

Effectivity of microscopic test as a simple diagnostic method to detect fat malabsorption in children

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ABSTRACT

BACKGROUND Lipid malabsorption causes many health problems, for example stunting, a major worldwide issue. There has not been any assessment on the effectivity of lipid microscopic test in diagnosing lipid malabsorption. This research was aimed to study the effectivity of lipid microscopic test in detecting lipid malabsorption in children.

METHODS This was a cross-sectional diagnostic study that evaluated the effectivity of lipid microscopic test using Sudan III against steatocrit test as the gold standard in diagnosing lipid malabsorption. The study was done in 68 children aged 6–60 months in Cipto Mangunkusumo Hospital, Jakarta. Results of lipid microscopic test were compared with that of steatocrit test among children with lipid malabsorption and normal children. The primary endpoints of this study are the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

RESULTS A total of 68 children consisting of 41 boys and 27 girls were included, with a median age of 14.3 months. The most frequently found stool consistency was mushy (50%). The most common result of microscopic test, found in 42% of subjects, was positive 1. Sensitivity, specificity, PPV, and NPV of lipid microscopic test were 49.15%, 66.67%, 90.63%, and 16.67%, respectively.

CONCLUSIONS Lipid microscopic test has a moderate sensitivity in diagnosing fat malabsorption and needs to be complemented with other methods such as steatocrit.

KEYWORDS children, feces, lipids, malabsorption syndromes

Stunting, a failure to reach potential growth, results from chronic undernutrition and is correlated with many aspects of children's life, such as cognitive, motor, and language development.¹ The most important causes of stunting is nutrient malabsorption.² Lipid, as a major constituent of macronutrients, has several benefits, such as source of energy, one of the main structural component of cell membrane and hormones, and media for lipid-soluble vitamins. Lipid also has an important role as the main source of nutrition for human brain development, so that it is important to detect lipid malabsorption accurately.

Various tests are available to detect lipid malabsorption in feces, consisting of qualitative, semiquantitative, and quantitative tests. In Indonesia, fecal analysis is the method commonly used to detect lipid malabsorption, and in Cipto Mangunkusumo Hospital, the numbers are approximately 70 tests per month or 840 tests annually. Lipid malabsorption is one of the most common results found in fecal analysis. The clinical implication of this test is quite significant because lipid is the main source of energy other than carbohydrate in children's growth and development. Lipid is also the main component of cell membrane and an essential part of children's brain development.

It is an important media for the absorption of lipid-soluble vitamins A, D, E, and K. Thus, it is essential to diagnose lipid malabsorption accurately.

van de Kamer test using a 72-hours fecal collection is the gold standard for evaluating lipid malabsorption. However, this test requires a relatively high cost, resources, and time to perform and thus is less practical to be implemented in daily practice. This test was replaced by steatocrit, which has 100% sensitivity, 95% specificity, and 90% positive predictive value (PPV) to detect steatorrhea and to evaluate fecal lipid quantitatively in more practical way.³ This is something that could not be evaluated by microscopic method, which is only semiquantitative. A similar study evaluating the value of steatocrit test was done by Satari,⁴ which results in 88.2% sensitivity and 88.9% specificity. It was then concluded that steatocrit test was valid and could be widely used to detect steatorrhea. Unfortunately, the steatocrit test is not used routinely, even though steatocrit is a simple, rapid, and accurate modality to detect lipid malabsorption. The semiquantitative method is the only test available in most hospitals, including the ones in the remote areas.

There has been no study about the effectivity of lipid microscopic test in diagnosing lipid malabsorption. Because of the high volume of this test, along with its clinical value and detrimental effect of lipid malabsorption, it is important to evaluate whether microscopic test is good enough as an alternative of steatocrit test to determine lipid malabsorption in children. If microscopic test is proven to be a liability, then reimplementing of steatocrit test as a routine examination to detect lipid malabsorption is a must. This study was aimed to evaluate the effectivity of lipid microscopic test in detecting lipid malabsorption in children.

METHODS

This study was designed to compare the accuracy of fecal lipid microscopic test against steatocrit test in detecting lipid malabsorption in children. This study was performed between April 1st and June 30th, 2015, in Cipto Mangunkusumo Hospital. Subjects within 6–60 months of age and indicated to undergo fecal analysis based on history and physical examination were included. Those who used suppositories or mineral oil within 24 hours before or during fecal material

collection, used oily substances in the anal area during fecal material collection, and with incomplete data were excluded. This study was approved by the Ethical Research Committee Faculty of Medicine, Universitas Indonesia on February 9, 2015 (No: 113/UN2.F1/ETIK/2015).

