Fecal Calprotectin in patients with Infantile Colic: A case-control study

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Abstract

Back ground

The precise etiology of infantile colic has not been elucidated after many years of research. In this study, we aimed to determine the association of fecal calprotectin with infantile colic.

Method

This case control study was performed on colicky infants referred to pediatrics clinics in Zanjan over a 6-month period. Infantile colic was clinically defined based on wessel criteria. The control group consisted of healthy infants matched for age, sex, weight, and type of feeding with the case group. Fecal calprotectin levels were measured in both groups by the ELISA method.

Results

Forty infants were divided equally into case and control groups. The cases consisted of 11 boys (55%) and 9 girls (45%) with a mean age of 63 days and in the control group there was 12 boys (60%) and 8 girls (40%) with mean age of 48 days. Fecal calprotectin was positive in 17 (85%) cases and 6 (30%) controls. This difference was statistically significant. There was no significant difference in fecal calprotectin regarding sex, age, weight and type of nutrition in both colicky infants and controls

Conclusion

Fecal calprotectin levels in colicky infants were significantly higher than healthy infants.

Background:

Infantile colic is characterized by paroxysmal inconsolable crying in an otherwise healthy infant and is accompanied with flushing and rising up the legs to the abdomen and fisting hands [1].

The disease is defined by Wessel “rule of threes” criteria and consists of crying for more than 3 hours per day, for more than 3 days per week and for more than 3 weeks in a healthy infant [2]. It is a common disease of infancy with a frequency of 20% that disappears usually around 12 weeks of age [3].This disease may cause fatigue and distress in families and may have negative impact on mothers mental health [4, 5]. The etiology is still unknown [6], and it is thought that it may be due to multifactorial causes. Although maternal stress, depression and anxiety, infants’ temperament, inadequate bonding and environmental issues such as tobacco exposure are proposed [5, 7], the role of intestinal microbiota, food hypersensitivity, intestinal function immaturity and dysmotility have been implicated [5, 7]. Gastrointestinal motility and hyper peristalsis induced by motilin may lead to abdominal pain and colic. It is thought that smoking may increase intestinal motilin level and cause colicky pain [8]. Moreover, different gut microbiota in colicky infants may cause inflammation, excess gas production and flatulence

[5]. Therefore; it may be hypothesized that the gastrointestinal inflammation may increase fecal inflammatory proteins such as Calprotectin in colicky infants.

Calprotectin is a protein of calgranulin family, secreted mainly from neutrophils with a regulatory function on inflammatory processes [9]. It may be used for diagnosis of Inflammatory Bowel Disease, Necrotizing Enterocolitis and intestinal Cystic Fibrosis [10, 11]. Few studies have investigated the amount of fecal Calprotectin in colicky infants. The aim of this study was to evaluate the fecal Calprotectin levels in infants with colic in comparison with normal infants.

Methods:

This case control study was performed on infants aged between 3 weeks to 4 months with diagnosis of colic, referred to the clinics of Moussavi hospital in Zanjan, Iran ,for a period of 6 months in 2019. This study has been approved by the ethics committee of Zanjan University of Medical Sciences (IR.ZUMS.REC.1398.191)

Infantile colic was clinically defined based on the Wessel criteria, which includes crying for more than 3 hours a day for more than 3 days a week and for more than three weeks. The case group was randomly selected from colicky infants aged 3 weeks to 4 months whose parents agreed to participate in the study. Patients with diagnosis of inflammatory bowel disease, infectious enterocolitis such as Shigella and Salmonella, and parental dissatisfaction were excluded from the study. The control group was selected from healthy infants without colic who were referred to the clinics for control and were willing to be tested. They were matched with the cases in terms of age, sex, weight and type of nutrition. Stool samples were taken in both groups to measure the level of calprotectin in the specimen. Fecal calprotectin measurement was performed by Calprest-Dynex Elisa laboratory kit at Ayatollah Mousavi Hospital. The cutoff point of ≥ 120 (µg/g) was considered positive.

