into their dairy meals. With respect to the type of delivery, no significant difference was found between the two years of study in the frequency of Cesarean sections, although the percentage of complications in pregnancy during the 2014 study was 70% among the pregnant women. Premature children born naturally have higher chances of survival in relation to those delivered by Cesarean section despite the complicated course of pregnancy [13]. The data also showed that the preterm infants from the 2014 study who received probiotics had the same number or somewhat fewer severe infections such as NEC and late sepsis, even though the result is not statistically significant. A study conducted 8 years ago suggests that probiotics reduce the frequency of NEC in the ICUs for the treatment of premature infants born before week 33 of gestation, which is similar to our results [14]. However, the introduction of probiotics did not result in a statistically significant decrease in the risk or mortality rate of NEC, as concluded by a study conducted in Denmark in 2016 [15]. In the course of 2014, 14% of the children had early-onset sepsis, which was confirmed by microbiological testing of blood culture, but in the same year significantly fewer children died as the outcome of treatment in relation to 2013. Results similar to ours with respect to a lower mortality outcome and lower frequency of infection in premature infants were obtained in studies conducted in the last two years [16, 17]. Furthermore, the most recent studies from 2016 indicate that giving probiotic supplementation reduces the risk of late-onset sepsis in preterm infants [18, 19]. Although these studies indicate the promising beneficial effects of probiotics, the long-term risks and health benefits of probiotic supplementation are still not clear [20].

Probiotics significantly affect the time of commencement of peroral feeding of the newborn during hospitalization [21]. This was also confirmed in our study: on average, the infants began feeding perorally without signs of feeding intolerance on the 3rd day of life in 2014 and on the 5th day in 2013. Most current research suggests that the impact of probiotics on the course, manner and outcome of treatment depends on the type of probiotics and the composition of microorganisms, which significantly affected our choice of the probiotics we administered to the premature infants at the ICU. However, the introduction of certain probiotics correlates with the development of sepsis in newborns, as shown by a research conducted in Italy in 2016 [22]. Research to date suggests the reduction of the use of antibiotic therapy in premature infants if the administration of probiotics along with protocol treatment begins on time [12], while our research shows the same use of antibiotics therapy during hospitalization.

Results show that premature infants in 2014 had a more severe clinical state of infection accompanied by significantly lower values of thrombocytes, a higher concentration of CRP and leucocytes compared to the children from the 2013 study, but spent significantly fewer days in the ICU. This implies a possible positive impact of the probiotics which were administered in the first days of life with small quantities of dairy meals. Considering the great difficulties encountered by prematurely born children, particularly invasive infections and an increasing number of multi-resistant microorganisms, probiotics appear to have high chances as a simple, safe and affordable health care resource for premature infants in the prevention of infections and consequently in the improvement of their survival rate. In recent years there has been significant progress in the treatment of preterm infants.

However, a study conducted in Germany in 2016 indicates a potential therapeutic manipulation in relation to the administration of probiotics in

the neonatal period [23]. Additional research is essential to to fill in the gaps in the knowledge of probiotics and their effects on reducing the costs of health care for the treatment of hospitalized children in ICUs [24]. Therefore, routine use of probiotics cannot be supported on the basis of existing scientific evidence as suggested by a study conducted in 2016. The safety of probiotics is also an important concern. On rare occasions, probiotics may cause bacteremia, fungemia, and sepsis in immunocompromised, critically ill children. More studies need to be conducted to answer questions on the effectiveness of multi-strain versus single-strain probiotics, the optimum dosage regimens and duration of treatment, cost effectiveness, and risk-benefit potential for the prevention and treatment of various critical illnesses in newborns [25].

Conclusion

The conclusion of this study is that probiotics may have a positive impact on the course and outcome of treatment in premature infants admitted in the ICU. Our newborns who received probiotics spent a shorter time in intensive care, they began a full peroral intake of milk sooner and received antiulcer medication for a shorter period of time, which is an important step towards the improvement of the treatment outcome of premature infants. This study showed encouraging results of the introduction of prophylactic probiotic therapy along with other treatment measures of newborns. However, further studies are needed in this field.

Declaration of interest

The Authors declare that there is no conflict of interest.

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