

Clinical Relevance of Faecal Calprotectin Level in Infantile Colic: A Cross-sectional Survey

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ABSTRACT

Background: There is limited knowledge on the potential applicability of fecal calprotectin (f-CP) as an inflammatory screening parameter in infantile colic (IC). This study aimed to evaluate f-CP in neonates with IC as a useful diagnostic indicator regarding this condition.

Methods: The present study was conducted on 100 cases, including 50 newborns with IC and 50 non-colicky neonates. The diagnosis of IC was fulfilled by the Wessel Criteria. The level of f-CP was determined by a specific enzyme-linked immunosorbent assay kit (Calprotectin ELISA, EuroImmun, Germany). The statistical analysis was performed in SPSS software (version 19).

Results: Out of 100 neonates, 57 cases were male and 43 subjects were female. The age spectrum ranged from 19-90 days (40.4±15.9). Colicky newborns were slightly younger ($P=0.06$) with higher birth weight compared to the infants without colic ($P<0.0001$). The level of f-CP was significantly higher in colicky neonates (113.7±98.2 µg/g) than non-colicky cases (71.4±45.5 µg/g) ($P=0.007$). Overall, 37%, 30%, 26%, and 7% of the newborns showed f-CP levels <50, 50-100, 100-200, and >200 µg/g, respectively. There was a significant difference regarding the distribution of these f-CP categories between neonates with IC and the cases without IC ($P=0.02$). There were no significant correlations between the f-CP and newborn age, pregnancy age, present or birth weights, and number of pregnancies. Receiver operating characteristic analysis rendered an area under the curve of 0.642 (95% CI: 0.534-0.748) ($P=0.01$). At the cut-off value of 74 µg/g, f-CP showed sensitivity of 60% and specificity of 59% for the detection of IC.

Conclusion: The results of this study revealed that the f-CP might be useful in the diagnosis of the IC.

Keywords: Calprotectin, Infantile colic, Intestinal inflammation, Leukocyte L1 antigen complex

Introduction

Calprotectin (CP) is an inflammatory mediator released by activated neutrophils during infectious and inflammatory conditions. With the estimated ratio of 60% of cytosolic proteins and 5% of total proteins, the CP constitutes a major compartment of neutrophil cytosol (1-4). In inflammatory states, the CP appears in gastrointestinal tract (GI) and stool. The levels of the fecal calprotectin (f-CP) have been elevated in childhood inflammatory bowel disease (5, 6), constipation (5) Crohn's disease (7), cystic fibrosis (8), as well as gastrointestinal

malignancies (9).

Moreover, the levels of this protein have been associated with non-specific inflammatory markers, such as erythrocyte sedimentation rate and C-reactive protein in intestinal inflammation (2, 5). Establishment of inflammation in intestine control methods in intestine requires visualization by endoscopy, which is an invasive procedure in pediatric and very young patients (2). On the other hand, the f-CP has shown promising evidences for becoming a reliable indicator of intestinal inflammation (10, 11).

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Infantile colic (IC) is a common ailment during infancy period. This is defined as excessive crying of the neonates without any apparent reason during the first three months of life. Although the IC is generally resolved with no need for serious clinical intervention, its psychological and financial impacts on families are remarkable (12). The etiology of the IC is not well characterized; nevertheless, intestinal inflammation seems to be a constant feature. The Role of the f-CP as an inflammatory marker in the IC has not been sufficiently addressed with few reports available on clinical usefulness of this protein in the IC. The present study assessed the potential role of the CP as a diagnostic marker in the IC.

Methods

This cross-sectional study was performed from March to December in 2017 in Pediatric Gastroenterology Clinic of Amir-Al-Momenin Hospital in Zabol, Sistan and Baluchestan province, Iran.

Study Population

50 Colicky and 50 non-colicky neonates were enrolled from outpatient clinic. The Wessel diagnostic criteria was used for identifying newborns with the IC (13). The criteria indicate excessive crying more than 3 h per day, for three days per week and for three weeks as a diagnostic recommendation for the IC. Accordingly, the neonates with less than 3 h of crying per day were recruited as the control group (14).

