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Comparison of the different kinds of feeding on the level of fecal calprotectin ☆

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ABSTRACT

Background: Controversial results have been reported on the effect of type of feeding on the level of fecal calprotectin in infants.
Objective: To assess fecal calprotectin levels in breast fed or nonbreast fed healthy infants.
Design: A study was conducted to compare fecal calprotectin in infants who were exclusively breastfed compared to those who were not breastfed in Shanghai, China. Stool samples were collected and analyzed, and the fecal calprotectin concentration was determined using a commercially available enzyme-linked immunosorbent assay. The infant's weight and length were measured. Parents were asked to fill in a brief questionnaire, with questions about several clinical and sociodemographic factors.
Subjects: This study included 105 healthy infants aged 0–5 months.
Results: Stool samples were obtained from 105 healthy infants (63 boys, 42 girls) with a median age of 2.86 months (range 1–5.88). The median fecal calprotectin concentration was significantly higher in breast fed infants (377 μg/g, range 35–937 μg/g) than that in nonbreast fed ones (233 μg/g, range 37–895 μg/g) ($p = 0.001$). A correlation was found that from 0 to 5 months, fecal calprotectin was negatively and significantly associated with age in both two kinds of feeding (breast fed: Spearman's rho −0.346, $p = 0.010$; nonbreast fed: Spearman's rho −0.478, $p < 0.001$).
Conclusions: Our findings show that the kind of feeding influences the fecal calprotectin concentration and breast fed infants have higher levels than nonbreast fed ones in the first months of life. This may represent that human milk influences the gut mucosa by immunomodulating factors.

1. Introduction

Calprotectin, a 36-kDa calcium and zinc-binding protein, constitutes approximately 60% of the cytosolic protein of neutrophils, monocytes and macrophages [1], composed of 8 and 14 kDa subunits [2]. Therefore, the presence of calprotectin in feces can be seen as proportional to neutrophil migration to the gastrointestinal mucosa. Calprotectin has immunomodulating and antiproliferative effects as well as an important role in neutrophil defense against bacterial infections [3]. Neutrophil transepithelial migration and accumulation at mucosal surfaces and within the gut lumen are a hallmark of digestive inflammatory pathology [4]. The simplicity of the methods, as well as the sensitivity, propose that fecal calprotectin (FC) may be an ideal routine screening tool for the diagnosis of organic intestinal disease [5]. Currently, the gold standard for assessing intestinal inflammation is endoscopy with biopsy sampling [6], but repeated endoscopic evaluations are not feasible, especially in children [7]. Fecal markers have been found to be an important clinic test in order to evaluate the flogistic state of the whole intestinal tract [8], more accurate than serum markers [9]. Measurement of fecal calprotectin is highly useful for the diagnosis of IBD and may serve as a surrogate marker of mucosal inflammation throughout the intestinal tract [10].

It is found a significant correlation between calprotectin concentration in gut lavage fluid and intestinal permeability, suggesting that increased intestinal permeability may be a consequence of increased transepithelial migration of neutrophils [11]. It is released from activated granulocytes and inflamed epithelia, as part of the initial innate immune response [10]. A high level of calprotectin in healthy neonates may be related to higher intestinal permeability, establishment of gut flora, and response to alimentary antigens [12]. Several factors, including postnatal age and feeding method, could play a role in the maturation process of the functional integrity of the small intestine mucosa resulting in a decrease in intestinal permeability [13]. The beginning of

Abbreviations: FC, fecal calprotectin.

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enteral feeding and to the type of ingested milk (maternal or formula), as well as determining its levels in cases of altered gastrointestinal function, would allow confirmation of the usefulness of calprotectin as an easy and reproducible marker of intestinal involvement [14]. Until now, controversial results have been reported on the effect of type of feeding on the level of fecal calprotectin in infants. On the one hand, Savino et al. found higher levels of calprotectin in breastfed term infants compared with formula-fed term infants [15] and Dorosko et al. observed higher calprotectin levels in healthy, exclusively breast fed infants aged 0–6 months compared to mixed-fed infants [16]. On the other hand, other studies suggest that there is no significant difference in fecal calprotectin concentrations between breast fed and formula fed infants [17,18]. The aim of our study was to determine fecal calprotectin levels in healthy infants exclusively breast fed or nonbreast fed in Shanghai of China.

2. Materials and methods

2.1. Study population

This study was performed at Department of Children and Adolescent's Health Care of Xinhua Hospital affiliated Shanghai Jiaotong University School of Medicine, where infants received routine physical examination. Parents of infants were consecutively recruited from April 2013 to November 2013, parents were asked to fill in a brief questionnaire, with questions about several clinical and sociodemographic factors. At enrolment, collected clinical parameters that included gestational age, birth weight, sex, Apgar score, mode of delivery (vaginal or cesarean section), postnatal age, type of enteral feeding (exclusively breast-feeding or formula-feeding), neonatal diseases, weaning foods, symptoms, and physical examination findings were recorded before each stool sample was collected. Inclusion criteria were as follows: infant age <6 months; gestational age >37 completed weeks; 5 minute Apgar score >7; birth weight appropriate for gestational age (2500–4000 g), and exclusively breastfed or nonbreast fed; no illnesses in the last month. The exclusively breastfed infants had to have received only their mothers' milk from birth until the time when they were recruited. Nonbreastfed infants were chosen by mothers who decided not to breast-feed at birth or formula fed infants had received a standard formula at least one month until the time of recruitment. Exclusion criteria were: any intake of steroidal or non-steroidal anti-inflammatory drugs, antibiotics or any other drug during the 2 weeks before recruitment; infants with history of any sign or symptom of infection or gastrointestinal disease (diarrhea, vomiting, fever) were excluded.

