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Fecal Microflora and Calprotectin in Infants with Colic

Soheir B. Fayed*; Radwa S. Ibrahim; Marwa Elhady*; Manal Tantawy***.**
*Pediatric department, **Clinical pathology department, Faculty of Medicine for girls -Al Azhar University,
***Pediatric department, Atfeeh central hospital, Egyptian ministry of health.
Corresponding author: **Manal Tantawy**, Email: dr_manaltantawy@yahoo.com

ABSTRACT

Background: Colic is a common distressing problem of infancy. The underlying etiology has not determined yet, however several hypotheses were suggested including gastrointestinal inflammation and disturbed gut microflora. The aim of this study was to explore the role of gut microorganism and fecal calprotectin in infantile colic.

Methods: Ninety healthy infants aged 14 to 90 days were included. Forty five of them have infantile colic. Detailed medical history and examination in addition to stool culture and measuring fecal calprotectin level by enzyme linked immunosorbent assay (ELIZA) were done for all included infants.

Results: Colicky Infants have significant higher rate of Escherichia coli infection than non-colicky infants (57.8% vs 17.8%) do. Fecal calprotectin was significantly higher in colicky Infants than non-colicky infants and in infants with Escherichia coli infection than non-infected infants. Vomiting, umbilical hernia, maternal stress, previous sibling with infantile colic, increased body weight and herbal intake were significantly associated with infantile colic. Types of feeding in first 3months of life were not related to infantile colic, E-coli infection and fecal calprotectin level. Regression analysis revealed that elevated fecal calprotectin, Escherichia coli infection, vomiting and higher weight were the main predictors for infantile colic.

Conclusion: Fecal calprotectin level and Escherichia coli infection are increased in colicky infants reflecting the role of gastrointestinal inflammation and infection in infantile colic. Advice the mothers to avoid stressful conditions, over feeding or introduction of any remedy food during the first 3 months of life.

Keyword: Fecal calprotectin, Escherichia coli, infantile colic, feeding.

INTRODUCTION

Infantile colic was originally defined by Wessel in 1954 as crying lasting three or more hours a day, at least 3 days a week and for at least 3 weeks [1]. In 2006, the Rome III criteria defined it as 'episodes of irritability, fussing, or crying that begin and end for no apparent reason and last at least 3 h a day, at least 3 days a week, for at least 1 week'[2]. The incidence of infantile colic varies between 5 and 30%, and it is reported to occur equally frequent in breastfed and bottle-fed infants and in both sexes [3].

The aetiology of colic is multifactorial. Multiple hypotheses have been proposed, including alteration in fecal microflora and higher levels of fecal calprotectin as a marker of gastrointestinal inflamation [4,5].

Recent advances in genetic sequencing technologies have provided a greater understanding of the molecular underpinnings of human microbial habitats and their interactions with host systems [6] . Of particular interest to infantile colic is the finding that microbes involved in intestinal colonization during the neonatal period demonstrate bidirectional communication with host cells to significantly impact and shape the immune system and inflammatory response [7]. The exact mechanism of how imbalanced colonization of the intestines by noncommensal bacteria, or intestinal dysbiosis, may contribute to symptoms of infantile colic is not fully understood. However, researchers have proposed that the presence of pathogenic bacteria in high numbers induces a chronic inflammatory response. In turn,intestinal epithelial tissue releases cytokines and chemokines both locally and systemically that further mediate immune cell migration and inflammation as well as sensitization of local nerve tissues [8].

Because of the much theories of infantile colic, it had been centered on the gastrointestinal habitat as a likely etiology, the potential influence of intestinal dysbiosis presents a plausible explanation for the expression of infantile colic symptoms and warrants further exploration.

Calprotectin, a calcium and zinc binding protein that belongs to the s100 protein family and is derived predominantly from neutrophils and, to a lesser extent, from monocytes and reactive macrophages .The functions of calprotectin are associated with the regulation of inflammation and apoptosis as well as

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its potent antibacterial, antifungal and anti-proliferative activities [9]. Fecal Calprotectin is a biochemical measurement of the protein calprotectin in the stool. Elevated fecal calprotectin indicates the migration of neutrophils to the gastrointestinal mucosa, which occurs during gastrointestinal tract inflammation, regardless of localization of inflammation in GIT. Fecal calprotectin is found elevated in children with various GI infections [10].

In pediatric patients, fecal calprotectin helped to distinguish inflammatory bowel disease from nonorganic disease, with a sensitivity of 89% and a specificity of 79% for identifying organic disease [11].However, the factors influencing calprotectin values in stool are not fully understood [12].

