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Original Article

Correlation between gut pathogens and fecal calprotectin levels in young children with acute diarrhea

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Abstract

Background In cases of acute diarrhea, it is difficult to distinguish between bacterial and non-bacterial causes. Increased fecal calprotectin (f-CP) level is a marker of neutrophil migration in the intestinal lumen and is associated with intestinal inflammation. Previous studies reported an increase in f-CP levels in children with acute diarrhea, which is caused by bacteria, but only few have studied the relationship between intestinal pathogens with f-CP levels in acute diarrhea.

Objective To assess for a correlation between gut pathogens and fecal calprotectin levels in children with acute diarrhea.

Methods We conducted a cross-sectional study between July to November 2012 on children aged 1-5 years with acute diarrhea, and underwent routine blood tests, stool microscopy, f-CP tests, and stool cultures. We used a simple linear regression and correlation analysis with a significance level of $P<0.05$.

Results Forty-two children enrolled in this study. The mean age of subjects was 2.27 (SD 1.34) years. Their mean f-CP level was 93.88 (SD 14.68) μg/g. On microscopic stool examination, 26 patients (61.9%) had positive leukocytes, 1 had *Ancylostoma duodenale*, 1 had *Ascaris lumbricoides*, and 2 had *Blastocystis hominis*. Positive stool cultures were found in 14 children (33.3%) with acute diarrhea. There was a significant positive correlation between gut pathogens and f-CP levels ($r=0.605$; $P<0.0001$).

Conclusion In young children with acute diarrhea, the average f-CP levels are higher in those with positive intestinal pathogens. **[Paediatr Indones. 2014;54:193-7.]**

Keywords: *intestinal pathogens, fecal calprotectin, acute diarrhea, children*

Acute diarrhea is a major cause of morbidity and mortality in children in developing countries. In most cases, acute diarrhea is caused by acute intestinal infection by viruses, bacteria or parasites.[1,2] According to WHO, an estimated 2.5 billion cases of diarrhea occur in children under 5 year old. More than half the cases of diarrhea that occur in Africa and Southeast Asia have poor outcomes or cause death.[3] In Indonesia, diarrhea remains a public health problem and is one of the highest causes of mortality and morbidity in children, especially those under 5 years of age.[2,4]

Early identification of the cause of diarrhea is important, particularly in diarrhea related to inflammatory processes, in order to determine the appropriate therapy. Doctors often have difficulty in differentiating between bacterial and non-bacterial acute diarrhea.[5] As such, a new, simple, fast, and accurate biomarker is needed to diagnose acute infectious diarrhea.[6] Currently, there is no such biomarker to detect bowel inflammatory processes. Fecal biomarkers may be interesting tools, as stool

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flows along the intestinal mucosa collecting molecules that may be used as markers of inflammation or mucosal damage.[7,8]

Calprotectin (CP), a calcium-binding protein, comprises 60% of cytosolic protein found in neutrophils.[9,10] Fecal calprotein (f-CP) concentration is an indicator of neutrophil migration into the intestinal lumen and is associated with intestinal inflammation. Invasive digestive tract bacteria often cause intestinal inflammation, but viruses, parasites, and toxin-mediated etiologies rarely cause inflammation.[11,12]

A previous study found that fecal calprotectin levels were higher in cases of acute diarrhea caused by pathogenic gut bacteria compared to cases of acute diarrhea due to other causes.[5] Studies on the relationship between intestinal pathogens and fecal calprotectin levels in children with acute diarrhea have been limited. The aim of this study was to determine the relationship between intestinal pathogens and fecal calprotectin levels in children with acute diarrhea.

Methods

We conducted a cross-sectional study at the Pediatrics Ward of Prof Dr. R. D. Kandou Hospital, Manado from July to November 2012. Acute diarrhea was defined as defecating more than 3 times per day, along with a change in stool consistency to a liquid, with or without mucous and blood, that lasted less than 14 days.[2,13] Inclusion criteria were all children with acute diarrhea aged 1-5 years and whose parents agreed to participate and were willing to fill the research forms. Patients with gastrointestinal bleeding and those receiving antibiotics, anti-inflammatory, or anti-diarrheal medications were excluded from this study. All children who met the inclusion criteria underwent routine blood tests, stool microscopy, f-CP tests, and stool cultures.

