Original Article

Selenium status and fungi in the protein-losing enteropathy of persistent diarrhea

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Background and Objectives: A vicious cycle of infection, malabsorption, and malnutrition has been implicated in the perpetuation of diarrheal disease. This study examined whether persistent diarrhea is associated with changes in selenium status and stool alpha-1 antitrypsin (AAT) concentration. Methods and Study Design: This cross-sectional study included 30 children aged 1–12 years with persistent diarrhea who were hospitalized in Cipto Mangunkusumo Hospital and Fatmawati Hospital, Jakarta, and 30 apparently healthy children who were matched by age and sex and lived in a rural area of Jakarta. Clinical examinations, blood routine tests, erythrocyte glutathione peroxidase (GPX) activity and plasma selenium levels as well as AAT in fresh stool samples were performed in all the subjects. Results: Of 30 children with persistent diarrhea, 17 had moderate malnutrition and 13 had severe malnutrition. The mean plasma selenium was significantly lower in children with persistent diarrhea than in children without diarrhea (86.0 µg/L [95% CI: 76.1–95.9] vs 110 µg/L [95% CI: 104–116, p<0.0001]). The mean stool AAT concentration was significantly higher in children with persistent diarrhea than in those without diarrhea (115 mg/dL [95% CI: 38.5–191] vs 16 mg/dL [95% CI: 4.0–13.5, p<0.0001]). Selenium correlated with AAT (r=0.05). Fecal fungi were persistently present. Conclusions: Although selenium status in both groups was optimal for the obtained plasma GPX activity, children with persistent diarrhea exhibited lower plasma selenium levels. This study suggests that the decrease in the plasma selenium level may be the consequence of protein loss and that fungi may be involved.

Key Words: malnutrition, enteropathogen, intestinal inflammation, protein-losing enteropathy

INTRODUCTION
A vicious cycle of infection, malabsorption, and malnutrition has been implicated in the perpetuation of diarrheal disease. Data of Indonesia Basic Health Research 2007 (Riset Kesehatan Dasar, Riskesdas, which are compiled by the Ministry of Health of the Republic of Indonesia) revealed that diarrhea remains the primary cause of death in babies and children younger than 5 years (31.4% and 25.2%, respectively).1 According to Indonesia Health Profile 2014, the national crude fatality rate of the diarrheal outbreak in 2014 was 1.14%, which is still higher than the expected target rate of <1%.2 Therefore Indonesia is still battling with this disease. These data reveal that most diarrheal episodes resolve within 1–2 weeks, this disease persists longer in some individuals (persistent diarrhea).3,4

Reactive oxygen species (ROS) and nitrogen species and depleted antioxidant defenses have been reported to play major roles in the pathogenesis of intestinal damage in malnourished children.5,7 Under normal conditions, a delicate balance exists between ROS production and antioxidant defenses that protect cells in vivo. Increased ROS generation may occur as a result of many conditions affecting children, including inflammation, infection, and malabsorption. Alternatively, increased ROS generation may occur because of inadequate antioxidant defenses.8 Selenium is an essential trace element that plays an important role in the redox balance through the antioxidant activity of selenoproteins.9 In individuals with selenium deficiency, the activity of selenium-dependent glutathione

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doi: 10.6133/apjcn.062017.s13
peroxidase (GPX), a major class of selenoproteins in the human body, is decreased. However, there is a hierarchy in the importance of selenoproteins in organs, and the selenium requirement for optimum immune function may be higher than that for GPX activity. In addition, the biochemical mechanisms underlying the association between a marginal selenium status and the increased risk of infection, inflammation, and immune disorders cannot be limited to the redox balance.

Because of its syndromic nature, persistent diarrhea leads to reduced absorption of all nutrients, with their subsequent loss in increased intestinal secretions and stool. Diarrheal disease is associated with abnormal transmucosal protein loss from the gut. This protein loss is a crucial mechanism through which diarrhea causes malnutrition. Protein-losing enteropathy (PLE) is a pathophysiological process that results in the loss of serum protein into the gastrointestinal tract, mostly because of mucosal inflammation. Alpha-1 antitrypsin (AAT) has been used as an endogenous marker, and determining the fecal clearance of AAT enables the diagnosis of PLE.