There are only a few studies investigating the relation between intestinal flora, fecal calprotectin and infantile colic with contradictory findings .There for **the aim of our work** was to determine factors associated with infantile colic and to clarify the role of gut microflora and fecal calprotectin in infantile colic.

METHODS

This cross sectional case control study was conducted on 90 infants aged from 14 to 90 days. They were divided into 2 groups; group I: forty five patients with colic according to Rom III criteria [2] and group II: forty five healthy babies age and sex matched as control. The studied infants were randomly selected from Pediatric Out Patients Clinic, Al Zahraa University Hospital and El-saff Primary Health Care Unit during the period from april 2016 to december 2016.

The study was approved by the Ethics Board of Al-Azhar University.

All studied infants were subjected to the following : complete history taking according to a specially desinged questionnaire with stress on crying, irritability and fussing time, through clinical examination lying stress on abdominal examination and labaratory investigations including : stool culture and measuring fecal calprotectin level by enzyme linked immunosorbent assay (ELIZA).

Data analysis

Data were analyzed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 21 (SPSS Inc., Chicago, IL). Numerical data were described as mean and standard deviation or median and range. Categorical data were described as numbers and percentages. Data were explored for normality using Kolmogrov-Smirnov test and Shapiro-Wilk test. Comparisons between two groups for normally distributed numeric variables were done using the independent Student's t-test. None normally distributed numeric variables were done by Mann-Whitney test. Comparisons between categorical variables were performed using the chi square test or fisher exact test as appropriate. To measure the independent effect of all factors that affect infantile colic, factors which had significant level ≤0.100 were selected to enter into stepwise logistic regression.A p-value less than or equal to 0.05 were considered statistically significant. All tests were two tailed.

RESULTS

The age of the studied infants ranged between 14 and 90 days with the mean age was (6.07 ± 3.54 and 6.2 ± 2.77) weeks for colicky and control groups respectively (table 1).

As regard sex distributions of the infants in our study, among 45 infants of colicky group, no sex predominance was found in relation to colic (table 1)

There was statistically significant difference between colicky and control groups as regard exposure of the mother to stress and positive family history of colic in previous sibling, herbal drink intake, prolonged crying and fussing time, presence of vomiting, prescence of umbilical heria, positive stool culture and higher level of fecal calprotectin (figure 1), while no differences between the two groups regarding other variables (table 1).

Table (1): Comparison between colicky and non-colicky infants as regard demographic data,feeding type,clinical manifestation and labaratory investigation:

Variables		Case (N=45)	Control (N=45)	P value
Gestational age (weeks)	,Mean ± SD	38.6 ± 1.0	38.7 ± 0.8	0.845
Age (weeks)	,Mean ± SD	6.07 ± 3.54	6.2 ± 2.77	0.897
Sex:	• Male	23 (51.1%)	23(51.1%)	1
	• Female	22 (48.9%)	22(48.9%)	
Mode of birth:	• CS	26 (57.8%)	25 (55.6%)	0.832
	• NSVD	19 (42.2%)	20 (44.4%)	
Order of birth :	• First born	14 (31.11%)	10 (22.22%)	0.346
	• others	31 (68.89%)	35 (77.78%)	
Birth weight (kg)	,Mean ± SD	3.21 ± 0.38	3.1 ± 0.25	0.146
Maternal age (years)	,Mean ± SD	28.6 ± 4.4	28.7 ± 4.8	0.737
Maternal illness	• Yes	3 (6.7 %)	2 (4.4 %)	1
	• No	42 (93.3%)	43 (95.6%)	
Maternal stress	• **Yes**	24(53.3%)	0 (0%)	<0.001
	➢ Family troubles	12 (50%)		
	➢ Lack of family support	3 (12.5%)		
	➢ Surgical stress	9 (37.5%)		
	• **No**	21 (46.7%)	45(100%)	
Maternal exposure to antibiotics	• Yes	2 (4.4 %)	2 (4.4 %)	1
	• No	43 (95.6 %)	43 (95.6 %)	
Family history of previous sibling with colic	• Positive	17 (54.84 %)	8 (22.86%)	0.008
	• Negative	14 (45.16 %)	27 (77.14%)	
Feeding type	• Breast	32(71.1%)	36(80.0 %)	0.483
	• Formula	3(6.7%)	1(2.2%)	
	• Both	10(22.2%)	8(17.8%)	
Herbal drink:	• NO	36(80%)	18(40%)	0.038
	• YES	103.9 ±45.8	27(60%)	
Crying Time (Min.)	Mean ± SD	201.7±85.7	29.4±9.1	<0.001
Fussing Time (Min.)	Mean ± SD	305.6±114	40.7±13.8	<0.001
crying and fussing time (Min.)	Mean ± SD	4.64±0.89	69.4±19.9	<0.001

