

BRIEF COMMUNICATIONS

MODIFICATION AND IMPROVEMENT OF THE HAEMADSORPTION IMMUNOSORBENT TECHNIQUE (HIT) FOR THE DETECTION OF DENGUE IgM ANTIBODIES

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Summary

A modified and improved version of the haemadsorption immunosorbent test (HIT) to detect dengue-specific IgM antibodies is described. No significant differences in titres were noted compared to the original HIT and 62.5% of single sera tested were shown to possess IgM antibodies by the modified HIT, as well as most acute-phase sera from secondary dengue infections. Only one acute-phase serum from primary dengue infection showed the presence of IgM by the modified HIT. A monotypic IgM response to dengue3 virus was observed in one acute-phase serum which was subsequently confirmed by virus isolation. Three acute-phase sera from suspected cases of viral encephalitis were shown to possess Japanese encephalitis virus (JEV)-specific IgM by the modified HIT. The significance and importance of these findings are discussed.

Keywords: Dengue diagnosis, IgM.

INTRODUCTION

Diseases caused by the dengue viruses, including dengue haemorrhagic fever and dengue shock syndrome (DHF/DSS), are still important causes of morbidity and mortality in tropical countries, including Malaysia. One important aspect in the management of DHF/DSS and in the prevention and control of outbreaks, is the availability of a rapid, sensitive, specific and economical laboratory diagnostic test to confirm dengue infection in suspected cases. Rational approaches to therapy can then be commenced, especially monitoring and prevention of shock. Relevant public health measures can also be rapidly implemented to prevent the outbreak from spreading.

Currently available tests for the laboratory diagnosis of dengue infections are by virus isolation and serology. These approaches are often slow, labour intensive, insensitive and, in the case of serology, often give inconclusive results. Another approach to serological diagnosis is the detection of IgM class antibodies. These generally appear early in the onset of disease and is indicative of recent infection. We have previously reported the development of a haemadsorption immunosorbent test (HIT) for the detection of dengue IgM antibodies.¹ The technique is based on the 'capture' of dengue IgM in human sera by

anti-human IgM immobilized onto a solid phase. The dengue-specific IgM is then detected by the addition of dengue antigen (haemagglutinin) and goose erythrocytes. Reaction of the goose erythrocytes with dengue haemagglutinins will produce haemadsorption. We present here results of further studies aimed at improving the HIT and making the test easier to perform.

MATERIALS AND METHODS

Serum Specimens

The following groups of sera were used in the study:

1. 62 single serum specimens from suspected dengue cases which had been previously tested by the original HIT procedure (see below). 32 of these sera had an inconclusive test result by the haemagglutination inhibition (HI) test.
2. 4 paired sera from primary dengue infections and 5 paired sera from patients with secondary dengue infections, as classified by the W.H.O. classification system.²
3. Three acute-phase sera (collected on days 1-5 of illness) from patients with a clinical diagnosis of viral encephalitis.

Antigens

Suckling mouse brain haemagglutinating antigens prepared to dengue viruses 1 to 4 and Japanese encephalitis virus (JEV) by the method of Clarke and Casals³ were used in the HI and HIT tests. The amount of antigen used was expressed as haemagglutinating units (HAU). The following virus strains were used: dengue-1 (Hawaii), dengue-2 (Trin 1751), dengue3 (H-87), dengue4 (H-241) and JEV (Nakayama).

HI Test for Dengue

The dengue HI test was performed by a modification of the method of Clarke and Casals.³

HIT Test for Dengue

This was carried out according to the method of Gunasegaran et al.¹ Briefly, polystyrene microtitration plates were coated with anti-human IgM, incubated overnight at 4°C and then washed. Human sera (extracted with acetone and absorbed with goose erythrocytes) were serially diluted in anti-human IgM-coated plates and incubated for 2 hours at 37°C and then washed. One HAU unit of dengue haemagglutinating antigen was added and plates incubated at 4°C overnight. A 0.06% suspension of goose erythrocytes was then added and plates incubated for 1 hour at 37°C. Plates were then read after centrifugation at 65 x g for 10 mins. A positive result was shown by a haemadsorption pattern, whereas a button indicated the absence of IgM antibody.

Modified HIT for Dengue

The following modifications were introduced into the procedures published previously¹ and described briefly above:

1. Sera was not extracted with acetone but absorption with goose erythrocytes was still carried out.
2. The addition of 4 HAU of dengue haemagglutinating antigen instead of 1 HAU.
3. Shortening the incubation time from overnight at 4°C to 1 hour at 37°C after the addition of dengue haemagglutinating antigen.
4. Reading of patterns without prior centrifugation at 65xg.

RESULTS

The results obtained from testing the 62

sera with the modified HIT showed no significant differences in titres with the original HIT. This group of 62 sera contained 32 single serum specimens from suspected dengue patients with an inconclusive HI result. Twenty of these 32 sera (62.5%) were shown to possess IgM antibodies by the modified HIT test (Table 1). Paired sera from primary and secondary dengue infections were tested and IgM antibody to dengue detected in all 9 convalescent-phase sera, 4 out of 5 acute-phase sera from secondary dengue infections and in 1 acute-phase serum from primary dengue infection (Table 2). Of particular interest is the one acute-phase serum from a primary dengue infection in which the IgM detected by modified HIT reacted only with dengue-3 antigen (Specimen No. 1, Table 2). A dengue3 virus was subsequently isolated from this specimen. Although some cross-reactivity was detected to JEV haemagglutinins the IgM titres to JEV were lower than the corresponding dengue titres (data not shown). Three acute-phase sera from suspected cases of viral encephalitis were also tested for the presence of dengue- and JEV-specific IgM by the modified HIT. All three sera were positive for JEV IgM and there was no cross-reactivity with dengue haemagglutinins (Table 3).