Sample size calculation

The sample size was estimated using the following equation:

$$N = \frac{(r+1)(Z_{\alpha/2} + Z_{1-\beta})^2 \sigma^2}{rd^2}$$

in which "N" indicated the total sample size in each group, "r" presented the ratio between the size of groups (considered 1), $Z_{\alpha/2}$ was considered as 1.96 (for 5% of significance level), and $Z_{1-\beta}$ was 0.84 (for 80% of power). The standard deviation (σ) was considered 71 $\mu\text{g/g}$ according to Rhoads et al. (14) for the detection of mean difference of the f-CP level between groups as 40 $\mu\text{g/g}$.

Exclusion criteria

The exclusion criteria were the neonates with apparent clinical illnesses, and the cases with the

symptoms of diarrhea, vomiting, very low birth weight (i.e., lower than 1800 gr), pre-term newborns (i.e., the gestational age of less than 36 weeks), no history of antibiotics treatment, and the subjects with fever.

Fecal calprotectin measurement

The level of f-CP was determined using a specific enzyme-linked immunosorbent assay (ELISA) kit (Calprotectin ELISA, EuroImmun, Germany) according to the manufacture instructions. Fecal specimens were put into sterile plastic containers with appropriate caps. The specimens were stored in -20°C until use. The preparation of the specimens was conducted as recommended by the kit provider.

Statistical analysis

The statistical procedures were performed in SPSS software (version 19). The descriptive statistics (i.e., means, standard deviations, and frequencies) were used to present the basic characteristics. Kolmogorov-Smirnov test was utilized to assess the normality of the data. The relation between categorical variables was checked by chi-square test. Mann-Whitney U test was used to evaluate any significant differences in the means of the CP between colicky and non-colicky neonates. Receiver operating characteristic (ROC) curve was utilized to assess diagnostic applicability of the f-CP in the IC.

Results

Out of 100 neonates, 57 cases (57%) were male and 43 subjects (43%) were female. The age of the neonates ranged from 19-90 days (40.4 ± 15.9 days). 62 mothers (62%) underwent vaginal delivery and 38 (38%) mothers had C-section. The mean of pregnancy age was 38.1 ± 1.3 weeks. From all neonates, 63 (63%), newborns were breast-fed, while 9 (9%) cases and 27 neonates (27%) had formula feeding and breastfeeding along with formula feeding, respectively. Family history of inflammatory bowel disease (IBD) was present in 33 (33%) cases. The mean scores of birth weight and present weight were 3.21 ± 0.62 Kg and 5 ± 5.08 Kg, respectively.

The IC was not related with gender, feeding pattern, or delivery route. Nevertheless, colicky cases were younger ($P=0.06$) with higher birth weight than subjects without colic history ($P<0.0001$). The mean of pregnancies was significantly higher in mothers that gave birth to the colicky neonates than mothers with non-colicky newborn ($P<0.0001$). Ultimately, 33 cases

Table 1. Relation of infantile colic with basic mother and infant characteristics

Parameters		Colicky Neonates N=50 N (%)	Non-colicky Neonates N=50 N (%)	P-value
Gender	Female	21 (42)	22 (44)	0.5
	Male	29 (58)	28 (56)	
Age (days±SD)		36.7± 12.3	42.3±17.3	0.06
Birth weight (Kg±SD)		3.5± 0.54	3±0.6	<0.0001
Delivery	Cesarean	20 (40)	18 (36)	0.4
	Vaginal	30 (60)	32 (64)	
Number of pregnancies	1-2	30 (60)	36 (72)	0.5
	3-4	15 (30)	11 (22)	
	5-6	5 (10)	3 (6)	
Mean number of pregnancies (±SD)		3± 1.2	1.7± 1	<0.0001
Feeding pattern	Breas-fed	34 (68)	30 (60)	0.5
	Formula-fed	3 (6)	6 (12)	
	Mixed	13 (26)	14 (28)	
Family history of IBD	Yes	33 (66)	0 (0)	<0.0001
	No	17 (34)	50 (100)	

IBD: inflammatory bowel disease

Table 2. Distribution of fecal calprotectin categories comparing colicky and non-colicky neonates

Categories of Fecal calprotectin		Colicky Neonates N=50 N (%)	Non-colicky Neonates N=50 N (%)	P-value
FC (µg/g)	<50	15 (30)	23 (46)	0.02
	50-100	17 (34)	14 (28)	
	100-200	11 (22)	13 (26)	
	>200	7 (14)	0 (0)	

out of 50 (66%) colicky infants had family history of IBD, while none of the subjects in control group stated a family history of IBD ($P<0.0001$) (Table 1).

