ORIGINAL ARTICLE

The Role of Laboratory Diagnosis in Children with Chronic Diarrhea

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ABSTRACT

Background: *Etiology of chronic diarrhea can be established through non-invasive examination such as stool analysis and stool culture. Colonoscopy is an invasive method, which is occasionally needed to discover the etiology of chronic diarrhea.*

Objective: *To recognize the characteristics of chronic diarrhea based on stool examination and colonoscopy results.*

Method: *Descriptive study on patients with chronic diarrhea who came to Cipto Mangunkusumo Hospital since 1 June to 31 August 2005. Laboratory tests were conducted in accordance with clinical indication. Data was presented in distribution tables.*

Results: *There were 41 patients with chronic diarrhea. Stool analyses were performed only in 38 patients with negative-Gram infection (86.8%). Stool cultures were performed in 27 patients with positive results of non-pathogenic E. coli (85.2%). Stool parasite analyses and concentration tests were performed in 17 patients, with 47.0% positive results as follow: Microsporidia 29.4%, Blastocystis hominis 11.8% and Giardia lamblia 5.9%. Colonoscopy examinations were performed in 6 patients and all patients indicated ulcerative colitis appearance with 50% histopathological impression of infective colitis.*

Conclusion: *Stool analysis in chronic diarrhea primarily indicates positive infection. Bacterial stool culture mostly includes non-pathogenic E. coli, while parasite stool culture largely includes Microsporidia. Biopsy examination tends to reveal infective colitis.*

Keywords: *chronic diarrhea, parasite stool, colonoscopy*

INTRODUCTION

Chronic diarrhea is defined as diarrhea which has occurred for more than 14 days and if it is caused by infection, then it is known as persistent diarrhea. The incidence rate of persistent diarrhea in some developing countries is ranged between 3-23%.[1,2] In order to establish diagnosis and to provide treatment of chronic or persistent diarrhea, we need a simple and non-invasive examination such as stool analysis. It should be continued with stool culture and stool parasite analysis to discover the etiologic pathogenic bacteria or parasite.[3,4] Invasive examination such as colonoscopy is occasionally needed in order to discover infective colitis grossly and to obtain colon biopsy at once to differentiate infection and non-specific inflammation.[5] This study is aimed to recognize the result of stool examination and colonoscopy in patients with persistent diarrhea in Department of Pediatrics Cipto Mangunkusumo hospital for three months period.

MATERIALS AND METHODS

This study is a descriptive study in patients with chronic diarrhea who were hospitalized at the Pediatric Gastroenterology Ward or had been cared at Pediatric Gastroenterology Outpatient Clinic in Cipto Mangunkusumo Hospital, Jakarta, since 1 June 2005 to 31 August 2005. Laboratory tests were conducted in accordance with proper clinical indication. Stool analysis and breath hydrogen test were conducted at Pediatric Gastroenterology Laboratory and were interpreted by Consultants of Pediatric Gastroenterology. Stool cultures were performed at Eijkman Laboratory and parasite stool analysis and concentration Test were conducted at Parasitology Laboratory. Colonoscopy and colon biopsy were conducted by Consultant of Pediatric Gastroentero-hepatology at Department of Pediatrics, Cipto Mangunkusumo Hospital. Biopsy results were sent to Department of Pathology Anatomy in medium of formalin solution.

Diagnosis of cow's milk allergy was established based on benzidine test, symptoms recovery, and increased body weight after elimination. HIV serologic examinations were based on risk factors in one or both parents and were conducted on permission of both parents. A patient was regarded as HIV patient if there was a positive result in three reactive screening examinations by using kit produced by Uniform, Behring and Abbot. Data was presented in distribution tables.