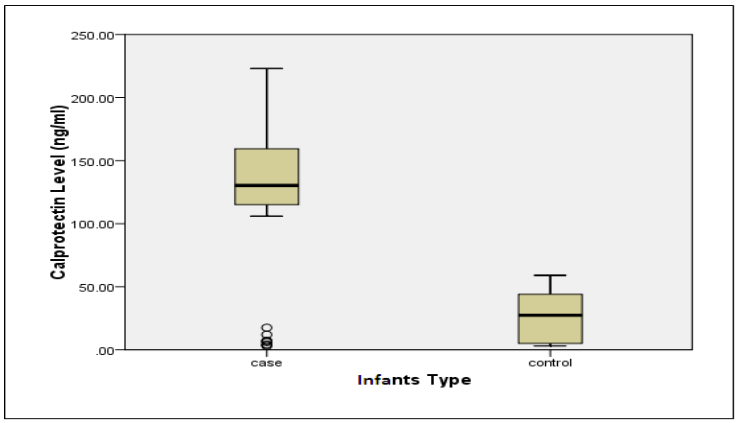

Figure (1): comparison between case and control infants as regard the fecal calprotectin level.

There was statistically significant association between higher fecal calprotectin level and E-coli infection (table 2, figure 2)

Table (2): Comparison between negative and positive stool culture infants as regard fecal calprotectin level.

Variables	Stool Culture						P value
	Normal			E coli			
	Median	Minimum	Maximum	Median	Minimum	Maximum	
Fecal calprotectin (ng/ml)	38.95	3	223	123.55	3	184.6	0.004

Figure (2): comparison between normal and E-coli stool culture results as regard the fecal calprotectin level.

There was no statistically significant association between feeding type and E coli infection (table 3) or feeding type and fecal calprotectin level (table 4).

Table (3): Comparison between negative and positive stool culture infants regarding feeding type

Variables		Negative (N=56)		Positive (E coli)(N=34)		P value
		Number	%	Number	%	
Feeding type	**Breast feeding**	43	76.80%	25	73.50%	0.723
	Formula	3	5.40%	1	2.90%	
	Both	10	17.90%	8	23.50%	

Table (4): Comparison between feeding types groups and fecal calprotectin level.

Variables	Breast feeding			Formula / or mixed			P value
	Median	Minimum	Maximum	Median	Minimum	Maximum	
Fecal calprotectin (ng/ml)	46.5	3	223	81.7	3	215	0.645

Table (5): Predictors of infantile colic based on clinical and laboratory parameters.

Variables	OR (Odds Ratio)	95% CI for OR		P value
		Lower	Upper	
Weight	**2.2**	**1.0**	**4.6**	**0.039**
Fecal calprotectin	**1.2**	**1.1**	**1.6**	**<0.001**
vomiting	**28.3**	**3.2**	**246.9**	**0.002**
Stool culture	**7.8**	**1.7**	**36.2**	**0.009**
Constant	**0.0**			**<0.001**

OR=Odds Ratio, 95% CI for OR = 95% confidence interval for the =Odds Ratio. P-value≤0.05 is considered significant

To study the effect of factors on infantile colic, all significant variables were entered into a stepwise logistic regression. The factors independently affecting the infantile colic were weight, fecal calprotectin, vomiting and stool culture results.

- Weight; with every increase 1 kg in infant weight there was 2.2 increase in probability to have infantile colic (95% CI: 1.0-4.6)
- Fecal calprotectin; with every increase 1 ng/ml in the level of fecal calprotectin there was 1.2 increase in probability to have infantile colic (95% CI: 1.1-1.6)
- Infants with vomiting (less than 8 times/day) **[4]** were 28.3 times liable to have colic (95% CI: 3.2-246.9).
- Infants with E-coli in stool culture were 7.8 times liable to have colic than those with normal culture (95% CI: 1.7-36.2).

DISCUSSION

In the present study, there was no statistically significant difference between colicky and control groups regarding age, sex, gestational age, infants age, order of birth, mode of birth, birth weight, maternal age, maternal illness and maternal exposure to antibiotic (table 1).

Perinatal stress had been shown to modulate the intestinal physiology by increasing gut permeability as well as influencing the microbiota composition**.** Furthermore, maternal stress during pregnancy had been found to be associated with the development of colic, suggesting possible causality between perinatal stress, gut microbiota and colic [13].