We used a descriptive analysis for the children's characteristics and laboratory data, while simple linear regression and correlation analysis were used to assess for a relationship between fecal pathogens and f-CP. Data were processed with *SPSS* 20.0. A P value of <0.05 was considered to be statistically significant.

Results

During the study period, we collected a total of 42 patients. The characteristics of the study subjects' are shown in **Table 1**.

The mean f-CP level in our subjects was 93.88 (SD 14.68) μg/g. On microscopic stool examination, 26 (61.9%) patients had positive leukocytes in the stool, 1 had *Ancylostoma duodenale*, 1 had *Ascaris lumbricoides*, and 2 had *Blastocystis hominis*. Positive stool cultures were found in 14 (33.3%) children with acute diarrhea. The species found were as follows: 8 non-pathogenic *E. coli*, 5 pathogenic *E. coli*, and 1 *Shigella sonnei*.

Table 1. Baseline characteristics of subjects with acute diarrhea

Characteristics	n=42
Mean age (SD), years	2.27 (1.34)
Gender, n (%)	
• Male	25 (59.5)
• Female	17 (40.5)
f-CP levels (µg/g)	
• Mean	93.88
• SD	14.68
• 95% CI	89.30 to 98.46
• Median	96.50
Mean diarrheal frequency(SD), times per day	5.95 (3.33)
Fecal leukocytes, n (%)	
• ≥ 1/HPF*	26 (61.9)
• 0 / HPF*	16 (38.1)
Mean blood leukocyte counts (SD)/mm^3	10,197 (3,922)

*HPF: high power field

Simple linear regression analysis with T-test revealed a significant correlation between the incidence of intestinal pathogens and f-CP levels (P= 0.0001). The correlation coefficient of the two variables was r = 0.605, suggesting that average f-CP levels were higher in those with positive intestinal pathogens (mean 100.77 μg/g) than in those who were negative for intestinal pathogens (mean 82.69 μg/g) (**Figure 1**).

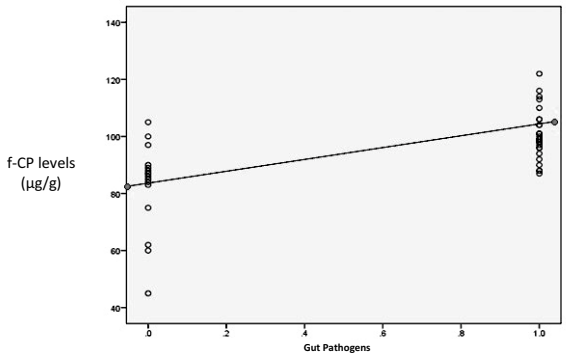

Figure 1. Correlation between intestinal pathogens and fecal calprotectin levels in children with acute diarrhea

Discussion

Acute diarrhea caused by intestinal pathogens may lead to inflammation of the bowel. Individual responses against intestinal infections determine the severity of disease, therefore, qualitative and quantitative measurements of bowel inflammation are needed to determine the type of early intervention therapy. Determining the cause of acute diarrhea is usually difficult. While one can look for cytokines and acute phase markers in stool specimens, the lack of diagnostic markers in early, acute diarrhea makes it difficult to predict the presence of intestinal pathogens.[14] In previous studies, an increase in f-CP levels in patients with acute diarrhea was mainly caused by bacterial intestinal pathogens.[5,6,15]

In our study, 33.3% patients with acute diarrhea had positive stool cultures resulting in bacterial growth. These findings were supported by a previous study which reported that only 35.7% of all patients with acute diarrhea had stool cultures positive for the presence of bacteria.[6] However, another study reported that from stool samples of 1,991 patients with acute diarrhea aged 0-15 years, only 260 (13.1%) showed bacterial growth.[16]

