

## Fecal alpha-1 antitrypsin concentration in protein-losing enteropathies caused by Rotavirus and enteropathogenic bacteria infection

D. Aulia<sup>1</sup>, I.S. Timan<sup>1</sup>, A. Firmansyah<sup>2</sup>

### Abstract

**Background** An increase in protein loss through the intestinal lumen is commonly found in children with intestinal inflammation. Measurement of fecal alpha-1 antitrypsin (FAAT) concentration has been used to detect the loss of protein through the digestive system. FAAT concentration increases in diarrhea patients due to *Rotavirus*, *Adenovirus*, *Shigella*, *Enterotoxigenic E. coli* (ETEC), and *Salmonella* infection.

**Objective** To determine the relationship between types of pathogen, acute diarrhea, and alpha-1 antitrypsin concentration in children with acute diarrhea caused by *Rotavirus* and enteropathogenic infection.

**Methods** Descriptive statistics and proportion difference between the two non-related groups were used to assess the proportion of protein-losing enteropathy (PLE) in children with acute diarrhea and was analyzed using chi square test.

**Results** In this study, PLE group comprised 25% (24/95) subjects without unknown cause of diarrhea, 50% (47/95) had one type of pathogen, and in 23% (22/95) subjects had 2 or more types of pathogens. The most common pathogen found in PLE group was *Rotavirus*, found in 67 (53%) subjects and *E. coli* in 41 (33%) subjects. In non-PLE group, we also found similar pathogen pattern. The mean alpha-1 antitrypsin (AAT) concentration in acute diarrhea group due to *Rotavirus* infection was significantly higher ( $P=0.003$ ) compared to acute diarrhea groups caused by non-*Rotavirus* infection. The mean AAT concentration in acute diarrhea group due to *E. coli* infection did not differ significantly ( $P=0.735$ ) compared to acute diarrhea group caused by non-*E. coli* infection.

**Conclusion** *Rotavirus* was a more significant cause of PLE compared to *E. coli*. [Paediatr Indones. 2009;49:315-21].

**Keywords:** alpha 1-antitrypsin, protein-losing enteropathies, diarrhea

Acute diarrhea in infants and children is still one of the main health issues in Indonesia due to its high morbidity and mortality rate. A survey in 1990-1992 showed the annual incidence of 60 million cases in Indonesia with diarrhea morbidity rate for all age groups was 230-330/1000 population.<sup>1-2</sup> It is assumed that each under-five children suffer from 1.6-2.2 episodes of diarrhea each year. In the Department of Child Health in Cipto Mangunkusumo Hospital, Jakarta, diarrhea ranked first place among all types of gastrointestinal infection from 2002 to 2004, with significant mortality.

Diarrhea in children could be due to disorders of both inside or outside of digestive system. The cause might be infection or non-infection. The infectious etiologies are viral, bacterial, or paracytic, whereas non-infective diarrhea could be caused by factors such as allergies to food components, food poisoning, or indigestion and malabsorption of nutrients.<sup>5-12</sup>

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From the Department of Clinical Pathology, Medical School, University of Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia (DA, IST).<sup>1</sup> From the Department of Child Health, Medical School, University of Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia (AF).<sup>2</sup>

**Reprint request to:** D Aulia, MD, Department of Child Health, Medical School, University of Indonesia, Cipto Mangunkusumo Hospital, Jl. Salemba 6, Jakarta 10430, Indonesia. Tel. 62-21-3907742. Fax. 62-21-3907743.

In children with intestinal inflammation, an increase in protein loss through the intestinal lumen will be found. The loss of protein can be used as a marker to determine the severity of the ongoing process. Measurement of fecal alpha-1 antitrypsin (FAAT) concentration has been used to detect the loss of protein through the digestive system.<sup>12-13</sup> The FAAT examination is a good marker to detect the loss of protein through the digestive system before hypoalbuminemia occurs. FAAT concentration increases in diarrhea caused by *Rotavirus*, *Adenovirus*, *Shigella*, *Enterotoxigenic E. coli* (ETEC), and *Salmonella*.<sup>14-16</sup> Alpha-1 antitrypsin (AAT) is a fraction of human plasma protein which is not digested in the intestines and not found in animal nor plant tissues. The presence of AAT in the stool itself is a good marker to detect protein loss through the digestive system.<sup>15</sup> Protein-losing enteropathies (PLE) was once detected by using radioactive chromium that is labeled with albumin and measured using radioactive methods in a 24-hour stool sample.<sup>17-18</sup> Now, AAT can be measured using nephelometric method or micro-Elisa in random stool samples.