Finally, the Calprotectin levels along with the demographic information were entered into the SPSS V22 software and analyzed. Normal data distribution was investigated using the Kolmogorov-Smirnov test. The independent T test was used to compare quantitative variables. Qualitative data were compared using chi square or Exact Fisher's test. $P < 0.05$ was considered statistically significant.

Results:

Forty infants (75.5% boys and 42.5% girls) were equally divided into two groups. In the colic group, 11 boys (55%) and 9 girls (45%) had a mean age of 63 days and weighed between 3700 and 6500 g. In the control group there were 12 boys (60%) and 8 girls (40%) with mean age of 48 days and their weight ranged from 3400 to 7200 g. The mean head circumference of colicky patients and controls were 38.32 ± 1.28 and 37.62 ± 1.31 respectively and the difference between the two groups was not statistically significant ($p = 0.095$). There were no significant difference regarding sex ($p = 0.749$), age ($p = 0.514$), weight ($p = 0.603$), and type of nutrition ($p = 0.887$) between the two groups.

Fecal calprotectin was positive in 80% of the colic group and 30% of the control group. The Chi-Square (Ch2) test showed that fecal calprotectin was significantly higher in infants with colic compared to the control group (p□0.001). Table 1 shows the relation between fecal Calprotectin and sex, age, weight and type of nutrition in both groups.

Table 1
comparison of fecal Calprotectin in cases and controls.

Variable		Colicky infants		pvalue	Controls		pvalue
		Positive Calprotectin	Negative calprotectin	0.660	Positive Calprotectin	Negative calprotectin	0.690
Sex	Boy	9 (81.8%)	2 (18.2%)		4 (33.3%)	8 (66.7%)	
	Girl	8 (88.9%)	1 (11.1%)		2 (25%)	6 (75%)	
Age	□ 1 month	5 (100%)	0 (0%)	0.99	2 (33.3%)	4 (66.7%)	0.359
	1–2 months	7 (100%)	0 (0%)		1 (14.3%)	6 (85.7%)	
	2–3 months	2 (50%)	2 (50%)		2 (33.3%)	4(66.7%)	
	3–4 months	3 (75%)	1 (25%)		1 (100%)	0 (0%)	
Weight	Low weight	4 (66.7%)	2 (33.3%)	0.316	2 (25%)	6 (75%)	0.788
	Normal Weight	12 (92.3%)	1 (7.7%)		1 (30%)	7 (70%)	
	Over Weight	1 (100%)	0 (0%)		1 (50%)	1 (50%)	
Nutrition	Breast milk	11 (84.6%)	2 (15.4%)	0.798	3 (25%)	9 (75%)	0.316
	Formula	2 (100%)	0 (0%)		2 (66.7%)	1 (33.3%)	
	Mixed	4 (80%)	1 (20%)		1 (20%)	4 (80%)	

As shown in Table 1, the Chi-Square (Ch^2) test showed there was no significant difference in fecal calprotectin regarding sex, age, weight and type of nutrition in both colicky infants and controls.

Discussion:

This study was designed to evaluate the fecal Calprotectin levels in infants with colic compared to normal infants. Our results showed that fecal Calprotectin was significantly higher in colicky infants compared to

controls but there was no significant difference in fecal calprotectin regarding sex, age, weight and type of nutrition in both groups.

Although the exact etiology of colic is not yet known [12], studies suggest that gut inflammation may play a major role in this issue [13]. Rhoads et al. exploring the pathophysiologic mechanism of colic, studied intestinal flora changes in 19 infants with colic versus 17 healthy infants and showed that the levels of fecal calprotectin in infants with colic were 2 times higher than in healthy infants [14]. A study conducted by Shahramian et al. in 2018 examined the level of fecal calprotectin in 100 infants and concluded that Calprotectin was significantly higher in the stool of colicky infants [15]. Rhoads et al. studied the factors influencing infant bowel inflammation in the United States in 2018. A total of 65 infants were included in the study, 37 of whom had colic and the rest were healthy. The results of this study showed that fecal calprotectin levels in colicky infants were significantly higher than healthy infants [13]. The concept of alterations in the gut microflora emerged several dietary interventions [12] A study by Savino et al. in 2015 evaluated the effect of treatment with Lactobacillus ruteri on the levels of calprotectin and the crying time in infants with colic. Forty-three infants, including 25 receiving probiotics and 18 receiving placebos were studied. The results of this study showed that fecal calprotectin levels were significantly higher in the colic group compared to controls and fecal Calprotectin as well as crying time decreased significantly after treatment with L Reuteri [16].

