Fecal Alpha1 - Antitrypsin in Healthy and Intestinal-Disorder Thai Children

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Objective: *Determine the normal FA1-AT level in random wet stool of Thai children using RID and NPL, and to study the correlation between RID and NPL methods for measurement of FA1-AT.*
Material and Method: *Random stool samples were collected from healthy children and intestinal-disorders patients. Alpha1-antitrypsin (FA1-AT) in wet stool samples was measured by nephelometry (NPL) and radial-immunodiffusion (RID) methods.*
Results: *Newborn infants had the highest FA1-AT level during the first day of life and declined to the same level as older children on day 3-4. Median and geometric mean of FA1-AT levels by NPL from healthy children aged 1 month - 15 years was 1.23 and 1.11 mg/dL respectively. FA1-AT levels by NPL from children with severe intestinal disorders, displaying median and geometric mean at 6.77 and 12.39 mg/dL respectively, were much higher than healthy children. The RID and NPL methods showed a correlation of* $r = 0.87$ ($p < 0.01$) *and* $R^2 = 0.75$.
Conclusion: *Random FA1-AT assay in wet stool is a non–invasive and simple test for supporting diagnosis of protein-losing enteropathy.*

Keywords: *Protein-losing enteropathy, Fecal alpha1-antitrypsin, Radial-immunodiffusion, Nephelometry, Gastrointestinal disorders*

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Protein-losing enteropathy (PLE) is a pathophysiologic process that results in the loss of serum protein into the gastrointestinal (GI) tract. The GI disorders that cause PLE can be either mucosal inflammation or lymphatic obstruction[1]. PLE has been determined by intravenous injection of ^{131}I- or ^{51}Cr-labeled albumin followed by measuring fecal excretion of the radioactive substances. However, this method poses hazards from radioisotopes and involves 48-72 hours of stool collection, which is a potential for urinary contamination. Recently, ^{99m}Tc-human serum albumin scintigraphy has been reported for detecting PLE and localizing the site of enteric protein loss[2].

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In 1977, Crossley and Elliott demonstrated a high level of alpha1- antitrypsin (A1-AT) in the feces of children with PLE[3]. A1-AT is a liver synthesized serum protein whose molecular weight is similar to that of albumin. As it is resistant to enzymatic proteolysis in GI tract, A1-AT is excreted in the feces without degradation[1]. Since it is not present in the diet, fecal level of A1-AT can reflect protein entering the intestine from the intravascular space. The original measurement of fecal alpha1-antitrypsin (FA1-AT) requires a 24-hour stool collection for analysis. Random FA1-AT measurement has been subsequently reported to be reliable for detecting PLE[4,5]. Further simplification of the method, using wet stool rather than lyophilized stool[6], made FA1-AT measuring more feasible in clinical laboratories.

Two methods of FA1-AT measurement, radial-immunodiffusion (RID) and nephelometry (NPL), have

been used; however, there were few studies comparing these two methods[7,8]. In addition, normal FA1-AT level in Thai children has never been reported. The objectives of the present study were to determine the normal FA1-AT level in random wet stool of Thai children using RID and NPL, and to study the correlation between RID and NPL methods for measurement of FA1-AT.

Material and Method

Subjects

A prospective study was conducted at Ramathibodi Hospital from November 2005 to July 2006. The present study was approved by the Ethics Committee of Faculty of Medicine, Ramathibodi Hospital. Parental consents were obtained. Random stool samples were collected from healthy newborn babies at Ramathibodi Nursery and from healthy children attending Ramathibodi Child Care Center. Children admitted to Ramathibodi Hospital without GI disorders were also included as controls. In addition, random stool samples from children with intestinal disorders: acute diarrhea, chronic diarrhea, enterocolitis, Crohn's disease, and systemic lupus erythrematosus (SLE) with suspected of PLE, were collected.

Method

All fresh stool samples were examined for parasites and eggs by direct smear and concentration techniques. Fecal samples were collected in stool containers and stored at -20□C until analyzed. At the time of analysis, 1 gram of feces was mixed with 5 ml of normal saline, vortexed for 60 minutes and centrifuged at 7,300 x g, 4□C for 20 minutes. The supernatant was used for measurement of FA1-AT by both RID and NPL methods.

