Research Note

Serological Screening of Tick-Borne Encephalitis (TBE) among Malaysian Encephalitis Patients.

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Abstract. Tick-borne encephalitis (TBE) is a viral infection of the central nervous system and is caused by tick bites, usually after travel to rural or forested areas. The disease is prevalent in Scandinavia, Western Europe, Central Europe and the former Soviet Union and East Asia including Japan. In Malaysia, so far there are no reported cases of TBE. In the present time, many illnesses have been attributed to traveling to other parts of the world. Thus it is important to carry out TBE prevalence study to determine whether the virus is present among Malaysian population. Samples (sera and CSF) from patients admitted to major MOH hospitals in Peninsular Malaysia and Sabah with a clinical diagnosis of encephalitis but is IgM negative for JE, were tested for TBEV IgM ELISA and TBEV IgG ELISA (DRG, Germany).Out of the 600 samples screened for TBEV IgG, all were non-reactive. In addition, out of the 100 samples screened for TBEV IgM, all the samples were also non-reactive. Our results indicate that currently TBE is not present in the Malaysian population. Among the reasons for this could be lack of the infection agent, absence of the suitable vector or subjects selected for the study did not fit the criteria of possible exposure to TBE infections. Hence we recommend that for any future study, the selection of subjects should include those who returned from tick-infested forested areas.

Emerging and re-emerging infections are a major threat to our population. Rapid urbanization, population growth and environmental changes are among the contributing factors that can trigger disease outbreaks. Among the recent emerging and re-emerging diseases worldwide are Nipah virus outbreak in Malaysia in 1999 and in Bangladesh in 2003, Rift Valley Fever in Saudi Arabia and Yemen in 2000, West Nile Fever in New York in 1999 and SARS coronavirus in China, Hong Kong and Taiwan in 2003. It should be noted that almost all emerging and re-emerging diseases are caused by RNA viruses and are zoonotic in nature.

Tick-borne encephalitis is a viral infection of the central nervous system and is caused by tick bites, usually after travel to rural or forested areas infested with ticks. The virus belongs to the Flaviviridae family which also includes 70 antigenically distinct viruses (Porterfield, 1980). The members of this family can be divided into mosquito and tick-borne viruses. Similar to mosquito-borne viruses like dengue and Japanese encephalitis, TBE virus is also a major human pathogen causing thousands of cases of severe neurological disease per year (Takashima *et al*, 1997). Based on antibody adsorption experiments, peptide mapping and nucleotide sequencing, two subtypes of the TBE virus have been identified and designated as western and eastern (Heinz & Kunz, 1981; Pletvev, 1990). The western subtype is endemic in large parts of Northern, Central and Eastern Europe including Germany and Sweden while the eastern subtype can be found in the European and Asian regions of the Commonwealth of Independent States (former Soviet Union), some parts of the Far East including Northern Japan.

Clinical presentation of TBE is typically biphasic. After an incubation period, which is usually between 7 and 14 days, the prodromal symptoms (uncharacteristic

influenza-like illness with fever, headache, malaise and myalgia) are followed by CNS involvement. After an afebrile interval of about 1 week, the second stage develops (Kaiser, 1999). TBE can also manifest as meningitis, meningoencephalitis or meningoencephalomyelitis (Duniewicz, 1976: Kaiser, 1995). Currently there is no specific anti-viral treatment. However a vaccine is available and normally can be administered to travelers intending to visit tick-infested areas in summer/spring.

In Malaysia, so far there are no reported cases of TBE. However this could be due to either the lack of awareness among the clinicians seeing patients displaying symptoms of TBE or simply to the fact that TBE was never investigated in Malaysia. In the present time, many illnesses have been attributed to traveling to other parts of the world. In addition, it was reported that there is an increase in the man-tick contact due to an increase in leisure and sporting activities that bring man into infected biotopes. Wandering and migrating birds also spread the ticks into new localities, thus increasing the risk of an emerging disease. Thus, it is important to continuously conduct surveillance for exotic diseases in the country as precautionary measures.