There has not yet been a study in Indonesia regarding AAT concentration in patients with infectious diarrhea caused by *Rotavirus* and microbes such as *E.coli*, *Salmonella*, *Shigella*, etc.

## Methods

This was a cross sectional study performed on the Laboratory of Clinical Pathology in Medical School in University of Indonesia-Cipto Mangunkusumo Hospital, Budhi Asih General State Hospital in East-Jakarta, and Koja Hospital in North-Jakarta. The study started from November 2007 to February 2008.

The study subjects were all children aged 6 to 24 month old that were admitted to the Budhi Asih General State Hospital or Koja Hospital with acute diarrhea accompanied with mild to moderate dehydration (WHO criteria) from November 2007 to February 2008. Selection of subject was done consecutively on patients who fulfilled the inclusion criteria and willing to participate in the study while the parents signed an informed consent.

We included hospitalized children aged 6 to 24 month old with acute diarrhea (mild to moderate

dehydration according to WHO standard), in slight malnutrition or in good nutritional status with deviation -2 SD (standard deviation) to +2 SD,<sup>12</sup> with watery stool.

We excluded patients with other disorders or other severe additional diseases such as septicemia, bronchopneumonia, seizure, etc. Patients that did not obtain consent from parents or possible difficulties in follow ups due to family or domicile reasons nor patients that already received antibiotics treatment in the past week were also excluded from the study.

Patients with diarrhea were treated according to the standard operational procedures of Gastro-hepatology Division in Department of Child Health in University of Indonesia (Cipto Mangunkusumo Hospital) as follows: acute diarrhea with mild to moderate dehydration to be given 75 ml/kg oral rehydration solution in the first 4 hours. Stool sample then to be taken in a stool container for viral examination, routine stool examination, and AAT concentration measurement. Stool for culture sample was taken using two sterile cotton sticks and put into Carry Blair media as a transport media for *Enterobacteriaceae* and *Vibrio*. The laboratory work up was done at the department of clinical pathology, medical school in University of Indonesia. Bedside pH examination was performed using Special indicator strip paper from Merck, which can detect pH ranging from 2.0 to 9.0.

Confirmation of nutritional status (body weight and height) were performed at Budhi Asih Hospital and Koja Hospital.

The routine stool analysis consisted of macroscopic (color, consistency, and presence of blood, mucus, pus, and worms), and microscopic examination (leukocytes, erythrocytes, mucus, and bacteria). Microbiologic examination analysis consisted of *Rotavirus Antigen*,<sup>19</sup> *Adenovirus* antigen and enteropathogenic bacteria: *E.coli*, *Salmonella spp*, *Shigella spp*, *Vibrio spp*.<sup>20</sup>

The principle for *Rotavirus* antigen in stool examination using immunochromatography methods was as follows: when the strip tests was dipped into the liquid phase of stool suspension, the conjugate would dissolve and migrate together with the *Rotavirus* antigen in the stool suspension using capillarity along the nitrocellulose strip, then contact will occur with the monoclonal antibody against *Rotavirus* which was already in the strip. If *Rotavirus* was present in

the stool sample then the conjugate and *Rotavirus* complex would bind with the monoclonal antibody and form a dark-colored line along the strip, which would be visible within 5 minutes. The complex would then continue to migrate and contact with the second antibody, which was the anti-mouse IgG that would bind any excess of conjugate and form the second dark line that served as control.

The stool culture examination was performed to obtain enteropathogenic bacteria such as *E. coli*, *Salmonella spp.* It was done by swabbing the stool that was previously put into Carry Blair transport media to various medias such as MacConkey agar, SS agar, and TCBS agar. All medias were incubated in 35-37 °C temperature overnight. If *E. coli* bacteria was found, then serological test was performed using reagents for pathogenous *E. coli* antisera from Microgen®.