Subjects were selected consecutively. After performing history taking and physical examination, the subjects' guardians were asked to fill out the informed consent and research questionnaire. Fecal samples were then collected and examined under the microscope using the Sudan III with Drummey method.⁵ Steatocrit test was also performed on the same fecal sample. The primary endpoints of this study were the sensitivity, specificity, PPV, and negative predictive value (NPV) of lipid microscopic test compared to steatocrit test as the gold standard.

The result of lipid microscopic test was categorized semi-quantitatively as positive 1 (+1), positive 2 (+2), or positive 3 (+3). Positive 1 category was defined as the presence of less than 100 small lipid cells per power field or if the lipid cells occupied one-third to half power field. Positive 2 category was defined as the presence of more than 100 lipid cells per power field or if the cells occupied more than half-power field. Lastly, positive 3 category was defined as the presence of lipid cells across all power fields. In addition to this, the result of lipid microscopic test was also further categorized into those without lipid malabsorption, which included negative and +1 results, and those with lipid malabsorption, which included +2 and +3 results.

The cut-off for the normal steatocrit test result used in this study was 0–4%. Those with >4–10%, >10–25%, and >25% results were categorized as mild, moderate, and severe lipid malabsorption, respectively as modified from Satari.⁶ Like the lipid microscopic test, the steatocrit test result was also further categorized into two groups: without (normal and mild lipid malabsorption) and with lipid malabsorption (moderate and severe malabsorption).

Univariate and bivariate analyses were also done in selected variable. The univariate analysis was done to obtain the frequency or proportion based on the analyzed variables. These variables were age, gender, and fecal matter consistency. The data were processed using SPSS software, version 13 (SPSS Inc, USA). Bivariate analysis was done using Spearman's test to study the correlation between the lipid

microscopic and steatocrit tests. Kolmogorov–Smirnov test was used to determine the normality of data distribution.

RESULTS

A total of 68 fecal analyses of children between April 1, 2015, and June 30, 2015, at Clinical Pathology laboratory, Cipto Mangokusumo Hospital, which fulfilled the inclusion and exclusion criteria, were included in this study (Figure 1). Of the 68 children, 55 had gastrointestinal symptoms and were indicated for fecal analysis, whereas the remaining 13 were healthy individuals that were included as a control group.

The ratio of men and women in this study was 1.5:1, and the median age was 14.3 (6–60) months old. Table 1 shows the demographic and clinical characteristics of the subjects. Most of these subjects (42%) were 6–12 months old. Among all subjects, 50% had soft and mushy feces, whereas the fecal consistency of all healthy children was solid.

The evaluation of lipid malabsorption by fecal analysis was done using the microscopic method,

in which around half of the subjects had lipid malabsorption. Based on the steatocrit test, the majority of subjects were found to have moderate lipid malabsorption (60%). Further classification of the result of the steatocrit test revealed that almost all subjects (87%) had lipid malabsorption.

Microscopic test result was compared against steatocrit test to study its diagnostic performance (Table 2). The sensitivity, specificity, PPV, and NPV were 49.15% (95% confidence interval [CI] = 35.89–62.5), 66.67% (95% CI = 29.93–92.51), 90.63% (95% CI = 74.98–98.02), and 16.67% (95% CI = 6.37–32.81), respectively. Spearman’s correlation test comparing both modality of examinations revealed no correlation between them ($r = 0.177$; $p = 0.148$).

DISCUSSION

Stunting, which occurs in one of five children, is a major worldwide problem. It is a condition caused by many factors, and one of those factors is malabsorption syndrome.^{1,2} Lipid, which plays a major role in malabsorption syndrome, could cause long-term problem, such as malnutrition, lipid-soluble vitamin