The present study examined whether persistent diarrhea is associated with a marginal selenium status, which was evaluated using two indices: a direct one (plasma selenium) and a functional one (erythrocyte Se-glutathione peroxidase [GSH]-Px [GPX] activity). This study also investigated whether these parameters are associated with the levels of stool AAT, which is a marker of PLE.

MATERIALS AND METHODS
This cross-sectional study included 30 children aged 1–12 years with persistent diarrhea who were hospitalized in Cipto Mangunkusumo Hospital and Fatmawati Hospital, Jakarta, and 30 apparently healthy children who were matched by age and sex and lived in a rural area of Jakarta. This study was conducted from November 2010 to December 2011. Before their enrollment into the study, informed written consent was obtained from the children’s parents. This study was approved by the Research Ethics Board of the Faculty of Medicine, Universitas Indonesia no: 484/PT02.FK/ETIK/2010. All eligible subjects underwent clinical examinations; their anthropometric measurements were obtained. The following parameters were assayed on the first day of admission: erythrocyte GPX activity, plasma selenium concentration, and stool AAT concentration; in addition, routine peripheral blood examination and stool analysis were performed on that day. Nutritional status was determined using the weight-for-height z-score according to the WHO growth standard. The mother’s education level was classified as “junior high school and lower”, “senior high school”, and “diploma and above”.

Venous blood was collected from each subject after 6-hour fasting to determine the complete peripheral blood count and erythrocyte GPX activity. The enzymatic reagent used was Ransel, and GPX activity was determined according to the method of Paglia and Valentine. GPX catalyses the oxidation of GSH by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form, with a concomitant oxidation of NADPH to NADP+. In this study, the decrease of NADPH absorbance at 340 nm was measured, and it is propotional to the activity of GPX. Blood samples for trace element (Se) analysis were collected in a trace element free-sodium heparin vacutainer. Plasma selenium levels were measured using inductively coupled plasma mass spectrophotometry.

Fresh stool samples were collected for a stool analysis; samples not used immediately were stored at −20°C until analysis of AAT by using ELISA kit. The stool analysis comprised a macroscopic examination (color, consistency, and the presence of blood, mucus, pus, and worms) and microscopic examination (leucocytes, erythrocytes, mucus, and bacteria). The analysis of stool AAT was performed using double antibody sandwich ELISA. The principle of this analysis is that AAT in the stool would bind to excessive rabbit polyclonal antibody on the surface of the well (solid), forming an antigen–antibody (Ag–Ab) complex. Subsequently, a peroxidase (POD) - labeled anti-AAT antibody (sheep polyclonal anti-AAT antibody) was added, followed by the addition of tetramethylbenzidine (TMB). The yellow Ab–Ag–Ab* complex was read on an ELISA reader. The colour intensity of the product corresponded to the AAT concentration in the stool. Moreover, a dose–response curve was plotted, with absorbance unit on the x-axis and the level on the y-axis. In this study, the cutoff point for the AAT concentration was 54 mg/dL.

Statistical analysis
The data were analyzed using IBM SPSS Statistics version 17. For statistical comparison, t test and one-way ANOVA were used for normally distributed data, and the nonparametric Mann–Whitney test and Wilcoxon’s signed rank test were used when the distribution was skewed. The chi-square test was used to compare frequencies. The association between AAT and selenium was evaluated using the Spearman correlation coefficient. Statistical significance was considered at the 5% probability level.

RESULTS
The children enrolled in this study were matched by age and sex. A predominance of boys (20 of 30) was noted. Regarding nutritional status, of 30 children with persistent diarrhea, 17 had wasted (moderate malnutrition), and 13 had severe wasted (severe malnutrition). Of 30 children without diarrhea, 20 had an appropriate nutritional status, and 10 had wasted (moderate malnutrition). Maternal education levels differed significantly between the two groups. This finding is because children without diarrhea were from a rural area of Jakarta, which is a middle–low socioeconomic area. However, Cipto Mangunkusumo Hospital and Fatmawati Hospital are located in central and south Jakarta, a middle socioeconomic area (Table 1).