Bacterial growth on SS agar was identified using RB system, followed with bacteria serogrouping test for *Salmonella* and *Shigella*.

If on TCBS agar yellow or bluish-green colonies of bacterial growth was found, bacteria was implanted to blood agar followed with oxidation test. When the oxidation test was positive, it was followed by identification and agglutination test for *Vibrio Cholerae* and other *Vibrio* species. If the oxidation test was negative, no further examination was performed.

The FAAT examination was performed using Elisa double-sandwich methods. The principle of this examination was that AAT in the stool would bind with the excessive rabbit polyclonal antibody on the surface of the well (solid), forming an Ag-Ab complex. Then a peroxide-labeled/POD anti-AAT antibody (sheep polyclonal anti AAT) was added. Afterwards, tetramethyl-benzidine (TMB) was added. The yellow Ab-Ag-Ab\*<sup>TMB</sup> complex then would be read by the Elisa reader. The color intensity of the product was parallel with the AAT concentration in the stool. Then a response-dosage curve was established with absorbency units as x axis and concentration as y axis.

The statistical methods used to assess the proportion for PLE based on the etiology (*Rotavirus* or enteropathogenic bacterias), was descriptive and the difference of proportion between the two non-related groups was analyzed using chi-square test.

Parents' subjects were asked for written informed consents after being explained about the

purpose, benefits, and possible side effects, and had the right to refuse or to participate and possibility of withdraw during the study at any time. The study was carried out after acquiring the Ethics Committee approval.

## Results

The study was done from November 2007 to February 2008 at Budhi Asih Hospital and Koja Hospital. The subjects' age ranged from 6–24 months. There were 72 (57%) subjects who were up to 12 months old, and 44 (43%) subjects were 12-24 month old. There were 18 (14%) subjects with good nutritional status (0 to +2SD), 46 (36%) subjects were very slightly malnourished (0 to -1SD), while 63 (50%) subjects were slightly malnourished (0 to - 2SD). All subjects had normal liver (confirmed by AST, CHE examinations) and kidney (creatinine test) function.

**Table 1** shows the characteristics of the study subjects as a whole and classified into PLE and non- PLE groups. We found no significant difference for age, weight and height parameters, nutritional status (weight age Z score/WAZ, height age Z score/HAZ), period of illness before admittance, and frequency of diarrhea between the two groups.

The results of the examination were as follows: unknown cause of diarrhea was 25% (24/95) in PLE groups, 50% (47/95) had 1 type of pathogen, *Rotavirus* or enteropathogenic bacteria (*E. coli*), and while 2 or more types of pathogens were found in 23% (22/95) subjects. In the non- PLE group, unknown cause of diarrhea was found in 47% (15/32) subjects, 1 type of pathogen was found in 34% (11/32), and 2 or more types of pathogens were found in 19% of the subjects (**Table 2**).

*Rotavirus* is the most common pathogen in children with acute diarrhea. The proportion of *Rotavirus* as a cause of acute diarrhea in all population studied was 53%. The proportion of *E. coli* as a cause of acute diarrhea in the studied population was 33%. In fact, from this study, we found that *Rotavirus* in acute diarrhea was significantly more frequent to cause PLE ( $P < 0.05$ ) than *E. coli*. It was also found that *E. coli* was not significant to cause PLE ( $P > 0.05$ ) in infected acute diarrhea subjects.