RESULTS

In 3 months period (1 June 2005 - 31 August 2005), we had managed 41 patients with chronic diarrhea. Stool analysis may have been performed only in 38 patients, with 86.8% intestinal infection of negative-gram bacteria, 42.1% intestinal fungal infection, 10.5% fat malabsorption, 7.9% lactose malabsorption, 18.4% possibility of overgrowth bacteria, and 34.2% possibility of cow's milk allergy. There were 83.3% positive benzidine test of 18 examined patients (table 1). Breath hydrogen tests were performed in 7 patients, which indicated results of no signs of overgrowth bacteria or lactose malabsorption (the table is absent).

Table 1. The result of stool analyses

	Stool Analyses	Total	%
Type of abnormality N = 38	Intestinal infection by negative-gram bacteria	33	86.8
	Intestinal fungal infection	16	42.1
	Fat malabsorption	4	10.5
	Lactose malabsorption	3	7.9
	Possibility of overgrowth bacteria	7	18.4
	Possibility of cow's milk allergy	13	34.2
Benzidine test N = 18	Positive	15	83.3
	Negative	3	16.7

Note: One patient may have more than one abnormality

Stool cultures were performed in 27 patients with 85.2% positive result, i.e. 29.6% *Enterobacter aerogenes*, 48.2% non-pathogenic *E. coli*, 3.7% *Proteus mirabilis* and 3.7% combination of non-pathogenic *E. coli* and *Proteus mirabilis* (table 2). Parasite stool analysis and concentration test were performed in 17 patients with 47.0% positive results, i.e. 29.4% Microsporidia, 11.8% Blastocystis hominis, 5.9% of both combination and 5.9% Giardia lamblia. (table 3). Colonoscopy examinations were performed in 6 patients with 100% macroscopic results of ulcerative colitis (the table is absent). The result of pathology anatomy examination of colon biopsy indicated 50% infective colitis result and 50% suspected results of infective colitis and eosinophilic colitis (table 4).

Table 2. The Result of Stool Cultures

The results of stool cultures	Total	%
Non-pathogenic *E. Coli*	13	48.2
Enterobacter Aerogenes	8	29.6
Proteus mirabilis	1	3.7
Non-pathogenic *E. Coli* and *Proteus mirabilis*	1	3.7
Negative	4	14.8
Total	27	100.0

Table 3. The Result of Parasite Stool Analyses and Concentration Test

Parasite Stool Analyses and Concentration Test	Total	%
Microsporidia	5	29.4
Blastocystis hominis	2	11.8
Both combination	1	5.9
Giardia lamblia	1	5.9
Negative	8	47.0
Total	17	100.0

Table 4. The Result of Pathology Anatomy Examinations

Pathology Anatomy Results	Total	%
Infective Colitis	3	50.0
Infective colitis with unexcluded possibility of eosinophilic colitis	3	50.0
Total	6	100.0

Of 8 HIV patients, only 6 patients had stool examinations because 1 patient had died before the examination performed and the other patient had no parents' permission. Stool cultures were positive in 5 patients and parasite stool examination were positive in 4 patients. (table 5)

Table 5. Stool culture and parasite stool analysis in HIV patients based on age

Age (year)	Stool Culture				Stool Parasite		
	Negative	*E. aerogenes*	*E. coli*	*P. mirabilis*	Negative	Microsporidia	B. hominis
0 – 1	-	1	1	-	-	-	2
1 – 3	1	1	-	1	2	1	-

3 – 5	-	-	1	-	-	1	-
Total	1	2	2	1	2	2	2

Of 13 patients who were suspected with cow's milk allergy, they all showed positive benzidine result and 5 patients had positive stool cultures of 6 examined patients, while the other 7 patients had not continued the examination because they were likely to have diagnosis of cow's milk allergy (table 6). Of 5 patients who had positive results, there were non-pathogenic *E. coli* bacteria in all cultures (the table is absent).