NPL

For NPL method, 20 μL of supernatant were loaded into a tube of the commercial kit (BN Prospec Antisera to human A1-AT, Dade Behring, Behring Werke AG, Marburg, Germany). Immunological precipitation was measured by nephelometer according to the manufacturer's instructions and the result was obtained within 20 minutes.

RID

For the RID method, 5 μL of supernatant were pipetted into the well of a commercial immuno diffusion agar plate (LC-Partigen alpha1-antitrypsin, Dade Behring, Behring Werke AG, Marburg, Germany). The plate was then kept in a moist chamber at room temperature for 18 hours and diameter of precipitation ring was measured. Concentration of FA1-AT was determined by standard curve created from diameter of reference standards.

The results of both methods were expressed as milligram per deciliter (mg/dL) of wet stool. For undiluted specimens, the range of FA1-AT concentration measured by RID was between 33.3 and 502 mg/dL while range of NPL was between 0.25 and 8 mg/dl, and more dilutions were required for detection of higher concentration.

Statistical analysis

Because of wide range and skewed distribution of result values, average FA1-AT levels from healthy children and all intestinal disorders patients were expressed as median and geometric mean. In addition, minimum value (min), maximum value (max), and third quartile value were also presented. Correlation between FA1-AT concentrations measured by NPL and RID were determined by Pearson correlation coefficient (r) and linear regression analysis (R^2). A p-value of less than 0.05 was considered significant.

Results

One hundred twenty-nine stool samples were collected but eight samples were excluded because of supernatant turbidity unsuitable for NPL technique. The remaining comprised of 28 samples from newborn infants aged 1-4 days, 72 samples from healthy children aged 1 month to 15 years, and 21 samples from patients with intestinal disorders. All 121 samples were negative for parasites and eggs.

FA1-AT levels for newborn infants were highest on days 1-2 and declined to the same level as older children on days 3-4 (Table 1). FA1-AT levels from healthy children aged 1 month - 15 years fell below the lower detection limit of RID (< 33.3 mg/dL); therefore, FA1-AT results from this group could be only obtained by NPL methods (Table 1). The median and geometric mean of FA1-AT levels from healthy children aged 1 month - 15 years was 1.23 and 1.11 (min 0.25, max 5.22) mg/dL, respectively, with the third quartile value of 2.16 mg/dL.

Seventeen of 21 children with intestinal disorders had FA1-AT values below 33.3 mg/dL and could not be detected by RID. Therefore, only FA1-AT levels measured by NPL method were analyzed for average values. The patients were subdivided into two groups according to severity of intestinal disorders. Group 1

Table1. Fecal alpha1-antitrypsin (mg/dL) in all samples obtained by NPL method

Group		Min	Max	Median	Geometric mean	Third quartile
Newborn Day 1	5	5.45	76.0	36.70	28.61	42.2
Newborn Day 2	13	4.26	66.0	34.40	24.12	47.50
Newborn Day 3	7	0.25	75.60	3.34	4.98	32.11
Newborn Day 4	3	0.25	6.62	0.97	1.17	3.80
Healthy children 1 month - 15 years	72	0.25	5.22	1.23	1.11	2.16
Mild intestinal-disorder patients (group 1)	12	0.38	2.72	1.67	1.36	1.98
Severe intestinal-disorder patients (group 2)	9	3.90	54.80	6.77	12.39	29.50
All intestinal-disorder patients (group 1 and group 2)	21	0.38	66.80	2.62	3.51	6.45

(n = 12) included the patients with mild acute diarrhea. Group 2 (n = 9) consisted of patients who had severe acute diarrhea, chronic diarrhea, enterocolitis confirmed by endoscopy, Crohn's disease, and one patient having SLE with PLE confirmed by ^{99m}Tc-albumin scintigraphy. Median and geometric mean of group 1 FA1-AT levels were 1.67 and 1.36 (min 0.38, max 2.72) mg/dL, respectively, with the third quartile value of 1.98 mg/dL. On the other hand, median and geometric mean of group 2 FA1-AT levels were 6.77 and 12.39 (min 3.90, max 54.80) mg/dL, respectively, with third quartile value of 29.50 mg/dL. FA1-AT levels of group 1 were not much different from healthy children while FA1-AT levels of group 2 were clearly higher than healthy children.