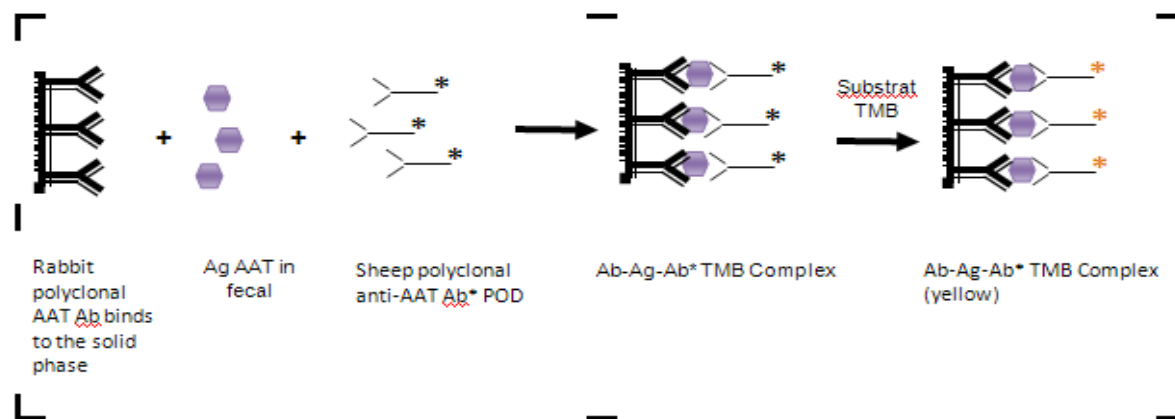


Figure 1. AAT examination principle

Ab= antibody; AAT= alfa1 antitrypsin; Ag= antigen; TMB= tetramethyl-benzidine; \*= labeled; POD= peroxidase

We found the mean AAT concentration in subjects with acute diarrhea due to *Rotavirus* infection was significantly higher ( $P = 0.003$ ) compared to the non-*Rotavirus* infection.

We also found that the mean AAT concentration in subjects with acute diarrhea due to *E. coli* infection was not significantly different ( $P = 0.735$ ) compared to those caused by non *E. coli* infection (Table 3).

The most common pathogen in PLE group was consecutively *Rotavirus* in 67 (53%) subjects and *E. coli* in 41 (33%) subjects. In the non-PLE group, we also found similar pathogen pattern. *Rotavirus* was the most common pathogen causing acute diarrhea and significant to cause PLE, while no significant difference was found in *E. coli* as pathogen (Table 2).

## Discussion

This study used 127 subjects aged 6–24 months who suffered from acute diarrhea. We included children of 0-2 years old because this age period is biologically susceptible to infection, nutrition, and diarrhea. Diarrhea episodes and mortality rate were highest at 0-2 years old.<sup>21</sup>

Study subjects were collected from 2 hospitals, Budhi Asih General State Hospital and Koja General State Hospital.

Fecal AAT was used as a marker for protein loss through the digestive system because AAT has a similar molecular weight as albumin, one of the important body protein components. Loss in the

Table 1. Characteristics of study subjects

Characteristics	Total population (n=127)		PLE (n=95)		Control group non-PLE (n=32)	
	mean	interval	mean	interval	mean	interval
Age (years)	10.2	7.2–13.2	10.6	7.6–13.6	9.43	7–12.2
Body weight (kg)	8.5	5.8–11.2	8.6	6.0–11.2	8.2	5.3–11.0
Height (cm)	72.9	55.9–89.9	73.2	55.2–91.3	72	58–86
WAZ	-0.86	-2.5–0.74	-0.82	-2.5–0.78	-0.95	-2.5–0.55
HAZ	-0.27	-2.7–2.16	-0.28	-2.5–1.92	-0.2	-3.1–1.7
Hemoglobin (g/dL)	10.9	8.3–13.5	11	8.1–13.9	10.8	8.2–13.4
Period of illness before admittance (days)*	2	1–10	2	1–10	2	1–10
Mean number of diarrhea/day *	5	3–20	5	3–20	9	3–11

\*Value as median

WAZ: weight age Z score, HAZ : height age Z score

Table 2. Pathogens causing acute diarrhea in PLE and non-PLE groups

Cause	Protein loss enteropathy (n=95)		Non protein loss enteropathy (n=32)		P
	Total	%	Total	%	
Rotavirus (+)	56	59	11	34	0.016
Rotavirus (-)	39		21		
E. coli (+)	31	33	10	31	0.885
E. coli (-)	64		22		
> 1 pathogen	47	50	10	31	
Non specific	24	25	13	41	

Table 3. Mean FAAT concentration in children with acute diarrhea due to Rotavirus (+) infection and Rotavirus (-), E. coli (+) infection, and E. coli (-).

