Original Article

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Diagnostic Value of Coproantigen for Detection of *Giardia* Infection in Stunted Children

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Abstract

Background: Giardiasis is a protozoa infection caused by *Giardia intestinalis,* which commonly infects children, impairing children's growth, development and cognitive function. Standard diagnosis is carried out by microscopic examination of stool. This study aimed to evaluate coproantigen examination in stunted children compared to microscopic examination.

Methods: A cross-sectional study was performed on stools collected from a survey among stunted children in Bandung in 2019. Stools were preserved in 10% formaldehyde and kept at -20°C until used. Direct microscopy examination with 2% Lugol solution and coproantigen ELISA test using *Giardia Cryptosporidium* (combo test) coproantigen test kit were performed in Parasitology Laboratory Faculty of Medicine, Universitas Indonesia.

Results: A total of 99 stools originated from stunted children aged 2–6 years, with boys predominant (52.5%). Microscopic examination showed that 12.1% (12/99) of the children were harbouring intestinal parasites, such as the protozoa *Giardia intestinalis*, *Blastocystis spp,* and *Entamoeba coli (E. coli)*. *Giardia* was the primary infection (9.1%), of which single Giardia infection (n= 8) and mixed infection of *Giardia* and *Blastocystis spp* (n= 1). Interestingly, coproantigen examination resulted in 6 positive samples, and 4 samples agreed with the microscopy result. With a sensitivity of 44.4% and a specificity of 97.7%. The positive and negative predictive values were 66.7% and 94.7%, respectively.

Conclusion: A moderate prevalence of *Giardia* in stunted children in Bandung regency has been observed. The combo coproantigen test method has high specificity and is suitable for use as a confirmation test to exclude Giardia infection.

Keywords: Coproantigen, diagnosis, *giardiasis*, stunting

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Introduction

Giardiasis is a gastrointestinal disease caused by *Giardia intestinalis* with clinical manifestations as chronic or acute diarrhea, nutritional deficiency, or asymptomatic and more frequently affected children, especially in developing countries.[1] *Giardia* infection occurs due to ingestion of mature cysts through fecal-oral contamination, person-to-person direct contact, animal-to-human, drinking contaminated water/food, and homosexuality.[2] Giardiasis in children may cause failure to thrive due to malabsorption of nutrients during diarrhea; malabsorption of fat, D-xylose, vitamin A, and vitamin B12 has been reported to be associated with *Giardia* infection.[3-6]

Malnutrition in the first 1,000 days of a child's life causes stunted growth and development, which will have an effect later in life when they become adults. The prevalence of stunting in Indonesia was 27.6% in 2019, and in West Java was 31.06% which was higher

than the global and Southeast Asia prevalence, which were 21.3% and 24.7%.[7,8]

Giardia is found in about 20% of patients with diarrhea, contributing to 2.5 million annual deaths worldwide from diarrheal disease. The most prominent symptoms, generally appearing 6–15 days after infection, are steatorrhea, weakness, weight loss, and stomach pain. Most of these are self-limiting, and 30–50% of patients become chronic. Steatorrhea, iron deficiency anemia, micronutrient deficiency, and malnutrition are long-term sequelae that can cause developmental failure and psychomotor retardation in children.[9]

Giardia intestinalis is one of the most easily recognized parasites on fecal examination. Microscopic examination was performed by directly examining of fresh stool or preserved in 10% formaldehyde or polyvinyl alcohol (PVA) for staining. Excretion of cysts into feces varies day by day, which makes microscopic examination have low sensitivity diagnostic value; hence, another detection method with higher sensitivity is required.[10]

The coproantigen examination has advantages compared to standard microscopic examination as it can examine large samples more quickly. This study aimed to evaluate the use of coproantigen examination in stunted children with and without symptoms compared to microscopic examination.

Methods

A cross-sectional study was carried out on fecal samples collected from stunted children in Bandung Regency in 2019 and preserved in 10% formaldehyde, then kept in the -20 °C freezer until used. Stool examination was performed in the Department of Parasitology Laboratory, Faculty of Medicine Universitas Indonesia. This study was part of the study on stunting children in Bandung Regency. The demographic data included age, gender, weight and height, history of persistent diarrhea, source of clean water, and habit defecation were obtained through questionnaires.

Giardia infection was detected by microscopy and examination of *Giardia* antigen in the stools. Microscopic examination by direct wet smear with 2% Lugol solution was carried out by two examiners at 400X magnification. *Giardia* antigen in the stool was detected with the *Giardia Cryptosporidium* (combo test) coproantigen kit (Cortez Diagnostics. Inc, Cat No: 8310-3) following the instructions in the kit manual. Briefly 100 uL faeces were placed in the microwell coated with *Giardia Cryptosporidium* monoclonal antibody and incubated for 60 minutes. After incubation, wells were washed 5 times with washing solution, and then enzyme conjugate was added and incubated again for 30 minutes. Chromogen was added following 5 times the washing step and incubated for another 10 minutes then, stop solution was added and read immediately with an ELISA reader at dual wavelengths 450 nm and 650 nm. Result was interpreted as positive for either *Giardia* or *Cryptosporidium* or both infections when optical density (OD) >0.08. All incubation was carried out at room temperature (25ºC); positive and negative controls were always included in every assay.