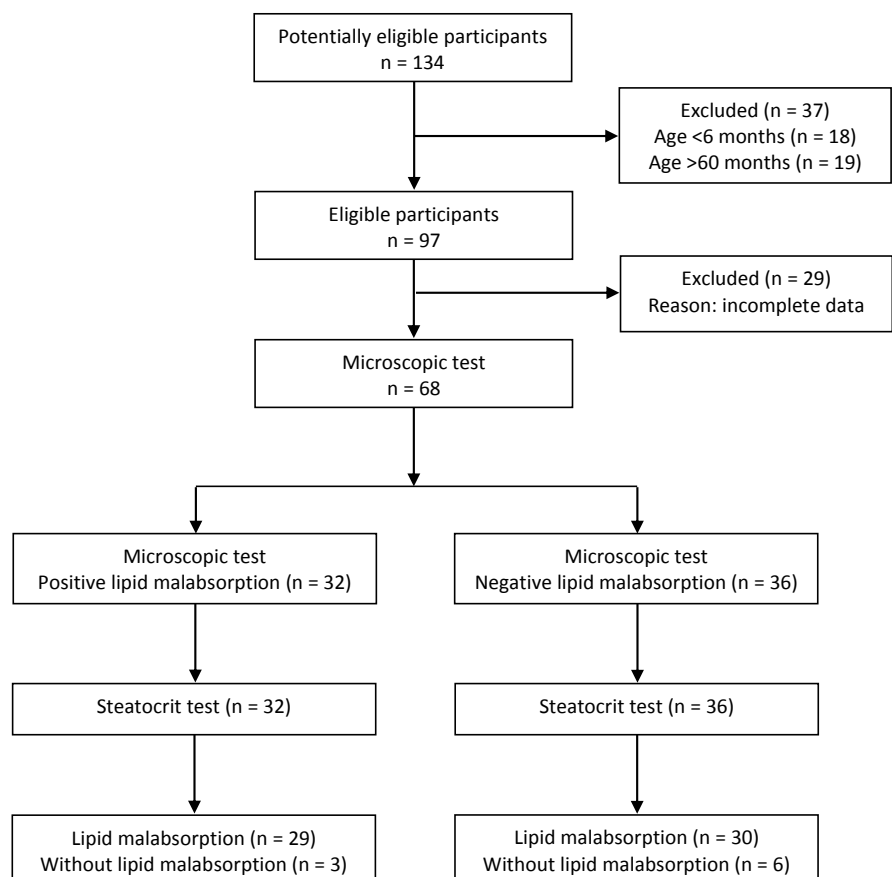


Figure 1. Subjects selection

Table 1. Demographic and clinical characteristic of research subjects

Characteristics	n (%) (N = 68)
Male sex	41 (60)
Age (months)	
6–12	29 (42)
12–24	15 (22)
24–36	12 (18)
36–48	8 (12)
48–60	4 (6)
Feces consistency	
Watery	8 (12)
Soft-watery	10 (15)
Soft	34 (50)
Soft-solid	1 (1)
Solid	15 (22)
Steatocrit test	
Normal	0 (0)
Mild lipid malabsorption	9 (13)
Moderate lipid malabsorption	41 (60)
Severe lipid malabsorption	18 (27)
Lipid microscopic test	
Lipid malabsorption	32 (47)
Without lipid malabsorption	36 (53)

Table 2. Diagnostic comparison of microscopic examination and steatocrit test (N = 68)

Microscopic	Steatocrit	
	Lipid malabsorption	Without lipid malabsorption
Lipid malabsorption	29	3
Without lipid malabsorption	30	6

deficiency, decrease in hormone production, and growth and developmental problem.⁷ Berstad et al⁸ said that intestinal malabsorption is a serious condition that often goes undetected because of methodological issues.

Quantitative biochemistry test of fecal lipid contents is a procedure that has been done since van de Kamer revealed the methods for the first time, by collecting all of the feces within 72 hours and quantifying the amount of lipid in the sample.⁹ This method was considered as the gold standard, although it has several shortcomings. It could be due to incomplete collection of feces or inconsistent patterns of defecation, which could yield a false-negative

result.¹⁰ A marker was developed to overcome this shortcoming, but it is still time-consuming and needs tight supervision, especially in children.

Fecal lipid microscopic test is a simpler method to detect steatorrhea in our daily clinical setting. Drummey et al⁵ demonstrated a good correlation between fecal microscopic test and quantitative measurement in adults. The Drummey method has been studied numerous times, and it resulted in various sensitivity and specificity levels. Amann et al³ showed that the qualitative (microscopic) lipid examination has a 78% sensitivity and 70% specificity. Teh et al¹¹ tried to modify the Sudan III coloration with Oil Red O, and the microscopic test results in 72.2% sensitivity and 95.4% specificity.

