THE PREVALENCE OF THE INDIRECT HEMAGGLUTINATION TEST FOR MELIOIDOSIS IN CHILDREN IN AN ENDEMIC AREA

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Abstract The indirect hemaglutination test for melioidosis was studied in 295 children who live in the northeastern part of Thailand. Sixty-seven children (22.7%) were healthy children who came to the well baby clinic. Two hundred and twenty-eight chilldren (77.3%) came to the hospital because of some illnesses other than melioidosis. Eighty-three percent of the children had an IHA titer of at least 1:10 or greater. Twenty-two percent had an IHA titer of 1:80 or greater. The prevalence of positive IHA titer and the mean titer were higher in the older age group. The age of children should be considered when interpreting IHA titer for milioidosis.

INTRODUCTION

Melioidosis, an infection caused by *Pseudomonas pseudomallei* is endemic in Southeast Asia and northern Australia (Howe *et al,* 1971). The disease can occur in all age groups. The clinical manifestations include septicemia and localized infection which can involve almost every organ and can mimic many other diseases. Apart from bacteriological culture which is the definite diagnosis, some serological methods have been utilized to provide rapid and presumptive evidence of infection (Alexander *et al,* 1970). The simplest and most widely used serological test is the indirect hemagglutination antibody test (IHA) (Nigg, 1963).

A study of United States soldiers in Vietnam indicated that the test was highly specific and sensitive (Alexander *et al,* 1970). In endemic areas the background prevalence of antibodies in the population was as high as 47% in one study, so the test should be interpreted carefully (Khupulsup *et al,* 1986). The interpretation of the IHA test in children is even more difficult because most of the serological survey studies were done in adults. Whether a background prevalence of antibodies is also present in children of different age groups is not known.

The purpose of this study is to determine the prevalence of IHA antibody for melioidosis in children who live in the northeastern part of Thailand where melioidosis is endemic.

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MATERIALS AND METHODS

Children age 0 to 15 years old, who came to the Department of Pediatrics, Srinagarind Hospital, Khon Kaen University, Khon Kaen during March-June, 1988 were enroled into the study. There were 2 groups of subjects. The first group was healthy children 0-18 months of age who attended the well baby clinic for routine immunization and at that time were enroled in either the hepatitis B vaccine or rubella vaccine trial projects. The blood samples drawn from these children were also tested by IHA for melioidosis. The other group of children were those aged 0 to 15 year-old who were admitted to the hospital due to various illnesses. The exclusion criterion was patients who were diagnosed as melioidosis by bacteriological culture results.

Data collection of both groups of children included age, sex, parents' occupation, source of utility and drinking water. The medical history and physical examination of children in the first group were done by the investigators (PC, PL). The same information including the bacteriological culture results of the second group were obtained from the medical records. History of previous illness within 1 year, immunosuppressive therapy and blood or blood products administration within 3 months were also noted.

The IHA test for melioidosis was performed by the method described by Khupulsup *et al* (1986). In brief, melioidin antigen was prepared from 25 strains of locally isolated *P. pseudomallei.* These strains were cultured in glycine broth at 37°C for 2 weeks before an autoclaved extraction. Melioidin antigen were diluted to the optimal concentration and coated on to sheep red blood cells (SRBC). To determine the IHA titer, serum was first inactivated at 56°C for 30 minutes and non-specific antibody was absorbed by non sensitized SRBC. A 0.05 ml aliquots of serial 2-fold dilutions of absorbed serum starting from 1:10 were mixed in U-tube microtiter plates with equal volumes of the sensitized SRBC. The IHA titer were read after the mixture was left at room temperature for 2 hours. Positive and negative controls were also included.

The statistics used were Chi-square or Fisher's exact test and simple regression analysis as required.

RESULTS

Two hundred and ninety-five children were enroled in the study. There were 172 males and 123 females. The ages ranged from 1 day to 15 years old. The mean age was 66 months. Fifty percent of the children lived in Khon Kaen Province. The remainder were from other provinces in the northeastern part of Thailand. Parents of 105 children (40.9%) were farmers. Fifty-one percent of the parents obtained their drinking water from natural sources *eg* rain water, digging wells, river, etc. Nineteen percent of them usually drank tap water. The sources of utility water were from digging wells, ditches and rivers in sixty-two percent while the rest used tap water.

There were 67 children (22.7%) in the first group. All were in good health as judged by history and physical examination. The second group comprised 228 (77.3%) children who had various illnesses (Table 1). Twenty-two percent and 17% of the children had previously received blood or blood products transfusion and immunosuppressive agents, respectively.

The distribution of IHA antibody titers in 295 children is shown in Table 2. Ninety-eight children (17%) had non detectable IHA antibody to melioidosis. Two hundred and 46 children (83%) had IHA antibody titers ranging from 1:10 to 1:640.

Table 1

Diagnosis of 228 hospitalized children*.