Data were interpreted as positive and

Table 1 Intestinal Parasites among Stunted Children in Bandung Regency

Code	Age (year)	Results	
		Microscopic	Copro Ag
10100109	4	*Giardia*	pos
112008303	4	*Giardia*	pos
112008406	5	*Giardia*	pos
132310001	4	*Giardia*	neg
152711603	4	*Giardia*	neg
152711901	3	*Giardia*	neg
173314231	3	*Giardia*	neg
234720606	5	*Giardia*	pos
152711631	5	Neg	pos
152711904	4	Neg	pos
132309803	3	*E.Coli*	neg
152812507	4	*Blastocystis*	neg
173214109	5	*Blastocystis*	neg
112008408	3	*Giardia+ Blastocystis*	neg

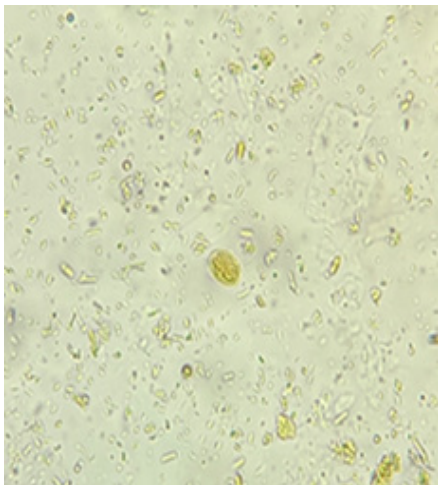

Figure 1 Wet Smear with 2% Lugol Solution Shows Cyst of *Giardia Intestinalis*, 400x Magnification

negative, counted, and presented in tables. Statistical analysis was done using SPSS version 20, with a significant p-value of p <0.05. This study had been approved by the Ethical Committee from the Faculty of Medicine Universitas Indonesia and Cipto Mangunkusumo Hospital No. KET-470/UN2. F1/ETIK/PPM.00.02/2021.

Results

There were 99 stools examined in this study taken from stunted children aged 2–6 years old who lived in Bandung Regency, West Java, Indonesia. Diagnosis of stunting was carried out by a pediatrician based on an assessment of the nutritional status and clinical examination. There were 52.5% and 47.5% stools collected from male and female subjects respectively. Most children were aged 3–5 years old, another two aged 2 years old and 5 children aged 6 years old (data not shown).

Microscopic examination showed 12/99 (12%) of the population harboring intestinal parasites consisted of the protozoa *Giardia intestinalis*, *Blastocystis spp*, and *Entamoeba coli (E.coli)*. *Giardia* was the main infection (9.1%); infected with single *Giardia* (n=8) as well as *Giardia* and *Blastocystis spp.* (n=1)

Coproantigen test showed (6.1%) (n=6) positivity with the OD ranges from 0.283 to 2.518. However, only four samples were in agreement with the microscopic finding of the *Giardia cyst* (Figure 1, Table 1, and Table 2). No *Giardia cysts* or other parasites were observed microscopically in the other two coproantigen positive samples. Other samples that were positive for *Blastocystis spp.* and *E.coli* on microscopic examination, also showed negative coproantigen. Most of the subjects with intestinal parasitosis did not have a history of persistent diarrhea (Table 1).

Further analysis on the diagnostic value of *Giardia Cryptosporidium* coproantigen test against microscopic examination resulted in 44.4% sensitivity, 97.8% specificity, 66.7% and 94.7% for positive predictive value (PPV), and negative predictive value (NPV), respectively.

Discussion

Giardia intestinalis is the common cause of parasitic diarrhea, malabsorption, and failure to thrive in children. However, the diagnosis is still unsatisfactory. This infection may also manifest without any symptoms, thus becoming a carrier. According to WHO, giardiasis is one of the neglected tropical diseases and is distributed more commonly in children in the tropics.[11–13] Most infections are asymptomatic, and when present, symptoms vary from diarrhea to abdominal pain to poor intestinal absorption, and the infection is responsible for retarding the growth and development of individuals. In children, recurrent or persistent giardiasis might lead to serious consequences due to malnutrition, including retarded growth and development and poor cognitive function.

