

Intestinal Lactobacillus Species: Is it Equal in Colicky and non-Colicky Breastfed Infants?

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Abstract

Introduction:

Infantile colic is one of the most common problems in the family in few weeks after birth. Pathophysiology of colic has not been explained yet. The aim of this study was to compare the intestinal microflora in colicky and non-colicky infants.

Materials and Methods:

Seventy breastfed infant aged 15-60 days were enrolled in this study and divided into two groups of 35 colicky and non-colicky according to Wessel's criteria. Stool sample were cultured on selective media. Lactobacillus species were defined by specific tests. Difference in the normal flora between two groups was analyzed by SPSS 16, t-test and a P-value less than 0.05 was considered being significant.

Results:

Among 35 colicky and non-colicky infant, 15 and 20 culture positive lactobacilli were detected respectively. Lactobacillus (LB) acidophilus grew in 7 (20%) non-colicky infants and none of colicky infants. (P=0.02).

Conclusion:

Absence of LB acidophilus in colicky infants may predispose growth of other bacteria which may play a role in the pathogenesis of colic and its symptoms.

Keywords:

Infantile colic

Introduction

Infantile colic is one of the most parental complaints and the cause of referring to pediatric emergency ward in the first few weeks of life (1-2). Depending on Wessel's criteria(3), colic was estimated to involve 5-40% infants (4). Palliative therapy of colic has not been successful.

Unfortunately, by this time, etiology of colic has not been clearly determined. Food allergy (5,6), gastrointestinal dysfunction, behavioral problem such as inadequate maternal-infantile collaboration, physiologic process of gastrointestinal development, were all theories related to pathophysiology of infantile colic (3,5-13). As in colic process, intestinal gas is prominent, it seems gas-producing

anaerobic bacteria is related to colic symptoms (14,15). According to few researches, intestinal bacterial microfloras are different in colicky and non-colicky infants (2). To find out the pathophysiology of colic we are interesting to know better lactobacillus species, one of the most abundant infant. The purpose of this study was to find out the association of intestinal microflora with infantile colic.

Materials and Methods

This prospective study was carried out in outpatient clinic of Amirkola Children's Hospital affiliated to Babol University of Medical Science, north of Iran from July

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2008 to May 2009. Seventy babies, 15-60 days old, exclusive breastfed, enrolled in two groups of colicky and non-colicky.

Colicky infants were selected by history, physical examination on the basis of modified Wessel's criteria (3). Control group was selected among the well babies who were visited by pediatrician in the well baby clinic. Exclusion criteria were: birth weight less than 2500 gr, receiving antibiotics and family history of atopy.

Data such as birth weight, mode of delivery and other variables were collected by a questionnaire. A urinalysis was done in order to rule out UTI as a cause of restlessness in colicky infant. One stool sample was obtained from all infants and then a serial dilution was prepared with distilled water for seven times in order to catch a 10^{-7} to 10^{-1} dilution. Afterwards the resulted sample was cultured on MRS (de Man, Rogosa and Sharpe) broth media (Merck company, Germany) and incubated for 48 hr in 37 °C in an aerobic and microaerophilic condition. After 48 hr, from each MRS broth media was culture individually in MRS agar media (Merck company, Germany) (16,17).

After autoclaving in 37 °C for 48 hr, the individual forming colonies were again cultured in MRS agar media in order to reach a pure culture. Cultured colonies were studied

on morphology, gram stain, motility, catalase, oxidase test, bile sculin test and also the ability to growing in 6.5% NaCl in order to confirm the lactobacilli. Then species of the lactobacillus were defined based on carbohydrate metabolism (18,19). Finally the results were analyzed using SPSS, χ^2 test and student's t-test were used when appropriate, and a P value less than 0.05 was considered significant.

Results

In both colicky and non-colicky groups, 35 infants were studied. They were matched for gender, birth weight, age and weight on visiting time (Table 1). Mean age in colicky and noncolicky were 39.23 ± 11.64 day and 41.57 ± 9.09 day respectively ($P=0.351$). In colicky babies 21 of 35 were boys and 14 of 35 were girl ($P=0.23$), that means there was not difference colic in two genders. There was no significant difference pertaining to the delivery mode (cesarean section or vaginal delivery). Lactobacilli grew in 20 out of 35 (57.1%) samples in non-colicky and 15 out of 35 (42.8%) of samples in colicky infant ($P=0.33$). Among culture positive lactobacillus in two groups some species grew only in one group (Table 2). The growth of each species in two groups was compared separately which showed a significant difference in *L.acidophilus*, ($P=0.02$).

Table1: Dermographic characteristic of colicky and non-colicky group

Characteristic		Colicky	Non-colicky	P-value
age \pm SD (day)		39.2 \pm 11.6	41.5 \pm 9.09	0.35
Birth weight \pm SD (gram)		3372 \pm 464	3290 \pm 606	0.52
referral weight \pm SD (gram)		4491 \pm 668	4627 \pm 866	0.46
Number of infant		35	35	-
gender	boy	21	21	-
	girl	14	14	-

Table 2. Frequency and percentage of lactobacilli species in colicky and non-colicky groups

Lactobacilli	Colicky	Non-colicky	P- value
Plantarum	2(5.7%)	0	0.12
Delbrueckii	6(17.1%)	2(5.8%)	0.05
Viridescens	3(8.6%)	1(2.8%)	0.19
Brevis	2(5.7%)	1(2.8%)	0.33
Halotolerant	2(5.7%)	0	0.12
Cusei	0	1(2.8%)	0.33
Sanfrancisco	0	1(2.8%)	0.33
Gasseri	0	2(5.8%)	0.22
Hilgardii	0	1(2.8%)	0.33
Acidophilus	0	7(20%)	0.02
Reuteri	0	2(5.8%)	0.22
Helveticus	0	2(5.8%)	0.22

Discussion

In this study we found that *L.acidophilus* was not isolated in any of colicky infant, similar to the results obtained by Savino et al in 2005 (2) *L.acidophilus* is one of normal flora of gut during infancy (20,21). There are few reported papers regarding the protective effect of lactobacillus acidophilus by enhancing the immune system, production of surface IgA and accelerating macrophage function in the intestine which play a role to destruct food antigen (22-24). The absence of this microorganism in colicky infant gut may provides a suitable environment for growth of anaerobic gram negative bacteria and resulting more gas production and colic symptoms. Some species of LB such as cusei, francisco, gasser, hilgardii, ruteri, helvitecus and acidophilus were not found in colicky infant. Furthermore in our study, LB delbrueckii, LB brevis and LB plantarum were more frequent in colicky group while in Savino's study, only LB brevis was isolated in this group (2). This difference may be related to race and maternal nutritional pattern, which could have an effect on content of breast milk. Same as Savino's study(2) we found that lactobacillus species in colicky and non-colicky infant are different, the most common lactobacilli species in non-colicky was LB acidophilus. Although the differences between LB species in colicky and non colicky infant are not significant except about acidophilus ,two groups showed difference in growing micro-organism(P=0.027).Nowadays, the use of pre-probiotic as symbiotic are common in

many of gastero intestinal dysfunctions (22,25,26). So using lactobacillus species as a probiotic in colicky infant may play a change in the intestinal microflora and bring it like as non-colicky infant (27). Further studies are needed to confirm these results.

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