The mean level of the f-CP was 92.56 ± 79.12 µg/g with a range of 5-478 µg/g. The level of f-CP was significantly higher in colicky cases (113.7 ± 98.2 µg/g) than that in non-colicky subjects (71.4 ± 45.5 µg/g) ($P=0.007$). Overall, 37 (37%), 30 (30%), 26 (26%), and 7 (7%) of the neonates showed f-CP levels lower than 50 µg/g, 50-100 µg/g, 100-200 µg/g, and more than 200 µg/g, respectively. There was a significant difference regarding the distribution of the f-CP categories between neonates with IC and the cases without IC (Table 2).

Among the colicky neonates, there was no significant difference in the mean level of the f-CP regarding the presence or absence of history of IBD in the family (Figure 1). There were no significant correlations between the f-CP level with the age of the newborn, pregnancy age, present or birth weights, and the number of pregnancies. ROC analysis rendered area under curve of 0.642 (95% CI: 0.534-0.748) ($P=0.01$)

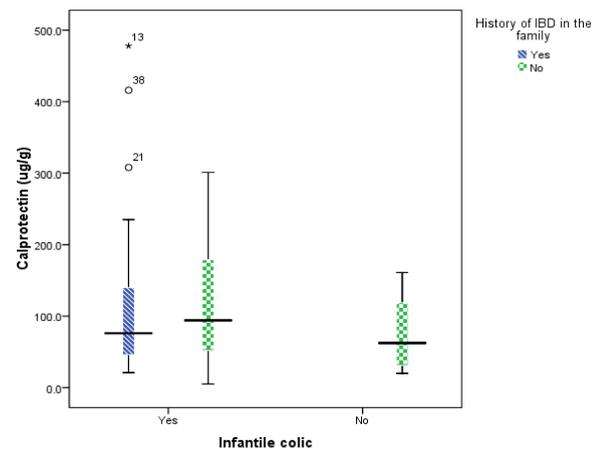


Figure 1. Fecal calprotectin level according to the presence or absence of infantile colic and family history of IBD, no significant relation, representing no neonate in non-colicky group with familial history of IBD

(Figure 2). At the cut-off value of 74 µg/g, the f-CP showed sensitivity of 60%, specificity of 59%, positive predictive value (PPV) of 36.7%, and negative predictive value (NPV) of 71.2%.

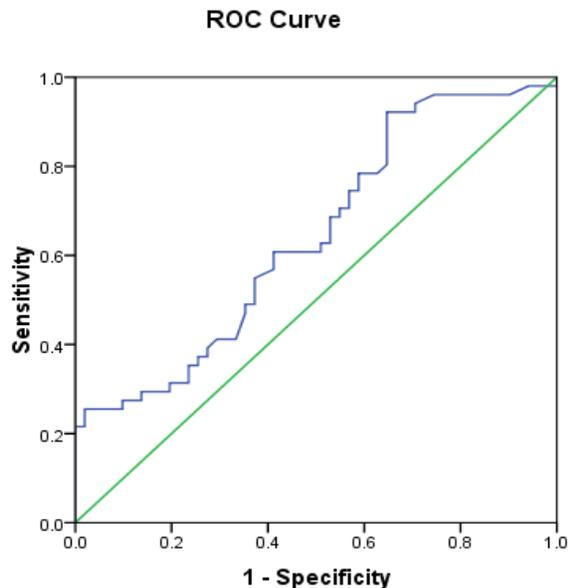


Figure 2. Receiver operating characteristic (ROC) curve analysis of fecal calprotectin for diagnosis of IC, area under curve: 0.642 (95% CI: 0.534-0.748)

Discussion

The CP is originated from the cytoplasm of neutrophils and released into gastrointestinal lumen during inflammatory and infectious conditions (1, 10). Accordingly, the f-CP is a representative of inflammatory conditions in the GI tract. The stability of the f-CP in stool specimens is nearly one week delivering this marker as a reliable indicator of both present and recent inflammation within the GI tract (1, 15).