Giardiasis is a waterborne disease, so drinking water plays a major role in *Giardia*

Table 2 Microscopic versus Coproantigen Detection to Diagnose *Giardia* Infection in Stunted Children

Coproantigen	Microscopic		n (%)
	Positive (%)	Negative (%)	
Positive	4 (44.4)	2 (2)	6 (6)
Negative	5 (55.6)	88 (98)	93 (94)
Total	9	90	99

transmission. Water quality is essential in studies of risk factors since studies conducted worldwide have found strong evidence that contaminated water is a risk factor for giardiasis.[10] In this study, a moderate prevalence of *Giardia* infection among stunted children in Bandung regency was evidenced by microscopic examination, while using the *Giardia Cryptosporidium* (combo) coproantigen method, it was even lower (6.1%), and only 4 out of 9 samples positive by both methods. The discrepancy could be due to the amount of antigen produced by the parasite being below or over the threshold limit. The moderate prevalence of *Giardia* infection also occurs among elementary school children in West Sumatra.[14] The nine subjects infected with *Giardia*, were more likely to suffer from chronic giardiasis and be carriers of the disease as they did not show any symptoms except for one person who had a history of persistent diarrhea.

Microscopic analysis is based on the morphological and morphometric characteristics of the parasite and is also related to the experience of the microscopist.[10,15] The diagnostic efficiency of *Giardia* can be improved by examining three fecal samples instead of one, which is difficult in practice. ELISA might be able to detect minimal amounts of antigen, even giving positive results when parasite load is low, by detecting antigens produced during the encystation or trophozoites and cysts stages, or in the prepatent stage before cyst excretion in stool. If the results of coproantigen examination are negative, the use of PCR is able to detect low numbers of *Giardia* up to 10 parasites/100 µL of stool and detect those who are asymptomatic or who have mild infections.[15]

By comparing microscopy as the gold standard, analysis of combo coproantigen ELISA for diagnostic value showed low sensitivity (44.4%) of detection for *Giardia* while the specificity was high (97.8%.). The sensitivity value is the ability of a diagnostic tool to detect positive results in sick subjects, meanwhile the specificity value was negative in non-diseased individuals, indicating that giardiasis could be excluded in 97.8% of patients with a negative result on direct microscopic examination.

Positive predictive value (PPV) is the possibility of someone suffering from the disease when the positive result was 4/6 (66.7%), and negative predictive value (NPV) is the possibility that someone does not suffer from the disease if the test results are negative by (94.7%). These results suggest that the combo coproantigen test is unsuitable for *Giardia* diagnostic screening purpose; however, it could be considered in surveillance or rapid screening of large stool samples and to exclude *Giardia* infection from those negative on microscopic examination. Coproantigen test cannot replace the microscopic examination of faeces for ova and parasites as a routine diagnostic test because the latter can detect other intestinal parasites.[15–17]

Microscopic examination of stool samples for the first examination detects the presence of parasites in only 50–70% of infected patients due to the intermittent release of cysts and trophozoites in the stool.[17] Repeated stool examination increases the percentage by >90%, but the examination is time-consuming and requires a trained microscopist. Furthermore, the sensitivity level can be lower in chronic giardiasis.

The antigen-capture ELISA method for detecting *Giardia* intestinal antigen in stool could help diagnose giardiasis. It was shown that ELISA coproantigen has higher sensitivity over microscopy for *Giardia* diagnosis when a single sample is analyzed.[17] ELISA has detected a higher number of positive samples than conventional microscopy, and it was more time-saving than microscopy when several samples needed to be examined. ELISA is a simple, practical, and highly specific diagnostic test for the detection of *Giardia* antigens. ELISA technique can be used instead of conventional microscopic techniques, and it does not require the observation of intact organisms.[19]

In this study, one-time stool samples were collected, then kept at -20ºC in formaldehyde for >18 months, which may affect the morphology of the parasites and furthermore the coproantigen detection.[20,21] There were two samples positive by coproantigen test. However, no parasites were found on microscopic examination. This could be due to *Cryptosporidium* infection, which was not detected on direct smear, while modified acid fast staining was not performed in this study.

Formalin solution is often used to preserve samples before transporting them to the laboratory. It is shown that there is a decrease in the sensitivity of the coproantigen tested when using formalinized stool, especially for a long time. In this study, pre-analytical steps were carried out following the instructions in the kit manual which allows the use of formaldehyde preserved samples, as well as fresh and frozen samples without affecting the reaction and

results. However, there is no mention of the time frame of preservative allowed without affecting the test performance.[19]

The limitation of this study are the use of long-term storage formalin stools at -20 ^{0}C and did not perform modified acid fast staining to detect *Cryptosporidium* infection, which could explain the discordance between negative microscopy and positive coproantigen result in two subjects.

In conclusion, the prevalence of *Giardia* is 9.1%, which is the main infection in stunted children in Bandung Regency. The combo test coproantigen method has high specificity and is suitable for use as a confirmatory test to exclude *Giardia* infection. This finding among the stunted children should be an alarm to overcome chronic giardiasis in children; therefore, early detection is recommended.

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