Children with persistent diarrhea had more bloody stools (p=0.0001), enteric infections (p=0.0001), fungal infections (p=0.005), fat malabsorption (p<0.0001) and less fiber maldigestion (p=0.020) than children without diarrhea (Table 2). The prevalence of lactose and carbohydrate malabsorption did not differ between the groups.
Table 1. Demographic data

<table>
<thead>
<tr>
<th></th>
<th>Case (n=30)</th>
<th>Control (n=30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>20 (67)</td>
<td>19 (63)</td>
<td>1.000</td>
</tr>
<tr>
<td>Girls</td>
<td>10 (33)</td>
<td>11 (37)</td>
<td></td>
</tr>
<tr>
<td>Aged (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–3</td>
<td>26 (87)</td>
<td>22 (73)</td>
<td>0.403</td>
</tr>
<tr>
<td>&gt;3–5</td>
<td>2 (6.5)</td>
<td>5 (17)</td>
<td></td>
</tr>
<tr>
<td>&gt;5</td>
<td>2 (6.5)</td>
<td>3 (10)</td>
<td></td>
</tr>
<tr>
<td>Nutritional status†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>0 (0)</td>
<td>20 (67)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Wasted</td>
<td>17 (57)</td>
<td>10 (33)</td>
<td></td>
</tr>
<tr>
<td>Severe wasted</td>
<td>13 (45)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Mother’s education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junior high school and lower</td>
<td>5 (17)</td>
<td>18 (60)</td>
<td>0.002</td>
</tr>
<tr>
<td>Senior high school</td>
<td>22 (73)</td>
<td>10 (33)</td>
<td></td>
</tr>
<tr>
<td>Diploma and above</td>
<td>3 (10)</td>
<td>2 (7)</td>
<td></td>
</tr>
</tbody>
</table>

†Nutritional status determined according to WHO z-score weight for height (zWH). Normal if -2<zWH<-2, wasted if -3<zWH<-2, and severe wasted if zWH<-3. If subjects aged more than 5 years old, nutritional status determined according to CDC 2000 and determined normal if 90-110% ideal body weight, wasted if 70-90% ideal body weight, and severe wasted if <70% ideal body weight.

Table 2. Stool analysis

<table>
<thead>
<tr>
<th></th>
<th>Case (n=30)</th>
<th>Control (n=30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bloody stool</td>
<td>12 (40)</td>
<td>0 (0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Enteric infections</td>
<td>28 (93)</td>
<td>0 (0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fat malabsorption</td>
<td>12 (40)</td>
<td>0 (0)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lactose malabsorption</td>
<td>1 (3)</td>
<td>0 (0)</td>
<td>0.317</td>
</tr>
<tr>
<td>Carbohydrate malabsorption</td>
<td>7 (23)</td>
<td>10 (33)</td>
<td>0.394</td>
</tr>
<tr>
<td>Fungi infection</td>
<td>7 (23)</td>
<td>0 (0)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Table 3. Antioxidant erythrocyte GPX activity, plasma selenium, stool alpha-1 antitrypsin, and Hb and ESR between groups

<table>
<thead>
<tr>
<th></th>
<th>Case</th>
<th>Control</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPX (U/lgHb)</td>
<td>38.0 (32.9-43.2)</td>
<td>40.2 (36.4-44.0)</td>
<td>0.357</td>
</tr>
<tr>
<td>Plasma selenium (ng/L)</td>
<td>86.0 (76.1-95.9)</td>
<td>110 (104-116)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stool alpha-1 antitrypsine (mg/dL)</td>
<td>115 (38.5-191)</td>
<td>16.0 (3.6-28.5)</td>
<td>0.010</td>
</tr>
<tr>
<td>Hemoglobin (mg/dL)</td>
<td>10.4 (9.6-11.2)</td>
<td>11.7 (11.4-11.9)</td>
<td>0.002</td>
</tr>
<tr>
<td>ESR (mm/hour)</td>
<td>18.8 (9.5-28.0)</td>
<td>15.7 (11.8-19.5)</td>
<td>0.530</td>
</tr>
</tbody>
</table>

Results are expressed as the mean (95% CI).

