VIROLOGICA SINICA

Electronic Supplementary Material

Seroprevalence of Dengue Virus among Young Adults in Beijing, China, 2019

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Supporting information to DOI: 10.1007/s12250-020-00285-4

Supplementary Materials and Methods

ELISA

Serum samples were tested at 1:11 (10 μL sera plus 100 μL dilution buffer) dilution for anti-DENV IgG by commercial ELISA kits (Beijing BGI-GBI Biotech Co., Ltd, China) according to the instructions of the manufacturer. The kit will not generated cross-reaction with other flaviviruses. Briefly, diluted sera were added to the plates and incubated at 37 °C for 30 minutes, followed by four washes. Each run included the respective positive and negative controls provided by the manufacturer. Bound antibodies were incubated with horseradish peroxidase-conjugated mouse anti-human IgG (H+L chains) monoclonal antibody at 37 °C for 30 minutes, followed by five washes. The reaction was visualized with substrate 3,3',5,5'-Tetramethylbenzidine and stopped by H_2SO_4. The optical density at 450 nm (OD_{450}) was measured by the ELISA plate reader (Thermo, USA). The cut-off value was also set based on the OD_{450} values according to the following formula: if the average value of negative controls (AVNC) $\geq$ 0.05, cut-off value = AVNC + 0.1; if the AVNC < 0.05, cut-off value = 0.15. If the OD_{450} values $\geq$ the cut-off value, the sample is anti-DENV IgG positive.

PRNT

A confirmatory test was performed on 14 anti-DENV IgG seropositive samples by using PRNT. Meanwhile, serotype-specific nAb titers within sera were determined. PRNT is recognized as the gold standard for detecting serotype-specific anti-DENV antibodies and has been commonly used, especially in studies evaluating the immunogenicity of dengue infection and vaccine (Capeding *et al.* 2011). The following viral strains including DENV1 (Hawaii), DENV2 (New Guinea C), DENV3 (H87), and DENV4 (H241) were used in this study. Sera were heat-inactivated at 56 °C for 30 minutes. A known amount of each of DENV1–4 (40–60 plaque focus units) was mixed with the same volume of serially 2-fold diluted sera at the range from 1:5 to 1:640 and incubated for 90 min at a 37 °C water-bath. Following this, 200 μL of the virus/sera mixture was added to fully confluent African green monkey-derived Vero cell monolayers (in 24-well plates) and incubated for 1 hour at 37 °C water-bath with gentle rocking every 15 minutes. Following incubation, the inoculum was removed from the cells and 4 ml of 1.2% methylcellulose overlay was applied to each well and the plates were incubated at 37 °C, 5% CO_2 for 6–8 days depending on the virus and visualized by crystal violet staining. The $PRNT_{50}$ titers were calculated as the reciprocal of the maximum dilution of serum that yielded a 50% plaque reduction in comparison with the number of plaques in the controls with DENV infection alone. The geometric mean titer (GMT) of $PRNT_{50}$ was defined as the reciprocal of the highest dilution. The virus-specific neutralizing activity was calculated according to the guidelines for PRNT of human antibodies to DENV (Roehrig *et al.* 2008), and $PRNT_{50}$ $\geq$ 1:10 was categorized as nAb seropositive.

Data analysis

The statistical analysis was conducted using SPSS Software version 17.0 (USA). Chi-Square test was performed to compare the difference in the seroprevalent status of antibodies against DENV across different socio-demographic characteristics. To compare the GMT of nAb between cohorts, Student's *t*-test was used. *P* values < 0.05 with two sides were considered statistically significant.

Table S1 Socio-demographic characteristics of seroprevalence of anti-DENV IgG antibody among all participants (n = 961)

Characteristic	Total	Seroprevalence of anti-DENV IgG antibodies		Chi-square	P^a
	n	n (%)			
		Positive	Negative		
Overall	961	14 (1.5)	947 (98.5)		
Gender					
Male	344	4 (1.2)	340 (98.8)		
Female	617	10 (1.6)	607 (98.4)	0.323	0.570
Age (Year of Birth)					
21 (1998)	236	3 (1.3)	233 (98.7)		
20 (1999)	725	11 (1.5)	714 (98.5)	0.075	0.784
Ethnicity					
Han	852	11 (1.3)	841 (98.7)		
Others	109	3 (2.8)	106 (97.2)	1.437	0.231

[a]Chi-square test was used to determine the difference of anti-DENV IgG antibody seroprevalence across different parameters.

References

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