Infection	n	FAAT concentration (mg/dl)	
		mean	interval
Rotavirus (+)	67	60.1	25.3-81.1
Rotavirus (-)	60	53.0	9.8-76.5
E. coli (+)	41	57.4	9.8-79.0
E. coli (-)	86	56.5	15.5-69.8

form of fecal AAT excretion can be considered equal with the possible loss of other protein with the same molecular weight or less. Fecal AAT examination is a non-invasive examination using only random stool sample that is easy to obtain and can be kept frozen for a long time, and can be sent to the local laboratory so that the examination can be widely available.<sup>22</sup> Fecal AAT examination can be done with proper micro-Elisa method using equipments that are commonly found in many laboratories in Indonesia today.

The FAAT concentration examination using micro-Elisa method is relatively new and has only been done for the past 2 (two) years. So far from the references, there has not yet been a reference value for AAT using micro-Elisa method in adults or children.

In this study the detected pathogens were *Rotavirus* and *E. coli*. In the examination to find the pathogens causing diarrhea, *Rotavirus* was the most common cause of acute diarrhea, followed by *E. coli*. Maki<sup>17</sup> in his study found that 20.3% of diarrhea was caused by *Rotavirus* and 8.85% was caused by bacteria (*E. coli*, *Klebsiella*, and *Shigella*). Tjitrastari<sup>23</sup> in her study found the proportion of *Rotavirus* as the cause of acute diarrhea in patients at the outpatient clinic in Department of Child Health in 2005 was

53.7%. Population-based study done by Setiyadi<sup>25</sup> at community health center (*Puskesmas*) in Bandung found *Rotavirus* as a cause in 48.8% of diarrhea. Hospital-based outpatient clinic study done by Hegar et al<sup>24</sup> at Cipto Mangunkusumo Hospital (1999-2001) found that *Rotavirus* proportion was 61.1%. The study that we conducted, the *Rotavirus* proportion was 52.8%. The differences of this result with studies by Setiyadi and Hegar were in population aspect and time of the study. Unlike this study, study by Hegar et al<sup>24</sup> was done at the peak of *Rotavirus* diarrhea incidence; the dry season. Meanwhile, study by Setiyadi<sup>25</sup> was conducted during one-year period, thus it might be slightly increased.

Indonesia is a country with two-peak seasonal variety; dry season and early rainy season.<sup>21</sup> This study was performed in the peak of rainy season, which was November 2007 to February 2008. Diarrhea due to *Rotavirus* can occur all year long with the peak incidence in the middle of dry season, while those caused by bacteria peaks in the middle of rainy season.<sup>5</sup>

Other risk factors that can increase enteropathogenic transmissions were lack of clean water and the water source was contaminated with stool. This study was done at Koja Hospital. The study subjects who lived near the North Shore of Java Island around the hospital were in difficulty to reach clean source of water. This study was also conducted at Budhi Asih Hospital. The study subjects mainly came from people living on the Ciliwung Riverside and also were having difficulties in getting clean source of water. Some risk factors on the hosts that can increase their susceptibility towards enteropathogens were malnutrition, low birth weight babies, immunodeficiency, and low level of gastric secretions.<sup>5-6</sup>

Pathogenic *E. coli* attaches to the intestine walls, multiplies, and colonizes in the intestines, causing



inflammatory reaction and epithelial degeneration changes upon bacterial attachment. From the results, we also found that the mean AAT concentration in subjects with acute diarrhea caused by *E. coli* infection did not differ significantly ( $P = 0.735$ ) compared to subjects with acute diarrhea caused by non-*E. coli* infection.<sup>26-28</sup> Darani<sup>30</sup> on his study found that fecal AAT concentration in patients with diarrhea caused by microbial infection were higher than non-infected diarrhea. The same thing was found by Fontana<sup>15</sup> in 1988 and Weizman<sup>29</sup> in 2002.<sup>29-30</sup>

Diarrhea due to *Rotavirus* infection can become chronic and causes malabsorption of disaccharides and weight loss, and plays an important role in the development of cow's milk enteropathy. Decreased lactase activity in the intestine is commonly found, however persistence of lactose malabsorption was rare because the activity of the disaccharidases will return to normal in a few days.<sup>31-32</sup>

In conclusion, the most common pathogen in PLE group are *Rotavirus* in subjects and *E. coli*. Similar pattern is found in the non-PLE group. *Rotavirus* is the most common pathogen for acute diarrhea that significantly causing PLE, while there is no significant difference in *E. coli* as pathogen.

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