In the current study, the maternal stress in the form of family troubles, lack of family support and surgical stress was significantly higher in colicky than in non-colicky group (table 1). As maternal stress, might affect gut microbiota that may be contributing to infantile colic. The mothers with perinatal stress were more likely to have babies with dysbiosis or gut bacteria imbalance with more proteobacteria (pathological bacteria) and fewer lactic acid bacteria (beneficial bacteria) in their GI tract. This means that they had a low diversity of beneficial bacteria. The presence of these bacteria is correlated with having a higher incidence of infantile colic. This was supported by **De weerth** and **Petzoldt** [14, 15] who found that the rate of infantile colic was higher among mothers with perinatal stress.

A complex interaction exists between colic and family dynamics, which are affected by pre- and postnatal factors. Families with colicky infants had more problems in family structure, functioning, and affective state. Associations between colic and dissatisfaction in the marital relationship, parental perception of stress, lack of parental self-confidence during the pregnancy, dissatisfaction with the delivery and levels of family stress have been reported [16].

The positive family history of previous sibling with infantile colic in our study was significantly higher in colicky than non-colicky group (table 1). This may be due to sharing same circumstances, which contribute to infantile colic like feeding malpractice, stressful condition to mother due to the close relation between

infant and mother as primary caregiver. Infantile colic seems to emerge in a dynamic interplay that can exacerbate to a vicious circle. Family stress, maternal anxiety, and transmission of tension from the mother to the infant, have been investigated as one potential vulnerability or risk factor [15].

This finding is in agreement with the study done by **Reinthal** [17] who found that 59% of the siblings of the affected children also had symptoms when they were infants.

This study showed that colic was not significantly associated with the type of feeding of the infants in the first 3 months of life (table 1). There was an evidence suggesting that breast-feeding *per se* had not been shown to provide a protective effect on the development of infantile colic, and the incidence of infant colic was similar among formula- and breast-fed infants [18].

This finding is in agreement also with **Rhoads and Asgarshirazi** [4, 19] who found that colic was not significantly associated with the type of feeding in the first 3 months of life.

In the present study, we found that herbal drink intake was significantly higher in colicky group than in non-colicky group (table 1). This may be related to contamination of herbal drink with bacteria, toxins, or particulate matter; unlabeled ingredients, such as alcohol, which leads to disturbance of the normal flora [20].

In the current study, colicky infants have prolonged crying and fussing time of 305.6 min. per day while it was 69.4 min. in non-colicky infants and this was statistically significant (table 1). This is in agreement with **Rhoads and De weerth** [4, 14] where the colic was significantly associated with prolonged crying and fussing.

In the present study, the weights of colicky infants were significantly higher than non-colicky infants (table 1). Stepwise logistic regression (table 5) showed that with every 1 kg increase in infant weight was associated with 2.2 increases in the probability to have infantile colic.

This finding may be due to the malpractice of over feeding and giving herbals sweetened by sugar as an attempt of the mother to calm the baby as most of mothers reflect infant cry as hunger. On the other hand, the study of **Rhoads** [4] found that infant weight was not significantly associated with colic.

The vomiting (less than 8 times) in our study was significantly associated with colic. 26.7% suffered from vomiting in colicky group versus 4.4% in non-colicky group (table 1) and in stepwise logistic regression infant with vomiting were 28.3 times liable to have colic (table 5). This may be due to malpractice of feeding such as over feeding which contributes to infantile colic. These finding indicated that vomiting might be related to infantile colic.

This study showed that the umbilical hernia in the infants was significantly associated with colic (table 1). This finding could be explained partially by the effect of strain, excess crying on increasing the intra-abdominal pressure predisposing these infants to umbilical hernia. Additionally, the presence of hernia leads to disturbed intestinal motility that exaggerates infantile colic.

There is accumulating evidence that the intestinal microbiota in infants with colic differs from that of healthy ones. In studies that were mostly based on traditional culturing approaches, the stools of colicky infants were found to display reduced diversity in microbiota, lower counts of lactobacilli and higher numbers of gram-negative bacteria. However, these reports described differences in infants already diagnosed with colic and usually of over 6 weeks of age [14].

In our study, the E-coli was significantly higher in colicky (57.8%) than in non-colicky (17.8%) group. This finding indicates that the colic was significantly associated with the presence of E-coli in stool of colicky infants (Table 1). Stepwise logistic regression showed that infants with E-coli infection were associated with 7.8 fold risk to have colic than those with normal culture (table 5). This finding could be explained by the fact that E coli bacteria are thought to be a possible cause of gut dysmotility and gas-forming and consequently to increase the intra-abdominal air load as well as aerophagia and pain, symptoms related to infantile colic [12].