2.2. Measurement of fecal calprotectin

Informed consent was obtained from each mother to collect a spot sample of feces from their infant. Parents were instructed to collect a sample of stools into a plastic container, and store it in a refrigerator at 4 °C–8 °C; the sample had to be taken or sent to the hospital within 1 day. About 1 g feces was collected from the nappy and stored immediately at −80 °C until analysis. Before analysis, frozen stool samples were thawed at room temperature, and the calprotectin concentration was determined using a commercially available enzyme-linked immunosorbent assay (Bühlmann Laboratories AG, Schönenbuch, Switzerland) that measures quantitative calprotectin as previously described in our study [19] and another study [20]. In each sample run were included blanks, standards and controls. The calprotectin cut-off level representing a positive value was equal or greater than 50 μg/g as stated by the manufacturer. Results were expressed as μg/g stool.

Informed consent was obtained by parents at enrolment. The study protocol was approved by the ethics committee of Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine.

2.3. Statistical analysis

The results are presented as median and range. All statistical analyses were performed with the Statistical Package for the Social Sciences for Windows software 17.0 (SPSS, Chicago, IL, USA). Statistical analyses between groups were carried out with Mann–Whitney U test and Kruskal–Wallis tests. Spearman's correlation test was used to evaluate the relationship between selected variables and fecal calprotectin values. Level of statistical significance was set at 0.05.

3. Result

3.1. Subjects characteristics

The study population included 105 infants (63 boys, 42 girls) born at a median gestational age of 38.9 weeks (range 37–42 weeks) with a median birth weight of 3262 g (range 2500–4000 g). Forty were born by cesarean section and 65 by vaginal delivery. Fifty four newborns (32 boys, 22 girls) were breast fed, and Fifty one newborns (31 boys, 20 girls) were nonbreast fed. The age of the infants was 2.86 ± 1.31 months (range 1–5.88) (mean ± SD, breast-fed group 2.77 ± 1.29 months and nonbreast-fed group 2.96 ± 1.35 months). Gestational age, birth weight, gender of the two groups, age at the time of fecal sampling, and weight at sample collection were similar. The characteristics of the subjects are presented in Table 1.

3.2. Fecal calprotectin concentrations

The median fecal calprotectin level calculated on all 105 infants was 288 μg/g of feces (inter quartile, 198-482 μg/g); high interindividual variations were observed, ranging between 35 and 937 μg/g. The fecal calprotectin concentrations (median: 288 μg/g) were mainly higher than the reference value. No significant differences were found between boys and girls ($p = 0.592$). Infants with breast fed ($n = 54$) had greatly increased levels of fecal calprotectin compared to infants with nonbreast fed ($n = 51$; $p = 0.001$). Breast fed infants had fecal calprotectin levels of 377 μg/g (range 35–937 μg/g) and nonbreast fed infants had 233 μg/g (range 37–895 μg/g). Fecal calprotectin levels for the two groups are displayed in Fig. 1.

There was no significant correlation between calprotectin level and birth weight ($r = 0.178$, $p = 0.059$) or gestational age ($r = 0.162$, $p = 0.084$). A correlation was found between fecal calprotectin and age, from 0 to 5 months; fecal calprotectin was negatively and significantly associated with age in breast fed infants (Spearman's rho −0.346, $p = 0.010$, seen in Fig. 2) and nonbreast fed infants (Spearman's rho −0.478, $p < 0.001$, seen in Fig. 3). Figs. 2 and 3 depicts fecal calprotectin concentrations according to age in the two kinds of feeding.

4. Discussion

In this study, we found higher fecal calprotectin levels in healthy, exclusively breast fed infants than in nonbreast fed ones in the first 5 months of life. This correlates similarly with previous research observing higher fecal calprotectin concentrations in breast fed infants compared to formula fed ones aged 0–3 months [15]. But in other study Rouge observed that fecal calprotectin levels were significantly higher in preterm infants who received formulas as their exclusive or predominant source of feeding ($n = 21$) than in those fed human milk ($n = 104$) [21].

It is well known that higher fecal calprotectin concentrations have been found in young infants compared with concentrations in adults and healthy children, showing wide interindividual and age-dependent variation [17,22]. Olafsdottir et al. [23] found that the mean fecal calprotectin levels were significantly higher in healthy infants (2–10 weeks) than those in healthy children aged >1 y (277