

**PROBIOTICS AND BREASTMILK ARE
ASSOCIATED WITH A DECREASED RISK OF
ATOPIC DERMATITIS IN VERY LOW BIRTH
WEIGHT PREMATURE INFANTS**

Study protocol and statistical analysis.
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STUDY PROTOCOL

BACKGROUND

Prematurity is associated with significant morbidity and mortality, including the prevalence of sepsis and gastrointestinal problems such as necrotising enterocolitis (NEC), among others. The aetiology is mainly due to immaturity, although numerous external factors play a role in aggravating its complexity. Some of these causes are increased gastrointestinal permeability, immaturity of the immune system, delayed colonisation and/or abnormal colonisation (dysbiosis), use of antimicrobials (sometimes prolonged), physiological limitation of the initiation of enteral nutrition... Some prenatal factors such as premature rupture of membranes or chorioamnionitis may also play a role, exposing them very early to pathogenic micro-organisms. These factors justify the higher risk of gut microbiota disruptions present in preterm infants compared to term infants.

The human microbiota plays a fundamental role in the development and maturation of the immune system. The symbiosis between microbiota and humans is essential in both innate and acquired immune maturation, intervening in the maturation of T cells and natural killer cells, proper regulation of the Th1-Th2 response, as well as in the tolerance of intestinal endotoxins.

Intestinal dysbiosis is conditioned by nutrition and healthy habits. In neonates, the main determinant of gut flora composition is diet and antibiotic exposure. Breast milk is the paradigm in neonatal nutrition, its microbiological content and macromolecules such as oligosaccharides in breast milk promote the growth of *Bifidobacterium* spp.

The use of probiotics in preterm infants, a population exposed to unfavourable environmental factors, may favour a more physiological development of the microbiota.

Numerous studies show that the use of probiotics in this population during the neonatal period reduces the development of NEC and late-onset sepsis in the short term. However, they also report other long-term beneficial effects on inflammatory, metabolic, neurological and gastrointestinal diseases, such as the reduction of allergic diseases throughout life, which is the focus of this study.

In recent decades, a significant increase in atopic-based diseases has been observed, in the pathophysiology of which metabolic activity triggered by gut dysbiosis seems to play a major role, as it has been observed that it may precede the onset of atopic dermatitis.

Atopic dermatitis is a chronic recurrent inflammation of the skin that leads to increased permeability, and therefore a loss of the skin barrier and increased risk of allergic sensitisation.

Several studies show the benefits of daily oral probiotics in the clinical improvement of atopic dermatitis and other atopic-based diseases with chronic inflammation, due to their effect on the development of immune tolerance. In the case of atopic dermatitis, a significant reduction in symptomatology and severity has been observed, as well as a reduction in the use of topical corticosteroids.

Factors involved in the early maturation of the immune system will condition the long-term development of atopic dermatitis. The use of oral probiotics composed of *Lactobacillus* spp. and *Bifidobacterium* spp. in the neonatal period may be a promising strategy against the development of atopic dermatitis due to their collaboration in the maturation and regulation of the immune system.

However, there is a lack of uniformity in the literature regarding the type of strains of choice, applicability of probiotics as prophylaxis or treatment, and in some of them even notable differences in results.

DESCRIPTION

In recent years there has been an increase in allergic diseases, which can be explained pathophysiologically by a dysregulation of the immune system. There are several lines of research into the cause of this increase, the best known of which is the "hygiene theory". Most agree that it could be due to a lack of immunological tolerance from a very early age.

The gut microbiota is directly involved in mediating immune tolerance to all antigens, including pathogenic and commensal mycobacteria, exposed at the level of the intestinal lumen. Currently, the use of probiotics is very popular in multiple diseases of an immunological and inflammatory nature, due to their interaction and modification of the microbiota, and therefore, with the immune system.

In neonatology, the use of probiotics is becoming increasingly widespread as a preventive measure against necrotising enterocolitis and late-onset sepsis. Based on theories about the interaction of probiotics with the immune system, it may be that the use of probiotics in this population may offer other medium- to long-term benefits in inflammatory diseases.

OBJETIVES

The main objectives are as follows:

1. To evaluate the association between nutritional factors in the neonatal period and atopic dermatitis, in relation to prevention.
2. To assess whether the use of breastfeeding and probiotic supplementation in very low birth weight infants as prophylaxis for necrotising enterocolitis is associated with a decrease in atopic dermatitis at preschool and school age.
3. To determine probiotic strains that offer benefits in reducing the occurrence of atopic disease.

The secondary objectives are set out below:

1. To assess whether the use of breast milk (both fresh and donated) offers a synergistic effect, when combined with probiotics, in the reduction of atopic dermatitis.
2. Impact of caloric and protein intake in very low birth weight infants on the development of atopic dermatitis later in life.

DESIGN AND METHODS

Observational retrospective cohort study in very low birth weight infants, <1500 grams and/or < 32 weeks, hospitalised in the Neonatal Intensive Care Unit at the Hospital Universitario Clínico San Cecilio between January 2009 and December 2021.

Enteral and parenteral nutritional data, type of feeding (fresh and/or thawed breast milk, donated breast milk or formula), probiotic administration and strain administered are recorded. Parental permission is available for all patients included in the study.

Inclusion and exclusion criteria:

All patients hospitalised in the Neonatal Intensive Care Unit with < 1500 grams birth weight and/or < 32 weeks at birth during the indicated time period. Deceased patients, incomplete or unavailable medical history and patients with severe congenital diseases are excluded.

Nutritional management:

The unit's nutrition and fluid management protocol, both enteral and parenteral, follows the recommendations of the Nutrition and Metabolism Group of the Spanish Society of Neonatology. Daily fluid (ml/kg/day), carbohydrate (mg/kg/min), protein (g/kg/day), lipid (g/kg/day) and calorie (kcal/day) intakes are calculated.

Given the characteristics of this group of patients, as described above, nutritional needs are often met with parenteral nutrition combined or not with enteral feeding. The objective is to cover the minimum needs to guarantee adequate growth and development of the newborn.

In relation to enteral nutrition, the first choice is fresh breast milk, sometimes it is necessary to freeze it for preservation, and it is thawed prior to use. In cases of unavailability, with parental consent, donated milk that has been pasteurised according to Holder's method is administered, or failing that, formula for premature infants.

Fortification with maternal or donated milk is started from one week of life, once water intake of at least 80 ml/kg/day has been achieved.

In our unit, the use of probiotics was initiated in December 2013 as a preventive measure against the development of necrotising enterocolitis. Two strains were available: *Lactobacillus Rhamnosus GG* and the combination of *Lactobacillus acidophilus* + *Lactobacillus bifidum*. The choice of the type administered depended on the preference of the responsible paediatrician. The probiotic was introduced after initiation of satisfactory enteral nutrition and continued until 35 weeks postnatal or until discharge from the unit.

Atopy registry:

Atopy is a state of abnormal hypersensitivity to exposure or contact with non-pathogenic antigens. We record a history of atopic dermatitis and first-degree family history.

STATISTICAL CONSIDERATIONS

In our study we have two types of descriptive variables: continuous variables and categorical variables.

- For continuous variables we will use the median and interquartile range, using the Mann-Whitney test for comparison.
- For categorical variables, distribution frequencies will be used, analysing them by means of Chi-square.

In order to study more than one variable on the desired effect, multivariate logistic regression analysis will be used.