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Case Report

URINE SPECIMEN IN DETECTION OF HEPATITIS A VIRUS ANTIBODY IN CHILDREN

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ABSTRACT

Hepatitis A is still a problem in developing country, especially in Indonesia. Twenty-three thousand cases per year in the world were reported by CDC in 1998. Detection of Hepatitis A Virus (HAV) antibody needs blood specimen, for which the drawing procedure is quite unpleasant for children. The purpose of present study is to examined the use of urine as an alternative specimen compared to blood in the detection of HAV antibody. A cross sectional study on children age 5–12 years who lived in Rusun Sombo District Simokerto Surabaya was performed in 2007. Blood and urine specimens were taken from all subjects, further tested with Microparticle Enzyme Immunoassay (MEIA) method using AxSym® HAVAB® 2.0. A total 74 children were included in the study, 43 (58.1%) were boys. Seropositive was found in 38 (51.4%) children and uropositive in 3 (4%) children. There are significant differences between blood and urine specimen, with sensitivity 7.9%, spesificity 100%, PPV 100% and NPV 50.7%. Urine could not replace blood specimen as the gold standart for the detection of hepatitis A antibody in children

Key words: *hepatitis A, antibody, urine, blood specimen, MEIA*

BACKGROUND

Hepatitis A is an endemic disease in developing country and it has close relationship with hygiene, sanitation, and viral transmission factors. Center for Disease Control and Prevention reported 23,000 cases of hepatitis A every year worldwide, with peak age 5 to 14 year.[1] Sulaiman (1993) found seroprevalence of anti-HAV at Papua was 100% in 4 year-old children. In Jakarta, Bandung, and Ujung Pandang, the seroprevalences were between 35–45% in 5 year-old children.[2] Dr. Soetomo Hospital had 23 cases of hepatitis A during 1999–2004 (unpublished data).

The process of taking blood sample from children who appeared healthy is somewhat hesitating especially in infant, considering that it needs agreement from parents and also qualified person to draw the specimen. Therefore, people search for a non-invasive solution using alternative body secretion in antibody detection for many diseases. Urine is an alternative solution, since it contains immunoglobulin even in low concentration and serologic assay could perform without centrifugation process with class-specific antibody capture assay. This assay has the ability to catch specific immunoglobulin in low concentration and to confirm diagnosis at the beginning of the disease.[3,4]

Total anti-HAV detection (IgG and IgM) is used to define immune status. Positive result of anti-HAV indicates infection or long exposure of hepatitis A that stimulate immunity. This assay can determined immune status in natural infection or caused by vaccination.[5]

This research was held at Rusun Sombo, a living location with very crowded population and low social-economic condition. Kitchen and toilet were shared together among several families, causing easier transmission of hepatitis virus. Clean water facility and waste product handling were not good. On the location, we tried to make a similar condition on social-economy status, hygiene and sanitation between samples.

The purposes of this research are to study the use of urine specimen for non-invasive detection of hepatitis A antibody (anti-HAV) and to study epidemiology of children with anti-HAV positive in Surabaya.

SAMPLE AND METHOD

This is a cross sectional study involving healthy-appeared children age between 5–12 years in Rusun Sombo District Simokerto Surabaya. Subjects were taken by

consecutive sampling. The research was held at September-October 2007. We collected 2.5 cc of blood from cubiti vein and 2.5 cc of fresh urine in the morning. Both specimens from every subject were tested with MEIA (microparticle enzym immunoassay) serologic assay to detect anti-HAV level. Result was analyzed with McNemar test and Kappa agreement.

RESULT

We collected 86 subjects met inclusion criteria, 12 were dropped out due to sample damage, with a total of 74 subjects.

From data analysis, 74 subjects consisted of 43 boys (58.1%) and 31 girls (41.9%), seropositive was found in 38 children (51.4%) and uropositive in 3 children (4%).

Thirty-one children (41.9%) age 10–12 years, 67 children (90.5%) were in elementary school. Based on the education of their parents, 28 children (37.8%) had parents graduated from elementary school. Socioeconomy status was dominated with middle-low status in 39 children (52.7%). Sixty children (81%) had their parents worked as pedicab driver, "Junkman", market trader, etc).

Almost 68 children (91.9%) stated that they never had history of jaundice before, and 71 children (96%) had no contact with jaundice patients in last 6 months. Sixty children (81%) regularly washed their hand before eat, and 68 children (91.9%) usually buy non-home-cooked meal or eating outside, 58 children (78.4%) liked to eat well-cooked meal. Almost every child stated that they never had contact with jaundice patients or had that disease before. However, hepatitis A in children is an asymptomatic disease. The environment condition in Rusun Sombo may support transmission of viral infection because they usually shared a same kitchen and toilet together on public facilities among 3–5 families.

All subjects stated that they never had hepatitis A immunization before. Each family lived in a 30 m^2 house with ceramic floor. About 33 children (44.6%) lived in a house with 4 persons with PDAM as clean water facility. Almost 73 children (98.6%) were nursed by their parents. Some children from 12 families in this research live together in the same house and neighbored with 5 families which were seropositive.

Based on nutritional status, we found 57 children (77%) with well-nourished condition, 4 children (5.4%) with malnourished condition, 8 children (10.8%) with under-nourished condition and 5 children (6.8%) in overweight condition.