DISCUSSION

In Indonesia, the prevalence of persistent diarrhea might have remained stable in the last 5 years. In Cipto Mangunkusumo Hospital, which was the main study center, the prevalence of persistent diarrhea from January 2009 to December 2010 was 19% (47/253). In this study, we included 25 children with persistent diarrhea who were hospitalized between November 2010 and December 2011. During this period, another five children with persistent diarrhea were recruited from Fatmawati Hospital. Our results reveal that persistent diarrhea remains an important problem that results in adverse effects on the nutritional status of children.

Persistent diarrhea can be the consequence of a broad spectrum of disorders. In children from developing
countries, such as Indonesia, intestinal damage by viral, bacterial, or parasitic agents is the current primary event in the majority of persistent diarrhea cases.\textsuperscript{25-27}

Selenium is an essential trace element with fundamental importance to human health, and it is known for its antioxidant and immune activity and its anti-inflammatory and antiviral properties, which may be involved in the occurrence of persistent diarrhea. However, only a few studies have reported the selenium status of children with persistent diarrhea.\textsuperscript{28,29}

In the present study, we found that plasma selenium concentration decreased in children with persistent diarrhea. By contrast, erythrocyte GPX activity remained within the range of reference values. This observation conflicts with the results of Chaudhary et al, in which no evidence of any modification of the plasma selenium was found in children with persistent diarrhea.\textsuperscript{26} This discrepancy may be explained by the population studied; Thomas et al reported that GPX activity in plasma and red blood cells and the plasma selenium concentration may decrease, increase, or remain within the reference range, depending on the cause of the gastrointestinal disorder.\textsuperscript{29} Notably, the selenium status of the studied Indonesian children was markedly higher than that observed in other countries.\textsuperscript{30} These high selenium concentrations explain why GPX activity remained within the reference range in children with persistent diarrhea in our study.\textsuperscript{13} The selenium concentration in this group was higher than the value proposed for optimum red blood cell activity.

In this study, we also observed a weak association between selenium and AAT. This finding suggests that the decrease in the plasma selenium may be the consequence of protein loss.

Stool AAT is used as a marker of protein loss from the digestive tract, and it allows the detection of protein loss from the digestive tract before the occurrence of hypoalbuminemia.\textsuperscript{17} This study found a significantly higher stool AAT concentration in children with persistent diarrhea than in those without diarrhea. The fecal protein loss was significantly higher in children with persistent diarrhea, which agrees with the results of a previous study.\textsuperscript{25} Enhanced fecal protein loss, as the consequence of intestinal epithelial damage, was observed in children with persistent diarrhea caused by various pathogens (bacterial, viral, or parasitic agents).\textsuperscript{25,26,31} This study should be extended by elaborating the enteropathogen causing persistent diarrhea, because this study found only one child infected by \textit{E. histolytica} (data not shown), without any data of enteric pathogens. However, signs of overgrowth of enteric bacteria (leucocytosis in stool) in children with persistent diarrhea were observed in this study.

Persistent diarrhea also adversely affects nutritional status and is often associated with malnutrition.\textsuperscript{31} This study demonstrated that children with persistent diarrhea were mostly undernourished; 56.7\% had moderate malnutrition and 43.3\% had severe malnutrition. Comparatively, children without diarrhea had an improved nutritional status; 66.7\% had an appropriate nutritional status, and 33.3\% had moderate malnutrition.

This study supports the concept of a vicious cycle of persistent diarrhea, protein losing-enteropathy, malnutrition, and selenium deficiency (Figure 1). Reactive oxygen species (ROS) are thought to play a key role in electrolyte loss and enhanced mucosal permeability occurring in chronic diarrhea.\textsuperscript{32} Malnutrition increases the infiltration of lymphocytes within the lamina propria, generating redox imbalance, Therefore intestinal water secretion increases which contributes to the persistence of diarrhea.\textsuperscript{33} A vicious cycle may also arise in the event of an infectious etiology as selenium deficiency may increase the virulence of microorganisms, particularly virus\textsuperscript{34} and induces immunodeficiency. However, in our study, selenium status was not deficient.