Disease	No. %
Hematologic disease and malignancy	97
Infectious disease	53
Neurological disease	37
Cardiovascular disease	11
Gastrointestinal disease	8
Respiratory disease	7
Urological disease	5
Miscellaneous	24

* Some children had more than 1 diagnosis

Table 3 shows the number of children in each age group who had IHA antibody titers $\geq$ 1:80. None of the 12 neonates had IHA antibody titers $\geq$ 1:80. Children aged 1-11 months old, 1-4 years old, 5-9 years old and 10-15 years old had IHA antibody titers of $\geq$ 1:80 in 5%, 23%, 24% and 36%, respectively. The means and standard deviations of titers of children in each age group are shown in Table 3. The mean titers and the percentages of children who had titers $\geq$ 1:80 were higher in older children with statistically significant differences ($p < 0.05$).

Table 4 shows the number of children who had an IHA titer of $<$ 1:80 and $\geq$ 1:80 according to the presence or absence of underlying diseases (tumor, hematologic diseases and malignancy), previous immunosuppressive therapy and blood or blood product administration during the past 3 months, There were no statistically significant associations between these factors and titers of $<$ 1:80 and $\geq$ 1:80 ($p > 0.05$).

Fifty out of 65 children who had IHA antibody titers for melioidosis $\geq$ 1:80 were followed-up. The duration of follow-up ranged from 3 days to 8 months. Eight children were in the first group, all were healthy at the time of follow-up. Forty-two children were in the second study group. Forty of them had no signs or symptoms suggestive of melioidosis. Two children died. The first one, who had an IHA titer of 1:160, was an 11 year-old boy who presented with bronchiectasis and pneumonia.

Table 2

The distribution of IHA antibody titer for melioidosis in children of different age groups.

Age	IHA antibody titer for melioidosis							
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	No^+/No tested (%)
0-29 days	1	-	1	-	-	-	-	2/12 (16.7)
1-11 months	8	4	7	1	-	-	1	21/39 (53.8)
1-4 years	35	35	12	22	5	-	1	110/124 (88.7)
5-9 years	13	18	20	11	5	1	1	69/174 (93.2)
10-15 years	7	11	9	9	5	1	2	44/46 (95.7)
Total	64	68	49	43	15	2	5	246/295

Table 3

Mean IHA titer, standard deviation, number of children who had titer $\geq$ 1:80 classified according to age groups.

Age	Mean titer* (log_{10})	Standard deviation	No. titer $\geq$ 1:80/Total no.(%)**
1-20 days	0.2168	0.5224	0/12 (0)
1-11 months	0.7469	0.7691	2/39 (5)
1-4 year	1.2537	0.5843	28/124 (23)
5-9 year	1.4287	0.5493	18/74 (24)
10-15 year	1.5651	0.5763	17/46 (36)

* Simple regression analysis, $p < 0.05$
** Chi-square test, $p < 0.05$

He died despite treatment with penicillin, cloxacillin and gentamicin. The second patient, who had an IHA titer of 1:640, was a 1 1/2 month-old boy who came to the hospital with high fever, lethargy and abdominal distention for 5 days. On admission he had cardiorespiratory arrest and circulatory failure. He died 6 hours after admission. The blood cultures of these 2 patients did not grow any organism.

DISCUSSION

In an endemic area, the cut off titer of IHA antibody for melioidosis at $\geq$ 1:80 is reported to have the specificity and sensitivity of 95% and 70%, respectively (Puapermpoonsiri *et al,* 1989). In our study only 2 out of 12 neonates had detectable IHA titers of 1:10 and 1:40. None had titers $\geq$ 1:80. A study of IHA antibody for melioidosis

Table 4

Children who had IHA titer < 1:80 and ≥ 1:80 classified according to various associated factors.

Associated factors		IHA titer	
		< 1:80	≥ 1:80
Underlying diseases :	Yes	184	58
	No	57	10
Immunosuppressive therapy:	Yes	37	11
	No	188	53
Blood and /or blood product administration :	Yes	53	8
	No	171	51

Chi-square test, $p > 0.05$

of cord bloods from southern Thailand showed that all cord bloods had titers < 1:20 (Appassakij *et al,* 1990). Children under 1 year of age rarely had IHA titers ≥ 1:80 (5%). Children over 1 years old had increasing positive IHA titers with titer ≥ 1:80 in 23-36%. This may reflect increasing exposure to the organisms which are abandant in soil and water in this area. The age of children might be an important factor when interpreting IHA titer. While other diagnostic approaches such as *P. pseudomallei* antigen detection are under investigation (Wongratanacheewin *et al* 1990), the IHA antibody test is still the only widely used serological method available. Further studies of larger numbers of healthy children in both endemic and non-endemic areas are needed in order to evaluate the prevalence of IHA antibody for *P. pseudomallei* in children.

Our study had some limitations, in that 77.3% of children studied had some kind of illness and many children had received immunosuppressive agents or blood and blood product transfusion. Whether these factors might interfere with the result of the IHA titer remains to be studied, but in our study there were no statistically significant associations between these factors and the IHA titer.

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