In the present study, significantly higher levels of the f-CP were detected in colicky neonates ($113.7 \pm 98.2 \mu\text{g/g}$) than non-colicky newborns ($71.4 \pm 45.5 \mu\text{g/g}$). In another investigation on the f-CP levels in the IC, the mean level was reported as $413 \pm 71 \mu\text{g/g}$, which was higher than that ($197 \pm 46 \mu\text{g/g}$) in non-colicky cases (14).

Although the findings of present study are in line with the abovementioned study regarding higher levels of f-CP in IC, the results of this study demonstrated lower levels of the protein. Comparable age and weight spectrums were found in the comparison of the population characteristics between the two studies; however, a significant ratio of the neonates in a study performed by Rhoads et al. (52% in colic group and 47% in control group) had been fed with cow milk, which might be the reason for higher f-CP in their population (14). On the other hand, Bremner et al. noted that the f-CP was significantly correlated with disease activity in children with

ulcerative colitis (5). This is also in line with previously noted higher levels of the f-CP in conditions with abdominal pain and inflammation (16).

Similar to the results of the present study, no relation was reported in the other studies between the f-CP level and pregnancy age or birth weight (17-19). Furthermore, no association was observed between the f-CP with the gender or age of the patients with cystic fibrosis (8, 10). Accordingly, no association has been reported between the f-CP level and the gender in healthy newborns (1).

No correlation was identified between the gestational age or birth weight and the f-CP in pre-term subjects (11). In line with the findings of this study, which revealed no association between the f-CP level and feeding patterns, no significant impact was noted on the f-CP level regarding feeding with prebiotic formula, regular formula, or breastfeeding in neonates during the first month of life (1).

In addition, it was noted that there was no significant difference in the f-CP level concerning the presence or absence of a familial history of IBD. In contrast, it has been mentioned that the f-CP could be significantly influenced regarding the type of delivery (18, 20), age of the neonates (18, 20-22), feeding pattern (20, 21, 23, 24), birth weight (11, 25), as well as history of antibiotics administration and microbial flora (14, 18). Nevertheless, high variation in the f-CP among the neonates might contribute to such differences (18, 20).

It seems that some of these factors (i.e. nutritional status, weight, age, term or pre-term, and antibiotics administration) might directly or indirectly impact the development of intestinal flora and intestinal immune compartments. Therefore, a multifactorial interaction between these factors seems necessary to counterbalance progression of a low-grade inflammatory status in intestine and the level of f-CP.

One possible approach to investigate the problem of high variations of the f-CP could be considered cut-off diagnostic values for the f-CP. In the present study, ROC curve analysis showed an AUC of 0.642 ($P=0.02$) indicating a potential role for the f-CP used as a diagnostic marker for the IC. It was noticed that the f-CP level of $74 \mu\text{g/g}$ delivered 60%, 59%, 36.7%, and 71.2% sensitivity, specificity, PPV, and NPV for diagnosis of IC, respectively.

Compared to this, the f-CP cut-off value of $363 \mu\text{g/g}$ was described with 65% of sensitivity and 82% of specificity for the diagnosis of mild and

severe enteropathy in pre-term neonates (11). The f-CP lower than 50 µg/g was suggested to rule out histological problems in children (26). Before the establishment of such diagnostic cut-off value for the f-CP in newborns, one should consider the effects of some factors that might affect the level of this marker such as phototherapy (27), the concentration of pancreatic digestive enzymes within the intestine (28), anemia and history of red blood cell transfusion (29), hyperbilirubinemia (30), and preparing measures for colonoscopy (31). More studies are warranted to establish a reliable cut-off value for the f-CP to diagnose the IC.

Conclusion

Based on the results, it was revealed that the f-CP might be useful in the diagnosis of the IC. However, the cut-off value for this should be adjusted considering a multifactorial approach, including both maternal and neonatal features. The Specificity of calprotectin for the detection of the IC was limited and it can restrict the applicability of the test as a definite diagnostic marker; nevertheless, measuring the CP in neonates with non-specific abdominal symptoms can obviate the requirements for invasive procedures in the cases with normal CP levels.

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Conflicts of interests

None declared.

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