DISCUSSION

The four important features of the modified HIT test are the ability to use unextracted (but goose erythrocyte-absorbed) sera, the use of haemagglutinins in excess of 1 HAU and washing off unbound antigen, shortened incubation times and reading of results without the need for centrifuging the microtitre plates. These modifications reduced significantly the time required to perform the test. In the original HIT test, the use of exactly 1 HAU of haemagglutinating antigen and the need to centrifuge the plates at low gravity forces¹ sometimes led to difficulties in reading the haemagglutination patterns and interpretation of test results.

The results of the present study also show that the performance characteristics of the HIT were not affected by the modifications; no significant difference in IgM titres were observed when a panel of sera were tested by both the original and the modified HIT. The sensitivity of the modified HIT was indicated by the finding that 20 of 32 (62.5%) sera from suspected dengue cases were positive in the present study. These observations confirm the usefulness of the HIT by demonstrating

that it is possible to obtain a rapid diagnosis on approximately 60% of suspected dengue infections based only on a single, acute blood specimen. A definitive diagnosis based on these single sera would not have been possible with the haemagglutination inhibition (HI) test. We have estimated that 70–80% of sera submitted for dengue diagnosis to our laboratory are single specimens.

Similar findings were also obtained in the present study with sera from suspected cases of viral encephalitis. All three of the acute-phase, single sera showed the presence of JEV-specific IgM antibodies with no cross-reactions to dengue haemagglutinins. The HI results of these three sera had been inconclusive. It is thus likely that the HIT is also useful in the diagnosis of JEV infections, which are often suspected but rarely diagnosed definitively in the laboratory. It is also of interest to note that two out of three sera which were JEV-IgM positive were completely

negative by the HI test. This would suggest a better sensitivity of the HIT compared to the HI method.

Our original report of the HIT test also showed that the dengue-specific IgM detected by the HIT was broadly cross-reactive with all four dengue serotypes and with other flaviviruses (e.g. JEV).¹ This fact would suggest that no conclusions could be made regarding the serotype of the infecting virus from the HIT results. Such an observation has also been made by other workers⁴ and is perhaps to be expected due to the sharing by flaviviruses of group-reactive antigenic epitopes. The present study, however, indicates that under certain rare circumstances (primary infection, early serum collection) a monotypic response to a particular dengue serotype could be demonstrated. Previous reports have also suggested that IgM antibodies formed in primary dengue infections, at least, may be monotypic.⁵

TABLE 1
MODIFIED HIT IN 32 SINGLE SERUM SPECIMENS COMPARED
TO HAEMAGGLUTINATION INHIBITION (HI) TEST*

Serum No.	HI titre	Modified HIT titre	Serum No.	HI titre	Modified HIT titre
1	<10	<40	17	160	320
2	<10	<40	18	160	<40
3	<10	<40	19	320	640
4	<10	<40	20	320	320
5	10	<40	21	80	<40
6	20	<40	22	160	2560
7	40	<40	23	640	1280
8	40	<40	24	640	5120
9	80	320	25	640	320
10	80	<40	26	640	20480
11	80	160	27	640	10240
12	320	640	28	1280	160
13	320	2560	29	1280	20480
14	320	1280	30	1280	2560
15	160	1280	31	2560	10240
16	160	<40	32	5120	2560

* HIT titre of ≥ 40 considered as positive.

TABLE 2
COMPARISON OF HI AND MODIFIED HIT IN PAIRED SERA

Serum No.	Phase*	HI titres to				Modified HIT titres to			
		D-1	D-2	D-3	D-4	D-1	D-2	D-3	D-4
1	A	10	20	20	40	<40	<40	160	<40
	C	320	1280	640	1280	1280	2560	2560	5120
2	A	<10	<10	<10	<10	<40	<40	<40	<40
	C	160	320	160	640	2560	2560	1280	2560
3	A	20	40	40	80	<40	<40	<40	<40
	C	320	320	160	640	160	640	640	1280
4	A	<10	<10	<10	<10	<40	<40	<40	<40
	C	640	640	320	1280	1280	2560	5120	2560
5	A	80	160	160	320	160	320	320	320
	C	2560	>5120	5120	>5120	2560	5120	5120	>5120
6	A	320	640	160	640	320	320	320	640
	C	1280	2560	1280	5120	5120	2560	1280	5120
7	A	40	80	40	40	<40	<40	<40	<40
	C	1280	2560	1280	2560	2560	5120	2560	2560
8	A	1280	1280	1280	2560	320	160	160	640
	C	>5120	>5120	>5120	>5120	>5120	>5120	>5120	>5120
9	A	2560	5120	5120	5120	>5120	>5120	>5120	>5120
	C	>5120	>5120	>5120	>5120	>5120	>5120	>5120	>5120

*Acute (A) phase = Sera collected between days 1-6 of onset.
Convalescent (C) phase = Sera collected between days 7-18 of onset.

TABLE 3
MODIFIED HIT TO DETECT JEV IgM IN THREE SERA FROM PATIENTS WITH SUSPECTED VIRAL ENCEPHALITIS

Specimen No.	Test	Antibody titres to*				
		D-1	D-2	D-3	D-4	JEV
1	HIT	<40	<40	<40	<40	160
	HI	<10	<10	<10	<10	<10
2	HIT	<40	<40	<40	<40	320
	HI	<10	<10	<10	<10	10
3	HIT	<40	<40	<40	<40	2560
	HI	40	80	80	160	320

* A titre of ≥ 40 in the HIT was considered positive for the presence of IgM

In conclusion, the present modifications and improvements in the HIT method confirms its usefulness in the rapid, economical, specific and reproducible diagnosis of dengue virus infections.

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