Evaluation of the Efficacy of Probiotics in the Treatment of Infantile Colic: A Randomized, Double Blind, Placebo Controlled Trial

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Abstract- The infantile colic is one of the most common complaints in the infancy; however, limited therapeutic approaches are described in the literature. Recently probiotics have been suggested as a potential strategy in the treatment of infantile colic. We conducted this study to investigate the efficacy of probiotics in relieving colic symptoms in Iranian infants. This double-blind clinical trial was performed among 70 infants aged 3 to 16 weeks with the presumed diagnosis of infantile colic according to Wessel criteria who were breastfed or formula fed. They were assigned at random to receive Pedilact® (Bifidobacterium infantis, Lactobacillus reuteri, and Lactobacillus rhamnosus) (N=33) or placebo (N=32). Demographic data were recorded in the questionnaires at the beginning of the study. The number of daily episodes of crying and fussiness, number of weekly crying days, and duration of crying were separately analyzed on 7, 21, and 30th days of investigation. Baseline demographic data showed no statistically significant difference between intervention and placebo groups. Infants given Pedilact® showed a significant reduction in daily episodes of crying, duration of crying, and the weekly number of crying days at the end of the treatment period compared with those receiving placebo (*P*=0.000). On 21th day of the study, daily episodes of fuss and crying (*P*=0.032) and duration of crying reduced significantly in the intervention group in comparison to the placebo group (*P*=0.000). Administration of Pedilact® drop significantly improved colic symptoms by reducing crying and fussing times in breastfed or formula fed in Iranian infants with colic.

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Introduction

Colic is a sudden and unexpected condition in infants which presents with mostly unstoppable attacks of severe fussiness and high pitch crying. Approximately 5-40% of infants experience colic in first 3 months of life (1,2,3). Wessel *et al.*, described "infantile colic" as an otherwise healthy infant who cries vigorously at least three hours a day, in at least three days of week, and this fussiness persist for at least three weeks (4). The exact mechanism and pathophysiology of infantile colic is still undetermined. Cow's protein milk allergy, motility disorders, gastro esophageal reflux are among the most significant differential diagnosis of an infant with fussiness that needs to be excluded in any infant with colic. Though colic has a reassuring prognosis, excessive crying can lead to caregiver frustration and may be a trigger for shaken baby syndrome (5,6). On the other hand, irritability due to colic causes lots of pediatrician referral and unnecessary visits. Therefore establishing a reliable method of treatment in infantile colic seems to be crucial. According to WHO definition, probiotics are " live microorganisms that, when administered in adequate amounts, confer health benefits on the host" (7). Enhancing the properties of intestinal flora and competitive adherence to the mucosa and

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epithelium are two probable mechanisms of action of probiotics (8). As several recent researches have been performed to examine the effectiveness of *L reuteri DSM 17938*, there are few clinical trials in literature evaluating the effect of Pedilact® *(bifidobacteriuminfantis, lactobacillus reuteri, and rhamnosus lactobacillus)* vs. placebo in treatment of infantile colic. So due to high psychological and economic burden of infantile colic on families and health system, we decided to conduct a clinical trial to evaluate the effectiveness of Pedilact® (bifidobacterium infantis, lactobacillus reuteri and rhamnosus lactobacillus) vs. placebo in a tertiary care children hospital in Tehran, Iran. Additionally, in order to investigate probable association between geographic region and probiotic responses in infantile colic, we conducted this study in our local region.

Materials and Methods

Study design and study population

This study was conducted as a randomized, double blind clinical trial in a tertiary hospital, Bahrami children hospital, during August 2016 to August 2017 in Tehran, Iran. Bahrami Children Hospital is a center in which approximately 30000 patients are seen in the emergency department every year. The study was approved by Research Deputy and Ethics Committee of Tehran University of Medical Science before initiation of the study. (Code: IR.TUMS.MEDICINE.REC.1395.10.15) All infants with diagnosis of infantile colic, according to Wessel criteria who presented to emergency department or outpatient general/gastrointestinal clinics, were enrolled. Participants were 3 to 16 weeks old, were breastfed or bottle fed or both, their gestational ages were ≥34 weeks, and their birth weights were ≥1960 grams.