Table 6. The result of stool culture and benzidine test in patients with cow's milk allergy based on age

Age (Year)	Stool Cultures			Benzidine Test	
	Positive	Negative	No	Positive	Negative
0 – 1	5	-	4	9	-
1 – 2	-	1	3	4	-
2 – 3	-	-	-	-	-
Total	5	1	7	13	-

DISCUSSION

When the stool sample is available, the wet specimen can be made by using NaCl solution or methylen blue, routine gram examination can also be performed. The existence of leukocytes, erythrocytes, trophozoites and parasite cyst should be evaluated. Positive leukocytes are evidence of inflammation component in stool.[6] Decreased pH and positive reduction materials in stool characterize carbohydrate/lactose malabsorption, normal stool pH > 5.5, and no carbohydrate/lactose in it. Positive occult blood may be a colitis because it is sensitized by milk protein.[3] In the present study, there was 86.8% intestinal infection of negative-gram bacteria and 34.2% of possible cow's milk allergy. If there is leukocyte in stool, the stool culture should be performed for intestinal pathogen. High fever (> 39^0C) in infants is another indication to perform stool culture.[7] Similar to stool culture, the parasite stool examination is indicated for patients with suspected parasite infection by the history taking such as immuno-compromized patient, history of traveling, homosexual, persistent diarrhea and diarrhea with no response to antibiotics.[8] A report of Alberta Children Hospital in 1995 indicated that culture examination and stool parasite examination in hospitalized patient or outpatient should only performed once. Most or 98% of child enterocolitis can be confirmed from the first stool culture and rarely need repeated cultures. In addition, 91% parasite infection can be found in the first parasite stool examination.[9] Valman HB wrotes that children less than one year of age with poor percentage increase of body weight should have parasite examination including Giardia and pathogenic stool culture such as *Escherichia coli.*[10]

Teo M et al in a study of chronic diarrhea causes in South Australia found that 48% cause of chronic diarrhea is overgrowth bacteria in intestinal lumen through duodenal fluid culture which obtained by endoscopy.[11] This study indicated that possible overgrowth bacteria was based on the result of stool analyses in patients with chronic diarrhea history, of 8.4%.

Breath hydrogen test using 20% lactose solution in 7 patients with chronic diarrhea in the present study showed no evidence of overgrowth bacteria or signs of lactose malabsorption. This may be caused by prior antibiotic treatment before admission to Cipto Mangunkusumo hospital. The breath hydrogen test is best performed in patients free of prior antibiotics treatment 2-3 days before test. A study in Adelaide Children hospital, South Australia found that of 9 patients with chronic diarrhea and abdominal pain in 2-34 months of age, all were caused by overgrowth bacteria and lactose malabsorption, proven by breath hydrogen test. After antibiotics treatment in accordance with duodenal fluid culture, the repeated breath hydrogen test showed normal result.[12] A study by Lee WS and Boey CCM in Kuala Lumpur showed that of 27 children with chronic diarrhea, there were 8 children with cow's milk allergy, 4 children with secondary lactose malabsorption and 7 children with viral, bacterial and parasite infection and the other children had other causes. [13] The result of other stool analyses in this study was 10.5% of microscopic fat malabsorption.