In this study, most of the patients tested for fecal analysis were children age 6–12 months (42.6%). Based on the lipid microscopic test, most of these results were +1 in 29 subjects (42.6%) and followed by +2 in 26 subjects (38.2%). These results were further classified into groups with and without malabsorption. The ratio between these two groups were quite similar (52.0% vs. 47.1%). Based on the steatocrit test, most of the subjects (41 subjects or 60.3%) were classified into moderate lipid malabsorption group. Further classification of the steatocrit test results (with or without lipid malabsorption) showed that most of the subjects fell into the lipid malabsorption category (87%). Fecal lipid microscopic test has 49.15% sensitivity and 66.67% specificity in detecting lipid malabsorption in children aged 6–60 months. The PPV and NPV were 90.63% and 16.67%, respectively. Thus, lipid microscopic test has a moderate sensitivity in diagnosing fat malabsorption.

Maranhão and Wehba¹² compared the result of the fecal lipid test by using the van de Kamer, Sudan III, and steatocrit in 50 children. Steatocrit showed a good correlation compared with the van de Kamer method, with 91% sensitivity and 87% specificity from feces sample taken before the 72-hours feces were homogenized. In a similar situation, the microscopic test with Sudan III shows 73% sensitivity and 69% specificity. The PPV of the microscopic test in this study is quite high, reaching 90.63%, which means that microscopic test can predict the presence of lipid in feces well; however, it has a low NPV (16.67%), and thus, it is not reliable enough to declare the absence of lipid.

Two of the most common results found in the steatocrit test were moderate (60%) and severe

malabsorption (27%). This trend may be caused by the selection criteria, which included patients who were indicated to have the test, and most of these patients suffer from diarrhea. However, no patient was found to have a steatocrit level of 0–4%, even in the control group. This showed that most of the subjects, including healthy children, have lipid malabsorption. Lipid excretion in feces is influenced by the intake of fat.¹³ Several studies showed that Indonesian children consumed more fat than the recommended dietary allowance.^{14,15} Thus, it is highly possible that the excess of fat contributes to the presence of lipid malabsorption in this study.

Even though we hypothesized that an excess of fat intake is the cause of lipid malabsorption in this study, the analysis of lipid intake was not performed. Bijoor et al¹⁶ used the acid steatocrit method in a random feces sample in 600 healthy adults in India and discovered that lipid excretion in Indian adults is higher compared with that of in the westerners (8.72 ± 1.86 g versus 7 g in 24 hours). However, the lipid microscopic test with the Drummey method in these 600 samples did not show any abnormality. It could be inferred that the lipid microscopic test might not be able to adequately represent the state of lipid excretion.

Nakamura and Takeuchi¹⁷ understand that healthy adults in Japan have an amount of fecal lipid <5 g/day. It is quite different from the findings of Bijoor et al,¹⁶ as stated earlier. This is probably caused by a difference in culture and ethnicity involving the type of food in their diet. For Indonesian, especially in children aged 6 to 60 months old, the difference in the lipid composition supposedly did not differ that much from the common diet.

Steatocrit is a semiquantitative method used to determine the amount of lipid in feces, as proposed by Phuapradit et al.¹⁸ This test shows excellent sensitivity and specificity and thus was used as a gold standard in this study. Amann et al³ then modified this method by adding acetate acid, and it produced a better result. The acid steatocrit test showed a linear correlation with quantitative fecal lipid in 72-hours fecal matter ($r = 0.761$; $p < 0.001$). The acid steatocrit test in random feces has 100% sensitivity, 90% specificity, and 90% PPV to detect steatorrhea compared with 72-hours quantitative fecal lipid test. Van den Neucker et al¹⁹ studied the excretion of fecal lipid and acid steatocrit in 42 subjects: half of it with lipid malabsorption and

the other half without lipid malabsorption. The acid steatocrit test has a good correlation with fecal lipid excretion ($p < 0.01$) or fecal lipid concentration ($p < 0.01$). The acid steatocrit sensitivity and specificity in diagnosing lipid malabsorption were 90% and 100%, respectively. Tran et al²⁰ also studied the effect of fecal acidification on steatocrit result in children. This study was based on the idea that centrifugation of fecal homogenates into lipid phase, water phase, and solid phase is a pH-dependent process. They proved that the result of the steatocrit test improved with the acidification of feces, and the optimum result was obtained with the lowest pH. The acid steatocrit test is better than the regular steatocrit test. Sugai et al²¹ compared the lipid composition of 148 feces with the conventional van de Kamer method and steatocrit. Steatocrit shows 87% sensitivity and 97% specificity with a 97% PPV and 87% NPV. However, when the evaluation was confined to feces with lipid excretion of >20 g/day, the sensitivity increased to 98%. A linear correlation was found between steatocrit and quantitative chemistry method ($r = 0.80$; $p < 0.001$). Sugai et al²¹ said that steatocrit is a simple, rapid, cheap, and reliable semiquantitative method to detect lipid malabsorption.

