

SUMMARY

Dengue is epidemic and endemic in tropical America, Africa and Asia where the principal vector *Aedes aegypti* is continuously present. The more severe form of the disease, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) was subsequently described and is seen in many parts of the world where it is now an important cause of endemic and epidemic morbidity.

Though Sri Lanka was fortunate to be a "silent area" for dengue haemorrhagic fever till 1988, the first epidemic was experienced in 1989. In 1990, there were 1121 clinically suspected cases of DHF with 345 serologically confirmed as dengue. Since then the cases have continued to occur in hundreds and in 1996 there were 1277 clinically suspected DHF cases with 392 serologically confirmed cases.

At the Medical Research Institute in Sri Lanka, traditionally the classical haemagglutination inhibition test (HI) is used in the diagnosis and surveillance of dengue. This is labour intensive takes 2-3 days to perform and also requires precise standardization of the reagents. The HI poses certain limitations in the

interpretation of results. The HI antibodies begin to appear at a detectable level (HI titre 1:10) in a person with a primary dengue infection by the fifth to the sixth day of the illness, and almost immediately in a secondary dengue virus infection. If paired sera are present (acute and convalescent) with a rising titre (four fold) or one sample with a HI titre of $\geq 1:2560$ the infection can be diagnosed as dengue but the majority of samples that are sent to the MRI for testing are only those with one (acute) sample. Those with a low HI titre ($< 1:1280$) fall into an inconclusive group as these levels can occur with a past infection.

The recent adaptation and application of a test for dengue specific immunoglobulin M antibody (IgM) the IgM enzyme linked immunosorbent assay (MAC-ELISA) has provided a method for more rapid serodiagnostic testing. These kits are either in-house or commercially available. This will provide a method of detecting the IgM which is present from about 5 days of onset of illness in both primary and secondary dengue virus infections even in a single sample.

The study group consisted of a total of 273 samples from clinically suspected DHF patients whose samples were sent to the MRI between March and September 1995. Out of these, 244 were acute samples and 29 were convalescent samples. These were grouped into the following. Group 1- consisted of 29 paired

sera clinically suspected of having DHF who had a four fold rise in the HI antibody titre, where there were 5 primary dengue infections and 24 secondary dengue infections. The acute samples were collected between 1-8 days of onset of illness and the convalescent samples were collected between 12-20 days of onset of illness. Group 2- consisted of 111 single sera with a HI titre of $\geq 1:2560$ collected between 2-8 days of onset of illness. Group 3- consisted of 64 single serum samples with a HI titre of $>1:20$ and $<1:1280$ with the collection of blood samples between 2-9 days of onset of illness. Group 4- consisted of 40 single sera with a HI titre of $<1:20$ and samples were collected between 1-9 days of the onset of the disease.

No laboratory based study has been carried out using both HI and IgM MAC-ELISA tests in Sri Lanka.

The serum samples were tested for both HI and IgM antibody. A commercially available IgM MAC-ELISA test kit (PanBio, Australia) was used.

From the 244 acute samples 128 (52.5%) were positive by the HI and 142 (58.2%) were positive by the IgM MAC-ELISA. There is no statistically significant difference (at 95%, SE=4.4). This is comparable with studies done in Puerto Rico and Malaysia where the HI results and the IgM results respectively

were 79% and 70% in Puerto Rico and 50.8% and 65.8% in Malaysia. The IgM MAC-ELISA test could therefore be used in the diagnosis of dengue virus infection.

In Group 1, the convalescent samples showed a 100% positive result by HI and a 89.7% positive result by the IgM MAC-ELISA. This is not statistically significant. (SE=6.1, $P>0.05$). The sensitivity of the test based on this is 89.7%. There were 3 (10.3%) samples which did not show a IgM response with the convalescent sample. As IgG can interfere with the detection of IgM and mask the presence of IgM these 3 samples could have shown a negative result. HI was positive in 58.6% and the IgM positive in 37.9% in the acute samples in this group. There is no statistical significance between the two proportions. (SE=12 $P>0.05$) A bigger sample size should be tested to arrive at a definite decision.

Group 2 which consisted of 111 acute samples of clinically suspected DHF cases with a HI titre of $\geq 1:2560$, showed a 80.2% positive result with the IgM MAC-ELISA test. This is statistically significant compared to the HI test results which was 100% positive (SE=3.7, $P<0.05$). IgG appears faster in secondary dengue infection and this is reflected in the HI test. IgM can be masked by the appearance of IgG and this, in addition to the timing of taking the sample can have an effect on the positive number in the IgM test.

Group 3 consisted of 64 clinically suspected DHF cases with single sera showing HI titre of $>1:20$ and $<1:1280$. In this group the IgM was positive in 54.7%. Therefore this 54.7% of patients who will normally be grouped as inconclusive by the HI test, now could be diagnosed as a current dengue infection with the IgM MAC-ELISA test. However this group must be further tested with paired sera and with virus isolation techniques and polymerase chain reaction (PCR) tests before drawing firm conclusions.

Group 4 consisted of 40 single sera of clinically suspected DHF cases with a HI titre of $<1:20$. There were 7% positive by the IgM in this group. This again would assist the clinician in the management of the cases, whom otherwise would have been grouped as inconclusive. As in group 3 further conclusions will depend on testing with paired sera as well as virus isolation and PCR tests.

The appearance of IgM was seen in all 4 groups at a higher percentage in the 5-8 day than the 0-4 day of the onset of illness ie; the figures for Groups 1, 2, 3 and 4, on the 0-4th day of illness respectively were 25%, 17%, 18.2%, and 12.5%. On the 5-8th day of the illness it was 81.8%, 85.7%, 73.8% and 20.8%. This shows a similar response as with other studies done in Puerto Rico and Brazil. Further studies should be done to see the persistence of IgM in the Sri Lankan population.