A study in Islamabad, Pakistan evaluated lipid absorption in patients with persistent diarrhea and normal children aged 3 months – 2 years by using moderate chain of triglycerides (trioctanoin), which was labeled by using atom ^{13}C. The study indicated that triglycerides intestinal absorption is not significantly affected in patients with persistent diarrhea.[14] Firmansyah et al, in microbiologic study in 136 patients aged < 5 years with persistent diarrhea, in 1991-1992 found that the main pathogen include 17.6% rotavirus, 2.2% salmonella, 5.1% enterotoxigenic *E. coli*, 1.4% shigella, 0.7% campylobacter and 2.2% *Entamoeba histolytica*.[15] Some pathology as the etiology of chronic diarrhea infection include virus (*rotavirus, adenovirus* and *norwalk virus)*, bacteria (*Campylobacter jejuni, Escherichia coli, Salmonella Enteritidis, Shigella, Clostridium difficile)*, parasites (G*iardia lamblia*, C*ryptosporidium*, M*icrosporidia,* B*lastocysis hominis)* and helminths.[3] Some etiologies that cause diarrhea in HIV patients include parasite (*Microsporidia, Cryptosporidium, Isospora belli*, *Cyclosporeae, Giardia lamblia* and *Entamoeba histolytica),* bacteria (*Salmonella, Shigella, Clostridium difficile, Adherent Escherichia coli),* fungi (C*andida spp)* and virus (*Cytomegalo, Herpes simplex, rotavirus, astrovirus and enterovirus)*.[16,17] The stool culture and parasite stool analysis in the present study indicated bacteria (*Enterobacter* a*erogenes, non-pathogenic E coli)* and parasites (*Microsporidia, Blastocystis hominis* and *Giardia lamblia).* Because of our limitation in technology, therefore it is difficult to discover other bacteria, parasites and other virus. Colonoscopy was indicated if the symptomatic treatment showed no response or no improvement of diarrhea symptom. Biopsy of colon mucosa should be evaluated against *Cytomegalo virus, M avium complex, Adenovirus*, *Herpes simplex* virus and fungi. Patients should also has gastroduodenoscopy examination and biopsy in order to evaluate virus, microbacteria, fungi and parasite.[16] Microsporidia, an intracellular obligate parasites, has spore form, which causes moderate to severe diarrhea in children. *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are 2 species of microsporidia, which commonly found in HIV patient. merupakan 2 spesies microspsoridia yang sering ditemukan pada pasien HIV. Similar to Cryptosporidium parvum, it is transmitted through fecal-oral transmission. It is reported that

microsporidia causes up to 7% infection in HIV children with acute or chronic diarrhea in Thailand.[18]

Walker-Smith applies the term of post-enteritis enteropathy for acute diarrhea which is more than 2 weeks, that causes persistent damage to the intestine. Such enteropathy may occur because of persistent infection by the same pathogens, re-infection by other pathogens and sensitization of food antigens, especially against cow's milk, which in some children there are delayed recovery because of cow's milk intolerance. Enteropathy caused by sensitivity against the cow's milk may be overlapped by infective enteropathy because both may occur concomitantly.[19] Persistent diarrhea usually correlates or concomitant with lactose or cow's milk protein intolerance, but the actual incidence rate is unknown.[20] Lactose and cow's milk protein intolerance may occur separately or concomitantly.[21] Both conditions are secondary to mucosa damage caused by infection, malnutrition or allergic reaction against cow's milk or other protein.[22] Some studies in India and Brazil which were conducted in hospitals found that about 28-64% malnourished infants with persistent diarrhea had lactose intolerance, and 7-35% had cow's milk allergy.[23,24] In the present study, there was 83.3% positive stool culture in patients with cow's milk allergy. Valman HB wrotes that the prevalence of cow's milk allergy varies among investigators but it is estimated that 1 among 1,000 infants has diarrhea before 6 months of age and the intolerance recover after 2 years of age. Establishing diagnosis by eliminating cow's milk product and provoking infants with 5 ml cow's milk should be conducted under doctor's supervision in hospital care unit. If diarrhea occurs in the first 48 hours then the diagnosis of cow's milk allergy can be established. Infants with cow's milk intolerance may also experience secondary lactose intolerance.[10]

CONCLUSION

Stool analysis in persistent diarrhea essentially indicated an infection (leukocytes). The most bacteria in stool culture is non-pathogenic *E. coli* and parasite stool examination mostly reveals Microsporidia. Colon biopsy tends to reveal infective colitis. In HIV patients, the stool culture reveals *E. aerogenes*, non-pathogenic *E. coli* and *P. mirabilis*, and parasites such as Microsporidia and *B. hominis*.