Tran et al²² in another study claimed that steatocrit value as a screening test for steatorrhea still showed different results. They modified the procedure by acidifying the feces and then comparing it to Sudan III (microscopic) with the Drummey method. They concluded that acid steatocrit shows a better result than the regular steatocrit because the acidification of the feces increased the extraction of lipid and thus the reliability of the steatocrit method to detect steatorrhea. We were initially planning to use the acid steatocrit method, but then, our initial study with 28 samples found no difference between regular steatocrit and acid steatocrit. Thus, we decided to use regular steatocrit, which is more practical, simpler, and cheaper in its material and execution.

The study sample consists of 41 boys and 27 girls. The median age of the research subjects was 14.3 months. The data distribution for this variable was quite heterogeneous, with the aforementioned age range between 6 and 60 months old. The lower limit of 6 months was chosen because in newborn babies, there may be some immaturity in the pancreas function, especially the production of lipase, which may result in neonatal physiologic steatorrhea.

Meanwhile, the upper limit of 60 months (5 years old) was chosen because it was the golden period for brain development, which could be affected by lipid malabsorption. A similar study was done by Ghosh et al²³ who tested the fecal lipid content by the microscopic method. This study included 100 children comprising 46 men and 54 women ranging from 11 days to 15 years old. To the authors' best knowledge, this was the first study to evaluate lipid malabsorption, specifically in children under 5 years old.²³

The birth weight, current weight, and diet or milk consumption variable were part of this study to be analyzed. Unfortunately, many of these variables were missing throughout most subjects. So, the variables mentioned above were not included in the final analysis. However, omitting the subjects with incomplete data will potentially cause selection bias. To prevent it, we decided against omitting the subjects, and thus, we did not analyze the incomplete variables.

Specifically, this study has an advantage as an operational study, so the result could be applied as part of hospital services. This study showed that using lipid microscopic test alone to diagnose fat malabsorption is not enough. The test should be complemented with other methods such as steatocrit. Fecal lipid test is often performed on a random stool sample, therefore explaining the relative inadequacy to diagnose fat malabsorption. It is also subject to observer variation, increasing the probability of a missed or false-negative result. With that in mind, we suggest performing multiple examinations of lipid microscopic tests by different people for every request of the test, even though it will be much more time-consuming. Further study could be done to evaluate whether this strategy will strengthen the value of lipid microscopic examination.

Another possible method to increase the diagnostic accuracy of lipid malabsorption is to reintroduce the steatocrit test as a routine examination. Steatocrit can be used to assess steatorrhea in pancreatic insufficiency (such as in chronic pancreatitis) and intestinal malabsorption. The test may uncover a large proportion of patients with subclinical lipid malabsorption, especially those who are affected as a result of chronic pancreatitis, therefore limiting the impact of lipid malabsorption in these groups of children.²⁴ Reimplementing steatocrit test in each hospital in Indonesia, especially the tertiary referral hospital, maybe one of the most important steps to

reduce the impact of lipid malabsorption, particularly stunting. The value of steatocrit as a quantitative test cannot be replaced by a microscopic test as a semiquantitative test. Besides the obvious superiority of its diagnostic value, steatocrit can also be used to evaluate the result of interventions.

This study has some limitations. First, part of the feces which was taken for lipid microscopic test and steatocrit examination could be varied, although taken from the same sample. This can cause variation in the result of the test. Secondly, diet analysis was not performed in this study, thus limiting the interpretation of the study data. For future study, we suggest including diet analysis as part of study protocol.

In conclusion, this study showed that lipid microscopic test has moderate sensitivity and specificity in detecting lipid malabsorption in children. Therefore, the test needs to be complemented by other methods of lipid examination, such as the steatocrit test. Future studies should focus on the evaluation of repeated lipid microscopic examination of a stool sample or the concurrent use of steatocrit and lipid microscopic tests in diagnosing lipid malabsorption in children.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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