Seropositive result was found commonly in higher age between 10-12 year-old (19 children), and according to education level, seropositive result was more common in elementary school children (35 subjects). Based on parents' education level the most common seropositive result was found in family which their parents graduated from

Table 1. Characteristic of subjects

Characteristic	Total subjects	
	N	%
Sex:		
Boys	43	58.1
Girls	31	41.9
Age:		
5–7 years old	19	25.7
8–9 years old	24	32.4
10–12 years old	31	41.9
Education:		
Kindergarten	5	6.8
Elementary school	67	90.5
Junior high school	2	2.7
Parents' education:		
No education	3	4
Elementary school	28	37.8
Junior high school	21	28.8
Senior high school	20	27
University	2	2.7
Income:		
< Rp. 750.000	25	33.8
Rp. 750.000-4.000.000	39	52.7
> Rp. 4.000.000	10	13.5
History of jaundice:		
Yes	6	8.1
No	68	91.9
History of contact with jaundice patients:	3	4
Yes	71	96
No		
Hand washing:		
regularly	60	81
sometimes	13	17.6
never	1	1.4
Eating outside/non home-cooked meal:		
Yes	68	91.9
No	6	8.1
Uncooked meal:		
Yes	16	21.6
No	58	78.4
Hepatitis A immunization:		
Yes	0	0
No	74	100
Number of person living together:	33	44.6
4 persons	30	40.5
5–6 persons	11	14.9
>7 persons		
Nutritional state:		
Malnourished	4	5.4
Under-nourished	8	10.8
Well-nourished	57	77
Overweight	5	6.8

elementary school (20 children) and 11 children (28.9%) had parents graduated from junior high school.

Seropositive result were common in family with middle to low family- income, about 14 children (36.8%) with income family < 750.000 rupiahs/month and 20 children (52.6%) had family income ranged from 750.000 rupiahs/ month up to 4 million rupiahs/month.

Based on history of jaundice in seropositive subjects, the majority of subjects never had the disease before (36 children or 94.7%) and all samples had no contact with jaundice patients before in last 6 months. Good hand washing habits was found in 31 children (81.6%), with 35 (92.15) admitted usually buy food outside their house, only 7 children admitted like to eat unwell-cooked meal from sea product. Seropositive result found commonly in well-nourished children, eventhough some literature stated that in children with malnourished condition usually had low level of immunoglobulin or antibody therefore seronegative result gained, but in this research we found 1 malnourished child with seropositive result. Uropositive result was found only in 3 children, 2 girls and 1 boy.

Chi square test was performed and found only one variable (parents' income) with positive association with anti-HAV level, where other variables had negative association. These results may be due to sampling process since we already make homogenous conditions of some characteristics such as socio-economy and environment which actually could influence hepatitis A viral transmission. In the other hand we must also consider the difficulties in objectivity of parents' disclosure in answering all questions which could interfere the result.

McNemar test was used to compare differentiation between two different independent tests in order to detect antibody level of hepatitis A, using anti-HAV serum as gold standard compared with anti-HAV level in urine. Kappa value in this study was 0.53.

Table 2. Statistical analysis of serology using with Mc Nemar test

Serology test	Anti HAV Serum		
Anti HAV urine	+	-	Total
+	3	0	3
-	35	36	71
Total	38	36	74

The result from McNemar test refused Ho which meant that there were significant difference between this two tests in order to detect anti-HAV level in children. Based on Kappa value we found that two tests had no good agreement.

We had 7.9% of sensitivity and 100% specificity with PPV 100% and NPV 50.7%, which described that only 7.9% urine samples could detect antibody to HAV and that positive result from urine can 100% exclude the negative result. PPV 100% means that the probability of subjects to have anti-HAV based on uropositive test result were 100% and NPV 50.7% means that probability of subjects to have no anti-HAV based on uronegative test result were 50.7%.

DISCUSSION

Study about anti-HAV level in Rusun Sombo District Simokerto Surabaya had found 51.4% children between 5–12 years old with seropositive result. This study tried to compare between blood (as a gold standart) and urine as specimen to detect anti-HAV level in healthy-appeared children with the result a significant difference between those two specimens.

Some literatures mentioned that IgA, IgM, and IgG can be found in external secretion of mucose of human body, such as in urine, saliva. It bounds to secretory component (SC), usually with FcRn. Therefore antibody to HAV can be found or can be detected too even in other body secretions like urine, even in low level compared to blood serum.[6-10] Since the level in urine is very low, it needed a sensitive serologic test with ELISA.[11,12]

The natural infection of hepatitis A actually was not at mucosal site of the human bladder, but with homing mechanism of the body, secretion of antibody happened in other site of external body mucose.[6–10] This study was performed in healthy-appeared children, based on the theory that IgM could be present or positive in external secretion until 3–6 months after infection and IgG may persist for years.[3,13–16]

The result of this study revealed 38 children with seropositive and 3 children with uropositive result. Since level of antibody in mucosal secretion is very low, and even with very sensitive serologic test, the result revealed very low positif result and showed significant difference. Therefore the use of urine as specimen for detecting anti-HAV was not applicable.

The result revealed sensitivity 7.9% and specificity 100% with positive predictive value (PPV) 100% and negative predictive value 50.7%, which means its sensitivity showed true positive result from all positive result was 7.9% and spesificity showed true negative result from all test that negative was 100%, therefore urine specimen which used as specimen showed big false negative result. PPV 100% showed probability of the sick from the positive result were 100%, with NPV which showed probability of the health from the negative result were 50.7%. It means urine specimen can not replace blood specimen as gold standart for detecting anti-HAV level in epidemiologic study.

This result was different from the study by Joshi (2002) and Rodriguez (2003),[3,4] probably due to the very low level of antibody from past infection in body secretion that can not passed through level of detection of the serologic test resulting in negative result in our subjects.

SUMMARY

Based on the result of this study, the application of urine specimen in detection of anti-HAV level can not be used. Compared to blood specimen as gold standart, it showed a significant difference.

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