FA1-AT levels from all 121 samples were used to assess correlation between RID and NPL methods. For attainable of calculation, the samples having RID value of < 33.3 mg/dL were assigned value of 0.00 mg/dL. The RID and NPL methods showed a correlation coefficient by Pearson analysis of r = 0.87 ($p < 0.01$) and by linear regression analysis showing $R^2 = 0.75$ (Fig. 1).

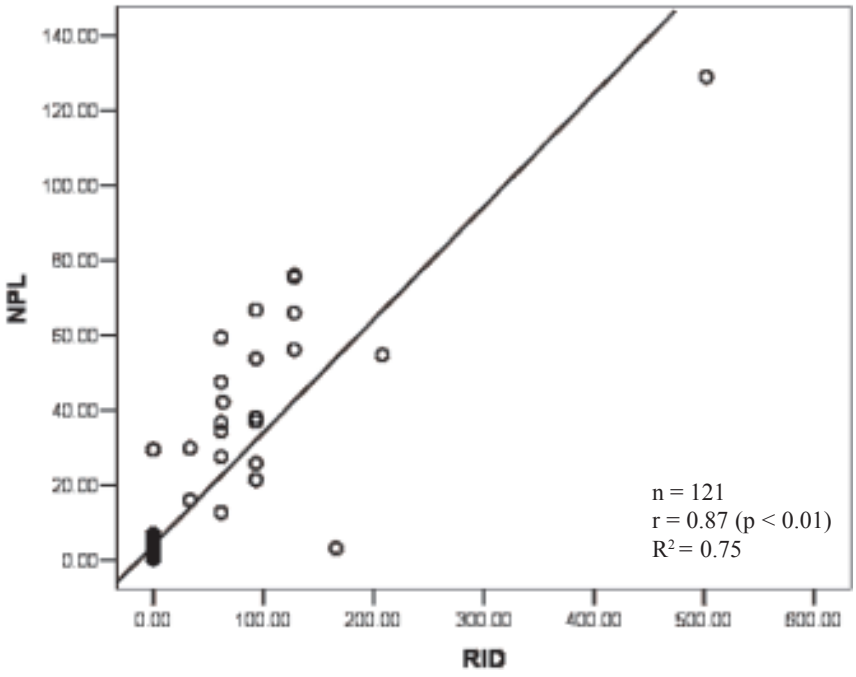

Fig. 1 Scatter plot and linear regression line showing correlation of FA1-AT levels (in mg/dL of wet stool) obtained by RID and NPL methods of all 121 samples

Discussion

FA1-AT has been widely used for diagnosis of PLE in various gastrointestinal disorders[5,7,9,10]. The advantage of FA1-AT measurement over radioisotope-labeled albumin excretion or scintigraphy is that patients particularly children are not exposed to radioisotope such as ^{51}Cr-albumin and ^{99m}Tc. Moreover, FA1-AT can also be used for monitoring and follow-up of the states of diseases causing PLE. However, to the authors' knowledge, this test has not been available in Thailand and normal FA1-AT level in Thai children has not been reported.

In the present study, FA1-AT levels from different age groups of healthy Thai children as well as patients with intestinal disorders were determined using random, wet stool samples. FA1-AT assay in wet stool is easier and more practical than using dried stool since lyophilization of stool is time-consuming and requires a vacuum freeze dryer. This is not generally available in many clinical laboratories[6].The FA1-AT levels in healthy, newborn babies were much higher than the older children. The level was highest on day 1 and gradually declined to the same level of older infants on the 4th day of life. The result agreed with the report by Keller et al[11] that meconium of normal newborn excreted during the first few days of life contained a high FA1-AT level which reflects meconium clearance rather than increased intestinal permeability. There was little difference of FA1-AT levels among healthy children aged 1month-15 years. The median and geometric mean of FA1-AT level using NPL method was 1.23 and 1.11 mg/dL wet stool. The results agreed with Lopez et al[7] who reported normal values of 1.19 ± 0.20 mg/dL and 1.23 ± 0.20 mg/dL wet stool by NPL and RID, respectively. There have been only a few reports of the normal FA1-AT levels in wet stool[5,6]. The other authors reported FA1-AT levels in dry stool of 3.47 ± 2.06[8], 0.58 ± 0.06[5], and < 2.0 mg/gram[12].

