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Bacterial enteric pathogens and serum interleukin-6 levels in children with acute diarrhea

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Abstract

Background Acute diarrhea is currently one of the major causes of morbidity and mortality in developing countries. A wide range of enteric pathogens, including bacteria, is responsible for the pathogenesis of acute infectious diarrhea. Recent studies have shown an increase in acute phase proteins, such as serum interleukin-6 (IL-6) levels, in patients with acute bacterial gastroenteritis. Thus, IL-6 may be a useful marker to differentiate bacterial from non-bacterial enteric pathogens.

Objective To assess for a correlation between bacterial enteric pathogens and serum IL-6 levels in children with acute diarrhea.

Methods We conducted a cross-sectional study from November 2013 to March 2014 in two hospitals in Manado. Subjects were children aged 1-5 years with acute diarrhea and good nutritional status. Subjects' provided stool samples for bacterial culture and microscopic examination, as well as blood specimens for serum IL-6 measurements. Data was analyzed by linear regression and Pearson's correlation tests for a correlation between bacterial enteric pathogens and serum IL-6 levels.

Results In children with acute diarrhea, those with bacterial enteric pathogens had significantly higher mean serum IL-6 than those with non-bacterial enteric pathogens ($r = 0.938$; $P < 0.001$).

Conclusion Serum IL-6 levels are significantly more elevated in children with acute diarrhea and bacterial enteric pathogens. Therefore, serum IL-6 may be a useful marker for early identification of bacterial gastroenteritis in children aged 1-5 years. **[Paediatr Indones. 2016;56:144-8.]**

Keywords: *bacterial enteric pathogen; serum interleukin-6; diarrhea*

Acute diarrhea is one of the most common diseases in children.[1] In developing countries, an estimated 12 or more diarrheal episodes per child per year occur within the first 5 years of life.[2] In Indonesia, household surveys revealed that diarrheal disease was the third leading cause of morbidity, with a morbidity rate of 7.8 per 1000 per year from 121,266 people surveyed. In addition, diarrheal disease in Indonesia was the leading cause of death in infants, causing 24.1% of all infant deaths and 40% of all deaths in the first 2 years of life.[3] A wide range of enteric pathogens, including bacteria, is responsible for the pathogenesis of acute infectious diarrhea. Severe complications such as septicemia can result from improperly treated bacterial gastroenteritis.[4,5] Therefore, antimicrobial agents play a vital role in the management of acute bacterial gastroenteritis. However, it is not always possible to distinguish between viral and bacterial infections based on clinical features and laboratory examinations, such as fecal occult blood and leukocyte

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tests. The gold standard to diagnose acute infectious diarrhea is by stool culture. The drawbacks of this analysis, however, are the extended amount of time needed and the high cost.[6]

A cascade of cytokines, including tumor necrosis factor-α (TNF-α), IL-6 and IL-8, coordinates the body's response to infection. Few studies have evaluated cytokine release into the serum during acute gastroenteritis.[7,8] In a study of children with complicated *Shigella dysenteriae* 1 infection, serum TNF-α and IL-6 were found to be increased. The TNF-α however, was only detected for up to 18 hours after stimulation with bacteria, whereas IL-6 concentrations were detectable in plasma for up to 44 hours after infection.[9] A previous study showed that there was an increase in acute phase proteins, such as serum IL-6 level, during acute bacterial gastroenteritis, thus it may be a useful marker to differentiate bacterial from viral gastroenteritis.[10] To date, serum levels of cytokines in acute gastroenteritis have rarely been studied in Indonesia. Therefore, the objective in this study was to assess for a possible correlation between bacterial enteric pathogens and serum IL-6 levels in children with acute diarrhea.

Methods

We conducted a cross-sectional study in two hospitals in Manado from November 2013 to March 2014 with a total 80 children, included by consecutive sampling. There were 41 boys (51.2%) and 39 girls (48.8%) enrolled. We included children with acute diarrhea, aged 1-5 years, with good nutritional status, and whose parents provided informed consent and agreed to fill the questionaires. Exclusion criteria were children who had consumed antibiotics within 3 days prior to admission, malnutrition, obesity, malignancy, or acute infections such as dengue fever, bronchopneumonia, or sepsis. Subjects provided stool samples for bacterial cultures and microscopic examinations. Blood specimens were centrifuged, and the sera stored at -70°C until the cytokine assay was performed. Serum IL-6 concentrations were measured by an ELISA method (R&D Systems). All measurements were performed at a reference laboratory.