Supporting our finding, **De weerth** [14] found that on studying bacterial content of stool of colicky and non-colicky infants aged 2 week, there was significant difference in the type of bacteria between both groups. Colicky infants had higher counts of gram-negative bacteria, especially coliform bacteria, mainly species belonging to Escherichia coli as compared to non-colicky infants. The association between colic and bacteria could be explained as early increased levels of pathogenic bacteria and reductions of lactobacilli, bifidobacteria or butyrate-producing bacteria leading to intestinal pain and inflammation in the infant, and this in turn causes excessive crying and colic.

This is in agreement also with previous studies done by **Savino** [12] who stated that there was significant association between E coli and infantile colic. **Rhoads,** [4] also reported that Klebsiella species were increased in colicky when compared to healthy infants.

Calprotectin is the only known antimicrobial manganese sequestration protein complex. Calprotectin comprises as much as 60% of the soluble protein content of the cytosol of a neutrophil, and it is secreted during inflammation. Increased translocation of granulocytes into the intestinal mucosa in conditions of inflammation might give increased levels of proteins from such cells in feces. [21]

In the current study, we investigated the relation between the calprotectin level and infantile colic of the infants aged from 14 to 90 days in colicky and non-colicky groups and we found that the calprotectin was significantly higher in colicky group (median 130 ng/ml) than in non-colicky group (median 27.4 ng/ml) as shown in table (1).

Additionally, Logistic regression showed that with every 1ng/ml increase in the level of fecal calprotectin there is 1.2 increases in probability to have infantile colic (table 5). This finding may be explained by the fact that the presence of calprotectin in feces is directly proportional to neutrophil migration towards the intestinal tract which reflects the presence of local intestinal inflammation and intestinal inflammations is one of the theories explaining colic [22].

Our results are in agreement with **Rhoads** [4] who found that colic was significantly associated with higher fecal calprotectin level.

Moreover, this study revealed that calprotectin levels were significantly higher in infants with E-coli infection than in infant with negative E coli culture (table 2).

It is important to consider whether an abnormal fecal flora is the cause of colic or colic is the result of the intestinal inflammation, caused by abnormal flora. However, a similar conundrum has existed to account for the abnormal flora seen in inflammatory bowel disease. Gut inflammation could produce differences in fecal osmolarity, pH, and concentration of luminal nutrients, variables that would affect the microbiota. Additionally, mucosally released inflammatory mediators such as interleukins, tumor necrosis factor-a, defensins, mucus, and trefoil factors could affect the composition of microflora, in the immunologically naïve gut of the neonate, high-level colonization by a few specific phylotypes could lead to an inflammatory response [23].

The deviations in gut microbiota composition might be one of the causes behind infant excessive crying and infantile colic. On the other hand, any inflammation such as food allergy or infection-related might alter gut microbiota colonization, and the possibility of an epiphenomenon must thus also be kept in mind, i.e. deviations in the gut microbiota might be cause or consequence of infant colic [24].

The current study showed that there was no significant association between positive stool culture of E-coli and feeding type (Table 3). This is in agreement with **Rhoads** and **de weerth** [4, 14] who addressed no correlation between the sources of nutrition and intestinal colonization of the newborns.

In the present study, the median level of calprotectin was higher in formula and mixed feeders (81.7ng/ml) than in breast feeders (46.5ng/ml) (table 4) however this difference was not statistically significant. This finding reflects that fecal calprotectin is mainly related to disturbed intestinal bacterial flora and inflammation but not to type of feeding.

This is in agreement with **Hayati** [25] who found that the mean of fecal calprotectin in formula fed group was higher than the mean of fecal calprotectin level in exclusive breast fed with no significant association between the level of calprotectin and feeding type.

In contrast to our finding**, Asgarshirazi** [19] found that the mean of fecal calprotectin in exclusive breast fed group was higher than the mean of fecal calprotectin level in formula fed with no significant association between the level of calprotectin and feeding type.

CONCLUSION

We observed that colicky infants had higher fecal calprotectin level and E-coli positive culture than non-colicky in the first 3 months of life. This may reflect the organic nature of infantile colic and the key role of pathological gut microbiota & intestinal inflammation in infantile colic although causality could not be assumed. We have identified an organism that might be pathologically linked to the condition: E- coli, which deserve further testing. Furthermore, we emphasized that the condition previously thought to be psychophysiological in origin may in fact be an inflammatory condition of the intestine of young infants and might be related to gut flora and calprotectin. The mechanism underlying the relationship between infantile colic & gut flora and

fecal calprotectin level in early infancy needs to be more clarified in further studies in light of advanced DNA technology.

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