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Serodiagnosis of asymptomatic dengue infection

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ABSTRACT

Dengue virus (DENV) is a mosquito-transmitted virus that is expanding across the world. The incidence of dengue infection, especially severe disease, has been increasing. DENV consist of 4 serotypes of single stranded RNA viruses (D1–D4) in the genus Flavivirus, family *Flaviviridae*. Majority of dengue infections are asymptomatic cases, which cause difficulty in disease control and are important in dengue surveillance. There is still no gold standard to diagnose asymptomatic dengue infection. Plaque reduction neutralization test (PRNT) has been developed for many purposes such as immunological study, clinical study, vaccine trial and is currently the most sensitive and specific method for serological surveillance. However, PRNT shows some degree of cross reaction among different dengue serotypes especially secondary dengue infection cases and to other flaviviruses. Moreover, various modification since the beginning make PRNT lack of inter-laboratory standardization which is an important issue. This paper discusses the important of asymptomatic dengue infection and its diagnostic method.

1. Introduction

Dengue virus (DENV) is a major arbovirus responsible for an estimated 2.5–3.0 billion people at risk worldwide. DENV has been expanding from a few South-east Asia countries to more than 100 countries across the world during this recent 60 year period. Dengue infection caused around 50–100 million cases of dengue fever (DF) and 250 000–500 000 cases of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), the severe disease that can cause mortality, each year. The incidence of dengue disease, especially severe forms (DHF/DSS), has been increasing since the 1950's [1–5].

DENV consist of 4 serotypes of antigenically distinct single stranded RNA viruses (D1–D4) in the genus Flavivirus, family *Flaviviridae*. Infection with one serotype can cause life-long immunity against the original serotype but transient cross protection to the other serotypes. The pathogenesis of severe disease is not completely understood [1,4–8]. Up to now, dengue prevention relies on vector control with limited success. There are many explanations for the unsuccessful disease control including rapid urbanization, insecticide resistance, national and international travelling [9–12].

2. Significance of asymptomatic dengue infection

Approximately, three-quarters of the dengue infections are asymptomatic [13–16]. Asymptomatic infection is therefore a major part of dengue burden and should be emphasized. In addition, asymptomatic cases may have a role in dengue transmission [17] although there has been no clear data on viremia in these asymptomatic cases as well as the impact of asymptomatic infection on dengue transmission. Study on asymptomatic infection may also provide insight on the epidemiology and pathogenesis of dengue. The incidence of asymptomatic dengue infection also reflects the quality of dengue control.

3. Diagnostic tests for dengue infection

The currently used diagnostic test for dengue infection can be divided into virologic/molecular/antigen based and serologic based. Since viremia occurs for only a short period [18] (i.e. 1–2 days before onset of symptom and upto 5–7 days after onset of symptom), the virologic/molecular/antigen based tests are applicable in only symptomatic infection which the disease onset is noted [19–21]. Serologic tests usually need two blood samples to detect rising antibody titer. However, sometimes these tests could not differentiate between acute and recent infection and there is cross reaction with other flaviviruses. Three serological tests have been used for detecting

