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Original Article

Effect of Probiotics on Infantile Colic Using the Quadratic Inference Functions

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ABSTRACT

***Background*:** Infantile colic is defined as episodes of extreme and excessive crying due to unknown causes. Various results have been reported regarding the management of colic with probiotics in terms of effectiveness, with no side effects or health risks in the infants. The present study aimed to evaluate the effect of probiotics on the infants with colic using the quadratic inference functions (QIF).

***Methods*:** This single-blind, randomized, clinical trial was conducted on 98 infants admitted to the pediatric gastroenterology clinic of Bu Ali Sina Hospital in Sari, Iran. The neonates were diagnosed with infantile colic by a pediatric gastroenterologist. Patients were randomly divided into two groups (49 subjects per each). In the control group, the infants received placebo, and in the case group, the neonates were administrated with BioGaia probiotic oral drops for 21 days. The QIF method was fitted to analyze the influential factors in the improvement of infantile colic.

***Results*:** According to the QIF results in data analysis, mean duration of crying had a significant difference between the infants in the case and control groups (P<0.001). Moreover, time (first, second, and third week) was determined as a leading variable in the improvement of infantile colic (P=0.001).

Conclusion: According to the results of longitudinal data analysis, use of probiotics in the evolving gut could reduce infantile colic and improve the quality of life in the studied neonates.

Keywords: Infantile colic, Probiotics, Quadratic inference functions

Introduction

Crying is considered a normal behavior during infancy, which is an awareness mechanism for newborns to meet their physiological needs, such as hunger, discomfort, and pain (1-3). In some cases, crying of infants might be abnormal and due to other causes, such as infantile colic. Infantile colic, also known as baby colic, is a common problem occurring in the first three months of birth, which affects 10-30% of the infants aged less than three months. Infantile colic applies to the neonates with prolonged crying without an evident cause during the first three months of life, which distresses parents and urges them to refer to pediatric emergency sections for treatment (4).

For the first time, Weasel described the infantile colic syndrome by the 'rule of three', which considers the following signs to be alarming: crying for more than three hours per day, three days per week, and continuing more than three weeks in the infants aged less than three months; otherwise, infants are not suspected of colic (5). Although colic disappears spontaneously at 2-3 months of age, the excessive crying associated with colic may incur anxiety in the parents, as well as unnecessary costs due to the frequent referrals to physicians, various clinical measures, and medications (6).

While the etiology of infantile colic is unknown, several theories have been proposed for the possible causes; such examples are the overproduction of intestinal gas, strong intestinal contractions, sensitivity to the protein in cow's milk, temporary shortage of lactation, and poor mother-infant bond (3, 5, 7, 8). On the other hand, some studies have implicated the potential role of gut bacteria in infantile colic, as the intestinal

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bacteria have been shown to be different in the neonates with and without colic (9-11).

Inadequate levels of bacteria (e.g., Lactobacilli) have been reported in the infants with colic, particularly in younger neonates. As such, recent studies have mainly focused on the use of probiotics (live microorganisms with various health benefits) for the treatment of infantile colic (12). In this regard, research suggests that the treatment of infantile colic with different types of probiotics could be effective with no side effects or health risks in the infants (13, 14). Other common treatments for infants with colic include detailed training on breastfeeding, preventing aerophagia, carminative drops (e.g., Dimetin), and occasional use of supplements (e.g., acetaminophen drop). However, the pain caused by infantile colic might persist in some cases despite the use of all these measures, leading to the excessive worry and distress of parents.

If probiotics are effective in colicky infants, using these bacteria could reduce the pain and anxiety of the family (14). Most studies evaluating the impact of probiotics on infantile colic have been clinical trials, with the follow-up continuing for several months (longitudinal studies). One of the important features of a longitudinal dataset is the repeated observation of the individuals, which allows the direct study of the changes and results in correlating the series of observations in a dataset. In fact, the independence of observations, which is one of the assumptions of the usual statistical methods, would be violated. Therefore, certain statistical methods are needed for the proper analysis of longitudinal data.