Increased FA1-AT concentration was observed in group 2 patients with severe diarrhea or enterocolitis. Two patients had Crohn's disease. The SLE patient with PLE had very high FA1-AT level, at 66.8 mg/dL. FA1-AT levels of patients in this group were obviously higher than those of acute diarrhea or healthy children. These results demonstrated that high FA1-AT level is found in patients with significant intestinal mucosal diseases. Lisowska-Myjak et al[13] reported an increase FA1-AT concentration in random, dried stool samples in chronic diarrhea patients, but there was no increase in FA1-AT concentration in the first 48 hours of acute diarrhea. However, Weizman et al demonstrated increased FA1-AT in random, dried stool samples in about 50% of 43 children with acute diarrhea without affecting serum protein[14]. A larger sample, size than the numbers of patients with acute diarrhea in the present study, would clarify these controversial results.

FA1-AT concentration measured by RID and NPL methods has a significant correlation. This agreed with previous studies reported by Lopez et al[7] and Buffon et al[8]. Although both methods can be used in routine laboratory services, NPL has a better analytical sensitivity. RID is more economical but the errors of determining precipitation ring diameter diminish precision of measurement. The RID plate used in the present study is the only commercial plate available in Thailand at the time. It can measure FA1-AT concentrations above 33.3 mg/dL. Concentrations below this lower limit need NPL assay. The RID plates used by Lopez et al[7] measured FA1-AT concentrations above 0.8 mg/dL, which cover low FA1-AT concentrations as well as the NPL method used in the present study that measures FA1-AT concentrations above 0.25 mg/dL. As shown in the present study, the FA1-AT levels in healthy children (1 month-15 years) could be only obtained by NPL but not by RID method because the levels in these children were lower than RID lower limits. NPL is also a more rapid method than RID. Therefore, it is more practical in clinical uses. The limitation of NPL is that it cannot measure turbid samples. There were eight samples in the present study that could not be measured by NPL due to the turbidity of the samples despite high-speed centrifugation. Lopez et al[7] suggested using 0.45 μm filter to purify the samples before being measured by this method.

Conclusion

The present study showed that random FA1-AT assay in wet stool is a simple and non-invasive method for supporting the diagnosis of PLE. The present study established normal FA1-AT values in Thai children, which can be used as references in clinical practice.

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การศึกษาค่าปกติของ fecal alpha1-antitrypsin ในเด็กไทยปกติและผู้ป่วยเด็กโรคทางเดินอาหาร

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ตัวอย่างอุจจาระเก็บจากเด็กปกติและเด็กที่เป็นโรคระบบทางเดินอาหารเพื่อตรวจ fecal alpha1-antitrypsin (FA1-AT) ด้วยวิธี radial-immunodiffusion (RID) และ nephelometry (NPL) ผลพบว่าค่า FA1-AT ในทารกแรกเกิดมีค่าสูงและลดลงจนเท่ากับเด็กโตเมื่อทารกอายุได้ 3-4 วัน ได้ค่าเฉลี่ย median และ geometric mean ของ FA1-AT ที่ตรวจโดยวิธี NPL ในเด็กปกติ อายุ 1 เดือน ถึง 15 ปี เท่ากับ 1.23 และ 1.11 mg/dL ตามลำดับ เด็กที่เป็นโรคที่ลำไส้อย่างรุนแรงมีค่าเฉลี่ย median และ geometric mean ของ FA1-AT ที่ตรวจโดยวิธี NPL เท่ากับ 6.77 และ 12.39 mg/dL ซึ่งสูงกว่าเด็กปกติมาก การตรวจโดยวิธี RID และ NPL มีความสอดคล้องกันด้วยค่า $r = 0.87$ ($p < 0.01$) และ $R^2 = 0.75$ สรุปได้ว่าการตรวจ FA1-AT ใน wet stool เป็นวิธีที่ง่ายสำหรับการช่วยวินิจฉัยภาวะสูญเสียโปรตีนในอุจจาระ