Serum and CSF specimens screened for the study were specimens that were sent from all hospitals in Malaysia to the Virology Unit, IMR for diagnosis of Japanese encephalitis. All these specimens were subjected to in-house JE IgM ELISA. All negative specimens for JE IgM ELISA were then included for the TBEV serology. Specimens selected were kept at 4°C before use. Commercially available TBEV IgG and IgM kits (DRG, Germany) were used to screen for TBEV antibodies in the selected specimens. The test was carried out as described in the in the kit protocol. Briefly 100 µl of diluted patient's specimen (either serum or CSF) and standards were dispensed into wells. Then,

the ELISA plate was incubated at 37°C for 1 hour. This was followed by 3 times washing by using 300µl wash solution after which 100 µl of TBE anti-IgG conjugate was added into each well. The plate was then incubated for a further 30 minutes at room temperature. Again the wells were washed thoroughly for 3 times after which 100 µl of TMB solution was dispensed into all wells. The plate was then incubated at room temperature for 15 minutes. The reaction was stopped by adding 100 µl of stopping solution. Finally the optical density of the specimens was read at 450 nm. In addition, TBEV IgM ELISA was also carried out on all TBEV IgG positive specimens and some selected specimens. The method for the test is similar to TBEV IgG ELISA.

Results indicate that all 600 specimens screened were negative for TBEV IgG antibodies. Also, all 100 specimens that were further screened for TBEV IgM antibodies were also negative.

Tick-borne encephalitis is a viral disease transmitted to human after being bitten by infected ticks. The disease is mainly found in temperate countries such as Central, Eastern and Northern Europe and some areas in Japan, China and Korea. Globalization has become the norm in many countries where many earlier travel and trade restrictions have been removed to enable easier travel and trading. Hence more people travel around the world, either for economic reasons or simply for leisure. As a result, the threat of spreading infectious diseases has become “globalized”. For instance if a person infected with the SARS Coronavirus were to take a flight to a different part of the world, this person is in a position to spread the infection to other parts of the world in a short time.

RNA viruses (in which TBE belongs) are more susceptible to mutations compared to DNA viruses. Hence these viruses have the ability to mutate at a faster rate in response

to changes in the environment. Some of the mutations are “silent”, meaning that the pathogenesis of the virus remains the same while other mutations can result in a more virulent virus with increased morbidity and mortality.

The diagnosis of TBE is normally derived from epidemiological (a stay in an area risk for TBE) and clinical (biphasic course of disease and neurological symptoms) and the demonstration of TBE-specific antibodies (Kaiser, 1999). Seroprevalence of TBE among a selected population could give an indicator of the prevalent of the diseases in the population. This paper discussed the findings of a TBE seroprevalence study among selected encephalitis patients. A total of 600 patients were selected for the TBE IgG ELISA test and out of these 600 samples, a hundred samples were further screened for TBE IgM ELISA. The samples selected were from 11 states with Kuala Lumpur and Perak accounting for almost 24% and 22% samples selected respectively. Our preliminary findings revealed that none of the patients with encephalitis have either IgG or IgM antibodies against TBE and thus TBE infection has not reached the Malaysian population as yet. However this result has to be taken with careful considerations, as there could be some explanations for the negative results. Firstly, a study done by Schuhmacher *et al* (1999) showed that not all patients bitten by ticks will come down with TBE or even produced antibodies. In their survey in the Lorraine region of France, a total of 1,777 subjects were studied, half of them lived in rural areas, and about 91% of them had occasional or regular contact with the forest environment and at least 21% of them had experienced tick bites. However only 19 subjects (1.6%) had showed IgG antibodies in their serum of which only nine were confirmed by Western Blot. In

addition, all those TBEV IgG positive subjects were negative for TBEV IgM indicating that the subjects did not have any recent infection (exposure).

In another study, Korenberg *et al* (2001) found that the risk for clinical illness of TBE is largely dependent on being bitten by a highly infectious tick (meaning ticks carrying high titre of the virus) and frequency of the bites. Schuhmacher *et al* (1999) also found that sero-prevalence was higher among subjects with past history of tick bites. Other seroprevalance study also revealed a low prevalence rate. Haglund (2000) reported that the seroprevalence of TBE among random samples of adults in Stockholm, Sweden or people attending TBE-vaccination was only about 2-4% even though many parts of Sweden is endemic for TBE.

Rodents play a major role in TBE, both in the life cycles of the major vector tick *Ixodes ricinus* and as an amplifying reservoir for a majority of the tick-transmitted pathogens (Granstrom, 2000). Even though rodents are known to be the most important carrier of the ticks responsible in causing TBE, Takashima *et al* (1997) reported isolation of the virus from sentinel dogs in Hokkaido, Japan. Thus the possibility of other carriers of ticks causing TBE cannot be ruled out.

The risk of TBE infections in Malaysia cannot be seen in a lighter picture as the disease is the most common arthropod-borne infection in Europe and America and seems to increasing, in both the number of cases and in geographical distribution (Granstrom, 2000). This is due to a number of factors including changes in environment and climate, which causes the ticks to adapt better and spread to new areas. Hence there is no assurance that Malaysia will stay TBE-free in the future due to many compounding factors which could trigger an outbreak.

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