REFERENCES

1. Departemen Kesehatan RI Ditjen PPM & PLP. Buku Ajar Diare 1995.h.93-8.
2. WHO CDD Programme and The Applied Diarrhoeal Disease Research Project (ADDR). Clinical Update: Persistent Diarrhoea 1992.
3. Bentley D, Lifschitz C, Lawson M. Acute and Chronic Diarrhea Pediatric Gastroenterology and Clinical Nutrition 2001.
4. Leung AK, Robson WL. Evaluating the child with chronic diarrhea. Am Fam Physic Chronic Diarrhea 1996:53(2);635-43.
5. Casburn-Jones AC, Farthing MJ. Management of infectious diarrhea. Gut 2004;53:296–305.
6. Hoshiko M. Laboratory diagnosis of infections diarrhea. Pediatr Ann 1994;23:570-74.
7. DeWitt TG, Humphrey KF, McCarthy P. Clinical predictors of acute bacterial diarrhea in young children. Pediatrics 1985;76:551-56.
8. Bitterman R. Acute gastroenteritis and constipation. In: Rosen P, Barkin RM, et al, eds. Emergency Medicine: Concepts and Clinical Practice. 3rd ed. St. Louis: Mosby Year Book 1992:1533-77.

9. Alberta. Clinical practice guidelines program working group. Laboratory guideline for ordering stool test for investigation of suspected infectious diarrhea. Infectious Diarrhea - March 1997 Reviewed 2002.
10. Valman HB. Chronic diarrhea. BJM 1981:282;2120-22.
11. Teo M, Chung S, Chitti L, Tran C, Kritas S, Butler R, Cummins A, Small bowel bacterial overgrowth is a common cause of chronic diarrhea. J Gastroenterol Hepatol 2004:19;904–09.
12. Davidson GP, Robb TA, Kirubakaran CP. Bacterial contamination of the small intestine as an important cause of chronic diarrhea and abdominal pain diagnosis by Breath Hydrogen test. Pediatrics 1984:74(2);229-35.
13. Lee Ws, Boey CCM, Chronic diarrhea in infants and young children: Causes, clinical features and outcome, J Paediatr Child Health 1999:35;260-63 .
14. Abbas KA, Bilal R, Sajjad MI, Latif Z, Mirza NH. Fat absorption in persistent diarrhoea using 13C-labelled trioctanoin breath test. J Tropical Pediatrics 1999:45(2);87-94.
15. Firmansyah A, Pujarwoto S, Siboro M. Penelitian mikrobiologi pada diare persisten. Konika IX Semarang 13-17 Juni 1993.
16. DuPont HL, Marshall GD. HIV-Associated diarrhea and wasting. Lancet 8/5/95, Vol.346 Issue 8971;352-57.
17. Liste MB, Natera I, Suarez JA, Pujol FH, Liprandi F,and Ludert Je. Enteric virus infections and diarrhea in healthy and human immunodeficiency virus-infected children. J Clin Microbiol 2000:38(8);2873–87.
18. Leelayoova S, Vithayasai N, Watanaveeradej V, et al. Intestinal microsporidiosis in HIV-infected children with acute and chronic diarrhea. South East Asian J Trop Med Public Health 2001:32;33–7.
19. Walker-Smith JA. Masalah Pediatric di bidang gastroenterologi tropis. Dalam: Problem Gastrenterologi daerah Tropis, Ed GC Cook. Edisi I. Jakarta 2003;EGC:133-41.
20. World Health Organization. Persistent diarrhea in children in developing countries. Memorandum from a WHO meeting, Geneva, WHO. Bull WHO 1988;66:709-17.
21. Harrison M, Kilby A, Walker-Smith JA, France NE, Wood CBS. Cowls milk protein intolerance: a possible association with gastroenteritis) lactose intolerance) and 19A deficiency. BMJ 1976;1:1501-4.
22. WHO/CHD, Persistent diarrhoea and breastfeeding. WHO division of Child Heath development Family and reproductive Health, Geneva 1997.
23. Arora NK, Bhan MK, Ghai OP. Protracted diarrhea of infancy: its etiology and management in 25 patients. Indian Pediatr 1981;18:272-8.
24. Fagundes-Neto U, Wehba J, Viaro T, Machado NL, da Silva Patricio FR. Protracted diarrhea in infancy: clinical aspects and ultrastructural analysis of the small intestine. J Pediatr Gastroenterol Nutr 1985;4:714-22.