Exclusion criteria were:

- History of antibiotic and/or probiotics consumption one week prior to initiation of the study
- Overt failure to thrive
- Past history of *gastroesophageal reflux*
- Present gastroenteritis symptoms or suspicion of other etiologies related to irritability

Data Collection and Randomization

A total of 70 infants were eligible to participate in the study that were randomized into 1 of 2 treatment arms, Pedilact® (bifidobacterium infantis, lactobacillus reuteri, and lactobacillus rhamnosus) and placebo group. An independent statistician prepared the computer generated randomization schedule using a block size of two to maintain balance between treatment arms within each stratum. As it was a double blind study, all treatment allocations were concealed from all study participants and investigators. A clinical pharmacist, who was independent and did not participate in the study, packaged all intervention and placebo drops identically so that all participants and investigators were blinded to their content. Randomization codes were concealed until final analysis of the outcome was completed. On enrollment, a standardized questionnaire was completed for every infant according to caregivers' information and physical examinations. The questionnaires contained demographic data, including infants' age, sex, birth weight, gestational age, and feeding methods. Maternal and familial contents of questionnaire included history of smoking, postpartum depression according to Edinburgh Postnatal Depression Scale (EPDS) , and family history of atopy. The treatment was Pedilact® (bifidobacterium infantis, lactobacillus reuteri and lactobacillus rhamnosus) (1×10^9 colony forming unit per drop) in an oil suspension including sunflower oil, medium-chain triglyceride oil, and silicon dioxide. The placebo included the same ingredients of oil suspension except for live bacteria.

Caregivers were instructed to administer 5 drops daily by oral route for one month, preferably at the same of time of a day. They were instructed to report any unusual symptoms to investigators during usage of drops. Primary and secondary outcome measures including number of daily episodes of crying and fussiness, number of weekly crying days, and duration of crying were separately assessed by investigators on study day 7, 21, and 30 during follow ups visits. Probable side effects like diarrhea, vomit, and constipation were evaluated by study investigator in follow ups. On 30th of the study, thorough physical exam was performed by the referring pediatrician.

Ethical considerations

The manuscript was approved by Research Deputy and Ethics Committee of Tehran University of Medical Science, Iran, before initiation of the study. (Approval Number: IR-TUMS-MEDICINE.REC.1395.1015)

Trial registration

The trial protocol was registered under the Iranian Registry of clinical trials (RCT Number: IRCT2016120818971N4).

Statistical analysis

The analysis was based on the intention to treat principle. A total sample size of 70 (35 participants in every study arm) provided 80% power to detect a small to medium effect size of differences (Cohen's $f = 0.25$, Cohen's $d = 0.5$) in the mean daily episodes of crying and fussiness between treatment groups with a significance level of $P<0.05$. Statistical analyses were performed using SPSS version 21 (IBM, Armonk, New York). Univariate comparisons for continuous data were made using Mann-Whitney tests and for categorical data using Chi-square or Fisher exact tests. Comparisons of clinical symptoms between intervention and placebo group were made using the non-parametric Friedman test. All tests were two-sided, and a $P<0.05$ was considered statistically significant for the primary analysis.

Results

This double blind randomized clinical trial was conducted on 70 infants with presumed diagnosis of colic according to Wessel criteria. On enrollment, 70 eligible infants were randomized, 35 to receive placebo, and 35 to receive Pedilact. Two participants in intervention group and 3 participants in control group were excluded from the study because of discontinuing of the drug. Finally, thirty three (94.3%) participants in intervention group and thirty two (91.4%) participants in control group completed the study and were included in the analyses.

Descriptive statistics

Demographic and clinical characteristics of both intervention and placebo groups were analyzed.

Intervention group

Intervention group consisted of 33 infants in which 16 infants (48.5%) were male, and 17 infants (51.5%) were female. At study entry, the mean±standard deviation (SD) of gestational age at birth was 38.58±1.03 weeks (37-41 weeks), and birth weight was 3377.88±511.89 grams (2000-4490 grams). Approximately 66 percent of infants were exclusively breast fed while the rest were bottle fed or combination of them. More than half of the infants revealed colic symptoms in early 30 days of life. Family history of allergy and atopy was evident in 51.5% of participants. According to Edinburgh Postnatal Depression Scale (EPDS) score, 12 mothers had low probability of depression.

Placebo group

Control group consisted of 32 infants, which 20 infants (62.5%) were male, and 12 ones (37.5%) were female. Mean gestational age and mean birth weight±standard deviation (SD) was 38.06±1.19 weeks (34-41 weeks) and 3239.38±642.02 grams (1960-4500 grams), respectively. Seventy five percent of infants were exclusively breast fed while others were bottle fed or utilized combination of them. 62.5 % of infants showed colic symptoms during 1 to 3 months old and only one infant revealed colic symptom after 3 months old. Positive family history of allergy and atopia was determined in 28.1% of participants. According to Edinburgh Postnatal Depression Scale (EPDS) score, 13 mothers had low probability of depression and 1 had signs leading to possibility of PPD. Comparison of demographic and clinical data in control and intervention group determined that there was no statically significant difference in all mentioned parameters between these groups at the study entry (Table 1).