The results of these studies are consistent with our study and suggest that fecal calprotectin is a good diagnostic and predictive agent for infantile colic. Calprotectin is secreted from the cytoplasm of neutrophils into the intestinal lumen during inflammatory and infectious conditions. As a result, fecal calprotectin is an indicator of inflammation in the gastrointestinal tract [17, 18]. Calprotectin lasts for a week in the stool which makes it a good marker for detecting inflammation in the gut [17, 19]. However, other studies with different results have been reported. In the study of Olafsdottir, there was not any significant difference in fecal Calprotectin of colicky infants compared to healthy babies and the level of Calprotectin decreased significantly with age [20].

The results of our study showed that both in the infants with colic and in controls, fecal calprotectin levels did not differ with sex. Similar results are found in other studies [9, 15, 20].

Our findings showed that the increase in age did not make a difference in the level of fecal calprotectin in the two groups. Shahramian and Asgarshirazi found the same results [9, 15], but Olafsdottir concluded that calprotectin levels is high in infancy and decreases with age [20]. In a study by Dorosko et al. in the United States, infants were assessed for the level of fecal calprotectin depending on their diet. In this study, 77 fecal samples were collected from 32 infants 3 days to 6 months of age. Fifteen infants were exclusively breastfed and 21 infants had a mixed diet. The results of this study showed that increasing age significantly reduced the level of calprotectin [21]. This study is not consistent with our study.

We concluded that infant's weight had no effect on fecal calprotectin levels. Some studies have shown the same result [15]. In the study of Karabayir et al. on 70 infants, fecal Calprotectin was significantly

higher in coliky infants compared to controls and similar to our study they showed that infant's birth weight had no effect on fecal calprotectin [22].

In our study there was no significant difference between calprotectin levels regarding dietary patterns. This finding is consistent with some studies [13, 17, 22].However, the results of Asgarshirazi's study showed that breastfed infants had significantly higher levels of calprotectin in their feces (9). Drosko studied 15 infants with exclusively breastfeeding and 21 infants with a combination feeding. The results of this study also showed that exclusive breastfeeding significantly increased fecal calprotectin levels [21].

Although the exact role of calprotectin is not yet known, its antibacterial and antifungal role has been proven in some studies [17, 23]. On the other hand, the secretion of calprotectin increases in cases of inflammation and intestinal infection. More researches are needed to determine the exact role of calprotectin.

Conclusion:

In conclusion, the present study demonstrated that fecal Calprotectin of colicky infants is significantly higher and therefore an inflammatory process should be considered in the etiology of this disease. By conducting well designed clinical trials to eliminate inflammatory agents, we may achieve brilliant results in treating the disease.

Abbreviations

ELISA
enzyme-linked immunosorbent assay

Declarations

Ethics approval and consent to participate

This study has been approved by the ethics committee of Zanjan University of Medical Sciences (IR.ZUMS.REC.1398.191)

written informed parental consent was obtained to participate in the study.

Consent for publication

Not applicable

Availability of data and materials :

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors have no conflicts of interest relevant to this article to disclose.

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Authors Contributions

Khoshnevisasl & Sadeghzadeh conceptualized and designed the study, critically reviewed and revised the manuscript. Mahmoudi rad undertook data collection and critically reviewed the manuscript. Motamed contributed to analyses and data interpretation. All authors approved the final manuscript.

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