Researchers often use marginal, mixed, and transition models to analyze longitudinal data (15, 16). Among the marginal models, generalized estimating equation (GEE) is an accepted method of statistical inference, which was provided by Liang and Zegerin in 1986 (17). Theoretically, if the correlation parameters are not estimated correctly, the GEE might be able to produce a consistent, but inefficient, estimator (18, 19).

Many advances have been achieved to overcome some of the problems in the GEE, such as the quadratic inference functions (QIF), which was first proposed by Qu et al. in 2000 (20). Despite the studies performed in the other parts of the world regarding the impact of probiotics on infantile colic, limited research has been conducted in this regard in Iran. In addition, the usual statistical methods that have been used to analyze the data from these studies are mainly in the form of clinical trials and longitudinal data. Longitudinal data analysis has not been applied in the previous studies performed in Iran and even other countries; therefore, the present study aimed to evaluate the effect of probiotics on infantile colic using the QIF.

Methods

Study Data

This single-blind, randomized clinical trial was conducted on 98 infants admitted to the pediatric gastroenterology clinic of Bu Ali Sina Hospital of Sari, Iran in the spring of 2014. The neonates were diagnosed with infantile colic by a pediatric gastroenterologist.

Inclusion criteria were as follows: 1) age of 15-120 days; 2) breastfed neonates; 3) failure to use antibiotics in the past two weeks; 4) crying for three hours or more than three hours per day and three days or more than three days per week, and 5) birth weight of 2500-4000 grams.

Exclusion criteria of the study were as follows: 1) consumption of powdered milk; 2) receiving antibiotics during the treatment with BioGaia probiotic; 3) definitive diagnosis of other causes for the infant's pain; 4) unwillingness of parents to continue the study; 5) underlying diseases, and 6) detection of symptoms mimicking infantile colic.

Infants who met the inclusion criteria were enrolled in the study after obtaining informed consent from their parents. Patients were divided into two groups of case and control by random blocks (49 subjects per each group). The control group received placebo, and neonates in the case group were administered with BioGaia probiotic oral drops containing *Lactobacillus reuteri* (made by Ferring Pharmaceuticals, Germany and Daya Teb Co., Iran).

In the infants of the case group, daily oral administration of BioGaia probiotic drops (5 cc) continued for 21 days. The placebo drops used for in the control group were prepared to be similar to the original drug in terms of the shape, size, and color. In both study groups, parents were assured of the safety of their infants and trained on the proper hugging and breastfeeding of the neonates. Training of the parents was provided prior to the study, consisting of the following concepts: 1) recording of the exact time of the beginning and ending of the infant's crying during the day and night and calculating the total duration of crying in hours and minutes on a daily basis for a month, as well as the number of colic attacks per day; 2) correct approaches for breastfeeding, exclusive feeding with breast milk, and their frequency depending on the tendency of the infants, and

burping after each feeding.

Treatment outcomes were determined through the clinical assessment of the patients and evaluated by a statistician on days one, seven, 14, and 21 of drug administration on a weekly basis in terms of the dosage and strain of BioGaia probiotic drops (not less than 108 CFU per five drops). In addition, timetables of the daily crying of the neonates were recorded as a weekly average by the mothers.

Based on the previous studies, sample size was estimated to be 36 infants per each group with 90% test power and 95% confidence level. Finally, 49 neonates were placed in each study group considering the possibility of attrition (14).

Statistical Analysis

Since the condition of the observational independence was not established according to obtained longitudinal data, the marginal model and QIF were used to analyze the observations. Mean marginal model was defined, as follows:

$$g(\mu_{it}) = X_{it}ß$$

where β represents the vector of regression coefficients, $X_{it}ß$ is the vector of covariates, and μ_{it} denotes the mean variable response of Y_{it} to the X_{it} condition.