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asymptomatic dengue infections: hemagglutination-inhibition test (HAI), enzyme linked immunosorbent assay (ELISA) and plaque reduction neutralization test (PRNT). For many years, HAI had been the most commonly used method for diagnosis of dengue infections due to its high sensitivity and can be used to differentiate primary from secondary infections. The hemagglutinating antigen of dengue virus can cause a complete agglutination when incubated properly with erythrocytes. The HAI test is performed by mixture of serial diluted sera with a certain unit of hemagglutinating antigen. After incubated with erythrocytes, the result is read as agglutination titer [22]. The HAI titer indicates the highest dilution of each serum which causes complete inhibition of hemagglutinating antigen. A titer of 1:10 or higher is seropositive. A four-fold rising in HAI titer of convalescent sera compared to acute sera is considered diagnostic for dengue infection. A titer of 1:1 280 or higher in sample is an indication of secondary dengue infection while the titer less than 1:1 280 in convalescent sera indicates primary dengue infection. The main disadvantages of HAI are its low specificity and inaccuracy in identifying the infecting virus serotypes [19]. Currently, ELISA has been widely considered the most commonly used test for dengue infection diagnosis due to its high sensitivity and feasibility [23]. The amount of antigen–antibody binding can be evaluated by ELISA. IgM and IgG antibodies can be separately performed and measured [24]. The intensity of color after incubation with substrate and enzyme reflects the antibody level. The seroconversion of IgM or IgG indicates acute infection. The high IgM/IgG ratio can be used to diagnose primary dengue infection [25]. ELISA is more specific than HAI and there is no need to pre-treat serum by kaolin to remove non-specific inhibitor as in HAI. However, ELISA is also unable to differentiate dengue serotypes and has low specificity. Plaque reduction neutralization test (PRNT) is the most sensitive and specific serological method for dengue diagnosis [26]. The PRNT is performed by incubating serially diluted sera with each serotype of infecting dengue viruses. The mixture then is added on monolayer cell line. After staining, the viral plaque can be visualized as a clear spot. The reduction of plaques compared to control is observed and reflect the antibody level [27]. There are many methods to interpret the result which may be quantitative or qualitative. For example 50% PRNT (PRNT50) titer is calculated as the highest dilution of each serum which causes 50% reduction of plaque numbers comparing to the control (no serum added). The titer of 1:10 or higher is considered as a PRNT seropositive. A four-fold rising in PRNT titer of convalescent sera compared to acute sera is diagnostic for dengue infection [28]. PRNT can be used to identify the infecting serotype in primary infection, since a relatively monotypic response is observed. In secondary infection, the antibody responses are cross-reactive and may be directed to previously infecting serotype and therefore determination of the infecting serotype by PRNT is not reliable [29]. In order to increase its specificity, 70% reduction or higher can be applied as a cut-off level. However, identification of infecting serotype still cannot be done by even PRNT70 or PRNT90 results [30]. For qualitative test, single dilution PRNT70 can be used. PRNT is currently recognized as the best method for serologic diagnosis of dengue infection. However, because PRNT is laborious and time consuming, it is usually only used in research and vaccine study, not in routine clinical diagnosis. PRNT is the most sensitive and specific test comparing to HAI and ELISA especially in primary infection. Nevertheless, the PRNT needs the highest cost, time and labor consuming and yields the highest inter-laboratory variation. All tests necessarily require paired serum. However, only ELISA test is a high throughput method.

4. Detecting asymptomatic dengue infection

Detection of asymptomatic infection is difficult and challenging. While symptomatic dengue can be clinically suspected and then confirmatory laboratory diagnosis can provide definite diagnosis, there is no clinical clue for asymptomatic infection. Detection of asymptomatic infection is therefore based on laboratory diagnosis.

Among various confirmatory laboratory tests, the tests that based on virologic or molecular or antigen detection are not convenient methods because the dengue viremia period is very short after infection [18] (approximately 1 week). This means that if these methods are being used, at least weekly blood samples are needed for surveillance of asymptomatic infection. Therefore serologic methods to detect rising in dengue antibody are more convenient.

After primary infection, the antibody response is characterized by a rise in IgM antibody after the 3rd day of disease onset. IgM persists for approximately 5 months. The IgG antibody can be detected after the 1st week of disease onset and may persist for a year. After secondary dengue infection, there is a lower IgM antibody response but more rapid and intense IgG response, which may persist for more than one year [31–34]. Therefore, the surveillance for asymptomatic dengue infection by measuring the rise in IgG titer may need at least yearly blood sample and need shorter interval if IgM titer is going to measure. More frequent blood sampling may result in more accurate result.

There are many study designs suitable for a surveillance of asymptomatic dengue infection. It depends on the objectives and accuracy needed for the surveillance and the diagnostic test being used. A prospective study by Burke *et al.* in school children in Bangkok, Thailand collected blood samples 6 months apart and used both HAI and PRNT [14]. Another prospective study was conducted by Endy *et al.* in 1998–2002 in Kampaeng Phet, Thailand. In this study, blood samples were collected 3 times (June 1st, August 15th and November 15th) and HAI was used to diagnose dengue infection. The investigators performed frequent blood sampling during rainy season because they believed that the incidence of asymptomatic dengue infection was highest during rainy season [15]. However, because the difference in frequency of blood samplings between rainy season and dry season, this study can not accurately demonstrate the difference in the incidence of asymptomatic dengue infection between these two seasons.

Another example of detecting asymptomatic infection is the study conducted by Mammen *et al.* This study aimed to detect asymptomatic dengue infection in high risk group (the people in the same household of dengue cases), not the general population as in the two studies previously mentioned. In this study, people in the same household of dengue cases were followed on day 0, 5, 10, 15 for any clinical sign and symptom. Blood samples were drawn on day 0 and 15 for determining by ELISA and because the time of infection could be estimated reverse transcription-polymerase chain reaction (RT-PCR) was also used for detecting dengue virus and its serotype [16].

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