Regression analysis and Pearson's correlation were used to evaluate the degree of association between bacterial enteric pathogens and serum IL-6 levels. We considered a P value of < 0.05 to be statistically significant. Statistical analysis was performed with *SPSS version 21.0* software. This study was carried out with the approval of Health Research Ethics Committee of the , Sam Ratulangi University Medical School/Prof. Dr. R. D. Kandou Hospital, Manado.

Results

Bacterial pathogens were positively identified in 39 of 80 children (48.8%), including 13 with enterotoxigenic *Escherichia coli*, 11 with *Salmonella enterica*, 8 with *Shigella species*, 5 with *Campylobacter jejuni*, 1 with *Enterobacter aerogenes*, and 1 with *Yersinia enterocolitica*. The clinical characteristics of subjects with and without bacterial pathogens are shown in **Table 1**.

Children with stool cultures positive for bacterial enteric pathogens had significantly higher mean values of IL-6 than children with non-bacterial enteric pathogens (9.98 pg/mL vs. 0.76 pg/mL; $P < 0.001$). Pearson's correlation test revealed a very strong correlation between bacterial enteric pathogens and serum IL-6 levels in children with acute diarrhea ($r = 0.938$; $P < 0.001$) as shown in **Figure 1**.

Table 1. Clinical characteristics of children with acute diarrhea on admission

	With bacterial pathogen (n=39)	Withoud bacterial pathogen (n=41)
Mean age (SD), months	42.69 (18.70)	26.24 (14.15)
Female, n (%)	19 (48.7)	22 (53.7)
Mean peak body temperature (SD), °C	38.8 (0.49)	37.4 (0.74)
Vomiting, n (%)	20 (51.3)	33 (80.5)
Mean diarrheal frequency (SD), times	5.38 (1.25)	6.95 (1.40)
Stomach ache, n (%)	24 (61.5)	1 (2.4)

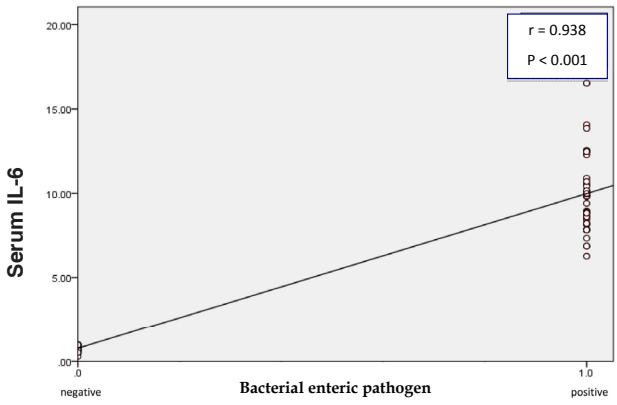

Figure 1. Correlation between bacterial enteric pathogen and serum

Discussion

Acute diarrhea remains a major worldwide public health problem. It contributes considerably to morbidity and medical expenses in developing countries. More than 700 million cases of acute gastroenteritis are estimated to occur annually in children less than 5 year old.[11] Our subjects were children aged 1-5 years. The reason for choosing this age group was based on the increasing number cases of diarrhea each year.[2,11] The World Health Organization (WHO) reported that more than 700 million diarrheal cases occurred in children less than 5 years of age.[4,12] National Health Insurance also reported that approximately 46,000 children were hospitalized each year and about 42% were children less than 5 years of age.[3]

Early identification of the agent causing acute diarrhea helps the health care provider to assess the disease and prevent unnecessary antibiotic treatment, community outbreaks, and nosocomial transmission. Clinical criteria and rapid fecal tests, such as occult blood and fecal leukocytes, may give contradictory results and are not as reliable as screening tests in infectious diarrhea.[13] Cytokines are known to be involved in infectious and non-infectious inflammation. Pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β are important mediators of inflammation and play a substantial role in the pathophysiology of infection. Pro-inflammatory cytokines also stimulate macrophages and neutrophils to produce large quantities of reactive and cytotoxic species of oxygen and nitrogen that support bactericidal function.[14,15]