Primary and secondary outcomes

Primary outcome measures in our study consisted of number of daily episodes of crying and fussiness while secondary outcome measures consisted of number of weekly crying days and duration of crying in 7^{th}, 21^{th} and 30^{th} days of follow ups. Analysis revealed a significantly great reduction in average of both primary and secondary outcome measures in intervention group in comparison to placebo at the end of the study period (Table 2 and 3). During the study, participants did not show any major side effects, including vomit, constipation, or skin reaction in placebo or intervention groups.

The comparison of primary and secondary measures in intervention and placebo group is comprehended as follows: Overall, intervention group exhibited a significantly greater reduction in weekly number of crying days in 7^{th} day of the study (P=0.043) (Table 4). Meanwhile, on 21^{th} day of the study, daily episodes of fuss and crying reduced significantly in intervention group in comparison to placebo group (P=0.032). Duration of crying also decreased significantly in intervention group on 21^{th} day of initiation of the study. (P=0.000) (Table 5). Eventually, on 30^{th} day of the study, there was a significant reduction in daily episodes of crying, duration of crying, and weekly number of crying days in intervention group in comparison to

placebo group. (*P*=0.000) (Table 6) .

Table 1. Demographic and clinical symptoms of study participants in intervention Pedilact vs. placebo groups

Demographic and clinical symptoms		Placebo group (N=32)	Intervention group (N=33)	*P*
Gender [Number (%)]	**Male**	20 (62.5%)	16(48.5%)	0.256 (NS)
	Female	12 (37.5%)	17 (51.5%)	
Gestational age (weeks)/Mean±SD		38.06±1.19	38.58± 1.032	0.068 (NS)
Birth weight (gram)/Mean±SD		3239.38±642.023	3377.78±511.89	0.339 (NS)
Age at entry (month) [Number (%)]	**<1 month**	11(34.4%)	17 (51.5%)	0.363 (NS)
	1-3 month	20(62.5%)	14(42.4%)	
	>3 month	1 (1.4%)	2(6.1%)	
Feeding Method [Number (%)]	**Breast feeding**	24 (75%)	22 (66.7%)	0.432 (NS)
	Bottle feeding	3(9.4%)	7(21.2%)	
	Both	5(15.6%)	4(12.1%)	
Family history of allergy or atopy/[Number (%)]		9 (28.1 %)	17 (51.5%)	0.054 (NS)
Maternal stress EPDS/[Number (%)]		12(37.5%)	14 (42.4%)	0.685 (NS)
Stool consistency [Number (%)]	**Constipation**	6 (18.8%)	4(12.1%)	0.459 (NS)
	Diarrhea	0 (0%)	0 (0%)	
	Normal	26 (81.2%)	29 (87.9%)	

N, number; NS, non-significant; SD, standard deviation
**P*<0.05 for the comparison between two groups with and without the specified characteristic

Table 2. Primary and secondary outcome measures in different follow up days during the study period, related to intervention group

Primary and secondary outcome measures	Baseline	7th day	21th day	30th day	*P*
Daily numbers of episodes of fussiness and crying(mean)	2.27±0.626	2.24±0.708	1.64±0.549	1.18±0.392	0.000* (S)
Duration of Crying (hours) (mean)	2.58±0.614	2.03±0.77	1.45±0.564	1.18±0.465	0.000 (S)
Weekly number of crying days (mean)	2.73±0.574	2.58±0.614	1.97±0.467	1.64±0.489	0.000 (S)

S: Significant
*Non parametric Friedman test.

Table 3. Primary and secondary outcome measures in different follow up days during the study period, related to placebo group

Primary and secondary outcome measures	Baseline	7th day	21th day	30th day	*P*
Daily number of episodes of fussiness and crying (mean)	1.91±0.689	2.03±0.647	2.03±0.538	2.16±0.574	0.301* (NS)
Duration of Crying (hours) (mean)	1.97±0.647	1.97±0.647	1.81±0.644	2.19±0.592	0.174 (NS)
Weekly number of crying days(mean)	2.00±0.672	2.13±0.707	1.89±0.641	2.19±0.592	0.062 (NS)

NS: non-significant
*Non parametric Friedman test

Table 4. Significant reduction of weekly crying days in intervention vs. placebo group on 7th day of the study

Outcomes		Intervention group N (%)	Placebo group N (%)	*P*
Weekly number of crying days	**1 Day**	8 (24.2%)	3(9.3%)	0.043
	2 Days	23(69.6%)	21(65.7%)	
	≥3 Days	2(6.2%)	8(25%)	

Table 5. Significant reduction of daily crying episodes and duration of crying in intervention vs. placebo group on 21th day of the study