Our study also reveals that 23\% (7/23) children with persistent diarrhea were infected by fungi. Several pathogeneses might be involved in this condition such as antibiotic treatment and immunodeficiency associated with diarrhea. There is also evidence that diet might contribute to a change in the structure of the gut microbiome as found here.\textsuperscript{35,36} Candida has been positively associated with carbohydrate ingestion.\textsuperscript{36} This finding is interesting and merits further epidemiological and experimental study.

The weak association between selenium and AAT can be explained by the previous finding that selenium-enriched yeast supplementation significantly decreased AAT concentrations in individuals with prostate cancer.\textsuperscript{37} However, in animal models, an increase in AAT has been observed with selenomethionylselenocysteine supplementation; the increase depended on the intake duration and dose of the supplement.\textsuperscript{38} Kelly et al\textsuperscript{39} demonstrated that in a cellular model, selenoprotein P downregulated numerous markers induced by the Z variant of AAT. The weak association observed in this study may be the consequence of the small sample size and the diversity of causes of persistent diarrhea; thus, this weak association must be confirmed in a larger cohort study.

**Conclusion and limitations**

In this study, which investigated a limited number of Indonesian children with persistent diarrhea, we found that a decrease in plasma selenium concentration was weakly associated with an increase in stool AAT concentrations. By contrast, given the high plasma selenium in children without diarrhea, GPX activity remained within the normal range. Finally, because of the small sample size, it was not possible to separately analyze the plasma selenium status and its association with AAT in subgroups of children defined according to the causes of persistent di-

**Table 4. Association between the number of children with normal and abnormal stool alpha-1 antitrypsin levels stratified by nutritional status and stool analysis**

<table>
<thead>
<tr>
<th>Nutritional status</th>
<th>Stool alpha-1-antitrypsin (≤54 mg/dL)</th>
<th>Stool alpha-1-antitrypsin (&gt;54 mg/dL)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>6</td>
<td>0.052</td>
</tr>
<tr>
<td>Wasted</td>
<td>16</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Severe wasted</td>
<td>18</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Stool leucocytosis</td>
<td>4</td>
<td>13</td>
<td>0.002</td>
</tr>
<tr>
<td>Stool erythrocyte</td>
<td>3</td>
<td>7</td>
<td>0.002</td>
</tr>
<tr>
<td>Stool fat</td>
<td>5</td>
<td>7</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Enhanced fecal protein loss, as the consequence of intestinal epithelial damage, was observed in children with persistent diarrhea caused by various pathogens (bacterial, viral, or parasitic agents).\textsuperscript{25,26} This study should be extended by elaborating the enteropathogen causing persistent diarrhea, because this study found only one child infected by \textit{E. histolytica} (data not shown), without any data of enteric pathogens. However, signs of overgrowth of enteric bacteria (leucocytosis in stool) in children with persistent diarrhea were observed in this study.

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Selenium and PLE in childhood persistent diarrhea

In particular, the persistence of fecal fungi requires that they be characterised. Therefore, these results must be confirmed in a larger cohort study to appropriately evaluate the effect of the selenium status and its association with AAT first in children grouped according to the different causes of persistent diarrhea (enteropathogen infections, intestinal inflammation, malabsorption, and malnutrition) and second in the population living in countries with a differential selenium status.

**ACKNOWLEDGEMENTS**
The authors thank Dr Nuraini Irma Susanti, SpA(K), and Dr Dedy Rachmat, SpA, for their support for collecting data from Fatmawati Hospital, Jakarta, as well as all cadres and children involved in this study.

**AUTHOR DISCLOSURES**
The authors declare no competing financial interests. This study was funded by the Ministry of Higher Education (DIKTI) of the Republic of Indonesia in regards to Hibah Kerjasama Luar Negeri dan Publikasi Internasional.

**REFERENCES**

5. Kukuruzovic R, Brewster DR, Gray E, Anstey NM. Increased nitric oxide production in acute diarrhoea is associated with abnormal gut permeability, hypokalaemia.

![Figure 1. The vicious cycle of persistent diarrhea - protein-losing enteropathy – malnutrition.](image-url)