Link function $g(\mu_i)$ connects the response expectations to the vector of the covariates, and the type of the link function depends on the type of the response variable (13). The correlation among the responses for each subject is an important feature of this model, which is included in the model along with various structures, such as the interchangeable, autoregressive, and independent correlation structures. Moreover, time was defined for the variable, and codes 3, 2, and 1 were used in the model to indicate days 17, 14, and 21 after the administration of the probiotic drop.

Mean crying of the infant during the week prior to the administration of the probiotic drop was considered as the baseline measure. Statistical modeling was carried out via QIF in the SAS software version 9.3 in order to evaluate the effect of probiotics on infantile colic in the patients.

Results

In total, 98 infants (49 in the case group, 49 in the control group) were enrolled in the study. In each patient, a single measurement was recorded for the mean crying of the infant before the intervention (administration of the probiotic drops), and three measurements were recorded for each infant after the intervention. Finally, 294 pieces of data were obtained for the mean crying of the infants in three replications, considering the three repetitions for each subject and absence of the missing data.

Table 1. Characteristics of Quantitative Variables

Variable	Mean±SD	
	Control	Case
Weight (g)	3.15±1.41	3.28±1.15
Age (day)	29.32±1.07	36.12±1.65
Baseline Crying (hour)	20±3.64	22.23±9.34

Table 2. Descriptive Characteristics of Qualitative Variables

Variable	Group	N (%)	
		Control	Case
Gender	Girl	25 (51)	23 (46.9)
	Boy	24 (49)	26 (53.1)
Mode of Delivery	Natural Vaginal	12 (24.5)	6 (12.2)
	Cesarean Section	37 (75.5)	43 (87.8)

Descriptive characteristics of the studied patients are presented in tables 1 and 2. Level of the changes in the mean crying during the three phases of the measurements over a one-week interval after the intervention in the case and control groups have been shown in Table 3 and Figure 1.

According to the information in Table 3 and Figure 1, based on the changes in the mean crying

Table 3. Mean Crying of Infants after Intervention

	Group	Mean Crying after Intervention		
		First Week	Second Week	Third Week
Mean±SD	Control	39.59±1.1	24.19±1.81	24.76±1.53
	Case	16.40±1.10	11.36±1.93	9.82±1.51

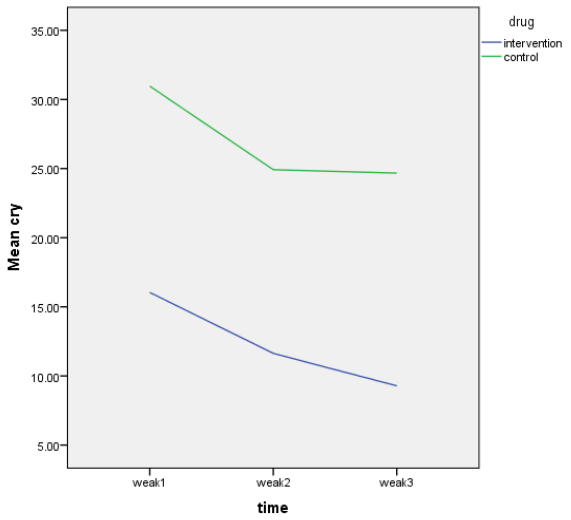

Figure 1. Changes in Mean Crying over Time in Case and Control Groups

Table 4. Final Model Parameter Estimates

Variable	Group	β	SE(β)	P-value
Constant		-1.6	3.52	0.6
Time		-3.13	0.07	0.0001
Baseline Crying		0.56	0.14	0.0001
Drug Administration	Case*	0	0	<0.0001
	Control	8.57	1.70	
Weight		0.0012	0.70	0.0001

Significance level=0.05; *basis of comparison

of the infants in the case and control groups, there was a more significant reduction in the mean crying over time in the case group compared to the control group. In fact, with the passage of time, the mean crying declined in both groups, while further reduction was observed in the treatment group.