Outcome		Intervention N (%)	Placebo N (%)	*P*
Daily number of episodes of fussiness and crying	3 times or less	13(39.3%)	6(18.75%)	0.032
	4-8 times	19(57.7%)	17(53.2%)	
	More than 8 times	1(3.03%)	9(28.1%)	
Duration of Crying (min)	<10 min	19(57.5%)	6(18.76%)	0.032
	10-30 min	13 (39.5%)	19(59.37%)	
	>30 min	1(3.03%)	7(21.87%)	

Table 6. Significant reduction of primary and secondary outcome variables in intervention vs. placebo group on 30th day of the study

outcome		Intervention N (%)	Placebo N (%)	*P*
Daily number of episodes of fussiness and crying	3 times or less	27(81.8%)	6(18.75%)	0.000
	4-8 times	6(18.2%)	16(50%)	
	8 times or more	0	10(31.2%)	
Duration of crying (min)	<10 min	28(84.8%)	8(25.07%)	0.000
	10-30 min	4(12.17%)	19(59.3%)	
	>30 min	1(3.03%)	5(15.6%)	
Weekly number of crying days	One Day	12(36.37%)	3(9.37%)	0.000
	2 Days	21(63.63%)	20(62.5%)	
	≥3 Days	0	9(28.12%)	

Discussion

Fussiness and crying of an infant is a distressing and exhausting event for parents, especially for unexperienced ones. The incidence of infantile colic (IC) differs between communities and depends on study design, region of sample recruitment, definition and method of data collection (2). IC incidence in literature has been reported between 5-40% (1,2,9) meanwhile according to a prospective study in Iranian population using Wessel criteria incidence of infantile colic in infants younger than 3 months old appeared to be around 20% (3). Recently, many researchers have been performed in order to evaluate the efficacy of probiotic supplementation in treatment of IC. The probable role of probiotics in treatment of IC is by modulation of microflora and altered balance of intestinal lactobacilli to provide a safe and sufficient microbial stimulus for the immature intestinal immune system (10). In our trial, we consumed Pedilact®, which is a combination of three strains of bacteria, *bifidobacter iuminfantis, lactobacillus reuteri, and lactobacillus rhamnosus*, in an oil solution for treatment of IC sufficiently severe for parents to seek medical care. The intervention and placebo group were similar at baseline risk factors including age, sex, birth weight, feeding method, and maternal stress. Our participants were breast fed and formula fed while most of the studies in literature evaluated exclusively breast fed infants. According to our knowledge, only one study in literature evaluated role of probiotics in formula fed infants which demonstrated formula fed infants in the probiotic group cried or fussed 49 minutes more than those in the placebo group (11). While in our study, there was not any significant difference in response to treatment between formula and breast feeding infants. Further prospective studies need to be performed to evaluate the exact role of probiotics in exclusively formula fed infants. According to our findings, there was a significant reduction in daily episodes of fuss/crying, duration of crying and weekly number of crying days in intervention group in comparison to placebo group on 30th day of trial. The achieved conclusion was aligned with several other studies performed (12,13,14). Only one study performed by Sung *et al*., revealed that treatment with *Lactobacillus reuteri* did not reduce crying or fussing, nor was it effective in improving infant sleep or quality of life (11). In some previous studies, mothers were instructed to refrain from using cow' milk protein products in their diet (14,12). We did not request breastfeeding mothers to adhere to a cow's milk protein elimination diet, and despite that, we

observed comparable improvements in daily crying and fussing times in the *bifidobacterium infantis, lactobacillus reuteri and lactobacillus rhamnosus*–treated group. It reveals that eliminating the cow's milk protein does not have significant effect on reducing crying/fussing time in infantile colic, as it was suggested previously (15). In present study, we evaluated multiple probiotic combination (*bifidobacterium infantis, lactobacillus reuteri and lactobacillus rhamnosus*) while only one type of probiotic *(Lactobacillus reuteri) was* used in the most of the previous studies (14,15,16,17). The safety and efficacy of a combination of probiotics were not evaluated in most of the published articles and therefore are unknown. Few side effects were demonstrated in our study while using Pedilact®. This fact is compatible with all previous studies in literature (14,15,16,17).

A potential limitation in our study, similar to most previous studies, is that our findings are based solely on mothers' report. Additionally, the exact consumption of Pedilact® by caregivers could not be evaluated precisely. Prompt weighing of drops before and after consumption should have been done in order to achieve the goal.

Supplementation with the Pedilact® administered at a dose of 10^9 colony forming unit per drop once daily to infants with presumed diagnosis of infantile colic resulted in significantly great improvement in colic symptoms at the end of treatment (30th days) compared to controls. With no prominent side effect, Pedilact® appears to be a safe treatment option in infants with infantile colic in Iranian population.

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