Stool specimens positive for leukocytes often indicate the presence of invasive bacteria or pathogens that produce cytotoxins.[17] The sensitivity of fecal leukocytes to inflammatory pathogens (*Salmonella, Shigella and Campylobacter*) as detected by stool culture varied from 45-95%, depending on the type of pathogen.[18] In our study of 42 children with acute diarrhea, 61.9% had positive fecal leukocyte as defined by a fecal leukocyte level ≥ 1 leukocyte/HPF. A study of 797 stool samples examined for fecal leukocytes and 473 stool cultures showed that stool leukocytes ≥1/ HPF had a 53% sensitivity and 88% specificity, and could be used to predict stool culture results (LR 4.2; 95%CI 2.7 to 6.5; P<0.001).[19] Another study found that in 25 patients with infectious gastroenteritis, 32% had positive fecal leukocyte results, and that using a cut off value of ≥1 leucokyte/HPF had a 92% specificity for detecting mucosal integrity.[20] A Bangladeshi study found at least 1 leukocyte/HPF in 3,487 of 3,558 (98%) of patients with bowel inflammation.[21]

In our study, the median level of f-CP in children with acute diarrhea was 96.50 μg/g, and the mean was 93.88 (14.68 SD) μg/g. Fecal calprotectin levels in this study were lower compared to a previous study in Italy that found the median f-CP in children with acute gastroenteritis was 110 (0.3-244) μg/g which significantly higher compared to control groups.[15] A study in Chech Republic reported that regardless of the cause of acute diarrhea, the overall median f-CP concentration in children with acute diarrhea was 163.3 μg/g.[5] These variations in f-CP levels may be due to differences in acute diarrheal patterns beween countries (Indonesian vs. Italy vs. Czech Republic) or in the f-CP testing methods used.

We observed a significant correlation between intestinal pathogens and f-CP levels in children with acute diarrhea (P=0.0001). Subjects infected with pathogenic intestinal bacteria had higher levels of f-CP than subjects infected with non-pathogenic gut bacteria. Similarly, Sykora *et al.* reported higher levels of f-CP in children with acute bacterial diarrhea.[5]

Elevated levels of f-CP in acute diarrhea caused by bacterial infection/intestinal pathogens can occur from products from bacteria such as *lipopolysaccharide* (LPS) that bound to toll-like receptor 4 (TLR-4) ba-

solateral which causes the production of interleukin 6 (IL-6) and IL-8, whereas lipoproteins through TLR-2 macrophage chemoattractant produce IL-1 and tumor necrosis factor-alfa (TNF-). In response to this cytokines production, polymorphonuclear neutrophils (PMNS) and neutrophils release calprotectin. The neutrophil migration and protein secretion causes epithelial damage or cell death (apoptosis), as well as CP secretion by damaged or dead epithelial cells, thus raising the f-CP levels. Simultaneously, CP may also trigger a positive feedback mechanism of macrophage activation through interaction with TLR-4. The destruction of intestinal epithelium and apoptosis also leads to reduced absorption, increased intestinal permeability and increased secretion of chloride ion (Cl^-), thus causing diarrhea. Gram-negative bacteria that cause diarrhea by cytotoxins can destroy the epithelium and cause inflammatory diarrhea, whereas enterotoxins cause an increase in cyclic adenosine monophosphate (cAMP) that leads to diarrhea.[22]

A limitation of this study was the result of stool cultures were not obtained growth of bacteria by the lack of bacterial growth in 28 (66.7%) stool cultures, while most specimens had high levels of f-CP. This result may be due to limitations of the culture media to detect only pathogenic *E. coli, Shigella*, and *Salmonella*.

In conclusion, increased f-CP levels are associated with bacterial pathogens that cause acute diarrhea. As such, this non-invasive laboratory test in children may help health care practioners to distinguish between bacterial and non-bacterial etiologies, in order to provide early and appropriate treatment therapy.

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