Initially, the normality and homogeneity of the variance assumptions in the response variable were reviewed and confirmed. Considering the correlations among the observations and information criteria, the interchangeable correlation structure was used as the correct correlation structure in the estimation method. Data fitted by the interchangeable correlation structure and QIF are presented in Table 4.

For the interpretation of the final model to investigate the effects of the probiotic drop and time on the improvement of infantile colic, all the variables were initially entered into the model, and a backward method was used afterward to exclude the least significant variables from the model. Finally, the significant variables were reported in the final model (Table 4).

In the statistical analysis (Table 4), a significant difference was observed between the case and control groups in terms of the mean crying of the infants ($P<0.001$), so that the mean crying was eight hours shorter in the neonates administered with the probiotic drops compared to the neonates receiving placebo. Furthermore, the time variable was reported to be a significant factor in improving infantile colic ($P=0.001$); accordingly, the mean crying reduced to 3.5 hours every week from the beginning of the intervention. Other contributing factors to the improvement of infantile colic were the birth weight and mean crying of the infants at the beginning of the study ($P<0.05$).

Discussion

According to the results of the present study, using probiotic drops in the neonates with infantile colic significantly reduced the duration of crying during the first, second, and third week of follow-up after the treatment. These findings are in line with the results obtained by Szajewska in Poland, who evaluated 80 infants aged less than five months in the two groups of intervention and placebo. In the mentioned study, it was observed that the administration of *L. reuteri* drops decreased the mean crying of the infants on days seven, 14, 21, and 28 after the treatment in the intervention group compared to the placebo group (21). Findings of the current research had slight differences with the studies conducted in Italy and Canada in this regard, which used the same definition for infantile colic and reported similar outcomes on days seven, 14, 21, and 28 after the treatment (22-24).

In the current research and previous studies, there was a significant reduction in the time of crying in the infants following the use of probiotics in the intervention group, while the downward trend in the duration of crying and other symptoms of colic was also observed in the control group. In their study, Savino et al. also reported the placebo effect in this regard (22). Furthermore, Szajewska (21) and Sung (23) observed high levels of recovery in the colic symptoms of the patients in the control group. In the study conducted by Sung, not only there was no difference in the improvement of the infantile colic symptoms between the intervention and placebo groups, but the improvement in the placebo group also had better scores compared to the probiotic group. A major part of the recovery process in the control group could possibly be associated with the improvement of the colicky infant over time regardless of the drug administration.

Several unknown factors may affect the results of the study (24). Acquisition and establishment of the gut microbiota during the neonatal period is a complex process, which stems from various factors (e.g., geographical region). Therefore, the patterns of the colonization and stabilization of the microbes in the infant's gut could be influenced by country and region (25, 26). Accordingly, the discrepancies between the present study and findings of Savino (22), Szajewska (21), and Chau (24) with the research by Sung (22) could be due to the differences in the geographical region of the study. Infants born in different regions might exhibit variable degrees of susceptibility to probiotic administration. Consistent with the present study, a similar research conducted in Babol (Iran) confirmed the effectiveness of probiotics in improving the symptoms of infantile colic (27).

Longitudinal survey of the impact of probiotics on the treatment of infantile colic was an important

aspect of the current research compared to the previous studies in this regard. Furthermore, the longitudinal survey and considering the correlations between the measurements over time resulted in the investigation of the improvement rate in infantile colic during a period of one month (four consecutive weeks). This clinical technique, along with the proper and efficient statistical model, could be used to assess the changes in the recovery process with higher accuracy and power.

One of the main limitations of the current research and other similar studies in this regard is using the reports provided by the mothers in measuring the outcomes, which might lead to certain differences in the measurements.

Conclusion

According to the results, using probiotics in the evolving gut could reduce infantile colic and improve the quality of life in the neonates. However, it is recommended that further investigation be conducted on larger sample sizes in different geographical regions in order to confirm these findings.

Acknowledgments

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Conflicts of interests

None declared.

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