The intestinal mucosa is known to be a site for the generation of acute phase proteins and various cytokines that may affect not only the gut mucosa itself, but also distant organs and tissues. Epithelial-derived cytokines, including IL-6 and IL-8 released after infection and inflammation, have been implicated as important factors in local and systemic immune responses in several studies. However, the sensitivity, specificity, and predictive values of positive and negative serum IL-8 levels in distinguishing bacterial gastroenteritis from viral gastroenteritis were lower compared to serum IL-6 (50%, 66.7%, 56.3%, 60.9% vs. 77.8%, 85.7%, 82.4%, 81.8%, respectively).[10,16]

Serum IL-6 is a multifunctional cytokine that responds to acute inflammatory reaction and has been suggested to be useful as an early indicator of bacterial infection.[17] A previous study found that IL-6 had better sensitivity and specificity than conventional inflammatory indicators such as C-reactive protein (CRP) and leukocyte count for detection of infection in hospitalized patients.[18] Gut mucosa and enterocytes increase the production on IL-6 during acute-phase inflammation. The cytokine profile found in inflamed bowel mucosa depends on the nature of the antigen presented to the host.[19,20]

In our study, serum IL-6 seemed to be a good indicator to differentiate between bacterial and non-bacterial pathogens. Mean serum IL-6 levels were significantly higher in children with stool cultures positive for bacterial enteric pathogens than in those with no bacterial enteric pathogens ($P < 0.001$). Linear regression analysis revealed a very strong positive correlation between bacterial enteric pathogens and serum IL-6 levels in children with acute diarrhea ($r = 0.938$; $P < 0.001$). Similarly, Lin *et al.* reported that serum IL-6 concentrations were high in children with bacterial gastroenteritis (45.29 ± 46.61 pg/mL).[10] Yeung *et al.*[6] also showed that IL-6 was significantly elevated in children with bacterial gastroenteritis (46.20 pg/mL) and was useful in differentiating between bacterial and non-bacterial origins. The best cut-off used in this study was 15 pg/mL, which gave a sensitivity of 75% and a specificity of 91% for confirming bacterial infection. Previous reports suggested that serum cytokines appeared in a temporal sequence after endotoxin injection[21] and Vaisman *et al.* indicated that there was no statistically significant difference in

serum IL-6 levels between groups in which blood was drawn earlier (0-36 hours) compared to those who were sampled later (36-72 hours).[20] This observation implies that IL-6 has a tendency to be present in the circulation for a considerable length of time following infection.[22] In our study, all serum specimens were collected within 72 hours of diarrhea onset, and all fecal specimens were obtained no more than 24 hours after drawing blood. Therefore, confounding by the timing of specimen collection may have had a limited effect on our statistical results.

The IL-6 secretion by gut mucosal dendritic cells increases during *Salmonella* infection. As such, IL-6 may play an important role in triggering systemic immune responses against *Salmonella*. The close association between elevated IL-6 concentration and bacterial infection may be a result of the sequential host response, that is, a local mucosal synthesis followed by a systemic release of pro-inflammatory cytokines.[7,16]

One of the limitations of this study was the shortage of the fecal cultures for obligate-anaerobic bacteria in our institution, so it was impossible to rule out this particular bacteria from all other diarrhea causing bacteria. Furthermore, we do not have the capability to culture viruses. Therefore, we could not definitively compare IL-6 concentrations in subjects with bacterial *vs.* viral etiologies.

In conclusion, we observed significantly increased serum IL-6 concentrations in children with acute diarrhea and stool specimens positive for bacterial enteric pathogens. Therefore, IL-6 may be a useful marker for early identification of bacterial gastroenteritis. A high serum IL-6 level suggests that bacterial infection is the likely cause of diarrhea and antibiotics should be commenced in order to shorten the duration of diarrhea and prevent other severe complications.

Conflict of interest

None declared.

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