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Kertas Asli/*Original Articles*

Emergence of Dengue Virus Type 4 during COVID-19 Pandemic in Patients Admitted to a Teaching Hospital in Malaysia

(Peningkatan Virus Denggi Serotip Keempat semasa Pandemik COVID-19 pada Pesakit yang Dimasukkan ke Hospital Pengajar di Malaysia)

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ABSTRACT

Prior to COVID-19, dengue was an important public health problem in Malaysia. Due to the movement control order imposed by the Malaysian government to curb the COVID-19 transmission, a study predicted that mosquito-borne diseases would increase during lockdown and partial lockdown seasons. Thus, this study aims to determine the current situation of dengue incidence during the pre-COVID-19 pandemic (2019) and during the COVID-19 pandemic (2020 and 2021). We compared the number of laboratory-confirmed cases in the pre-COVID19 year (2019) and during the COVID-19 pandemic (2020 and 2021). In addition to that, we characterized the clinical manifestation, dengue serotype and viremia levels of dengue patients that were admitted to the Hospital Cancelor Tuanku Muhriz. We found a significant decrease in the number of laboratory-confirmed cases between COVID-19 pandemic and the pre-covid period (p2020=0.064; p2021<0.001). In this study, we found DENV 4 serotype was the most common serotype in dengue patients admitted to our hospital. There was no significant correlation between DENV serotype/viremia level with clinical manifestation of dengue fever and dengue with warning signs. However, patients infected with DENV4 had the highest viral load compared to patients infected with other serotypes. We also found high viremia levels were significantly associated with the febrile phase.

Keywords: Dengue; clinical manifestation; serotyping; dengue virus; Malaysia

ABSTRAK

Sebelum COVID-19, denggi adalah masalah kesihatan awam yang kritikal di Malaysia. Semasa perintah kawalan pergerakan yang dikenakan oleh kerajaan Malaysia untuk membendung penularan COVID-19, satu kajian meramalkan bahawa kes bawaan nyamuk akan meningkat semasa musim kawalan pengerakan. Kajian ini bertujuan untuk menentukan keadaan semasa kejadian denggi sebelum dan semasa pandemik COVID-19. Kami membandingkan jumlah kes yang disahkan oleh makmal pada tahun pra-COVID-19 (2019) dan semasa wabak COVID-19 (2020 dan 2021). Selain daripada itu, kajian ini juga melakukan memperincikan simptom klinikal, serotip virus denggi dan tahap viremia bagi pesakit denggi yang dimasukkan ke Hospital Canselor Tuanku Muhriz. Kajian mendapati terdapat penurunan yang signifikan bagi jumlah kes denggi yang disahkan makmal pada sebelum wabak COVID-19 dan semasa wabak COVID-19 (p2020=0.064; p2021<0.001). Selain daripada itu, kami mendapati bahawa DENV 4 adalah serotip yang paling dominan di kalangan pesakit denggi yang dimasukkan ke hospital kami. Tiada korelasi yang ketara antara tahap denV serotip/viremia dengan manifestasi klinikal demam denggi dan denggi dengan tanda-tanda amaran. Walau bagaimanapun, pesakit yang dijangkiti DENV4 mempunyai beban virus tertinggi berbanding pesakit yang dijangkiti dengan serotip lain. Kami juga mendapati tahap viremia yang tinggi dikaitkan dengan fasa demam.

Kata kunci: Denggi; manifestasi klinikal; serotipe; virus denggi; Malaysia

INTRODUCTION

Prior to the COVID-19 pandemic, dengue (DEN) had become a major public health threat in tropical and subtropical regions including in Southeast Asia, Africa, South Pacific, Central and South American, and Eastern Mediterranean (M. G. Guzman and Harris 2015). It was estimated that there are 400 million cases of dengue virus (DENV) infection annually (Stanaway et al. 2017). DENV belongs to the genus flavivirus of the family Flaviviridae. There are 4 serotypes of DENV: DENV1-4 that are antigenically different but serologically similar between serotypes. The definition of serotype is based on the observation that infection against homologous serotype confers full protection but limited protection against heterologous serotype (Weaver and Vasilakis 2009; Katzelnick et al. 2016). Infection with any serotype of DENV induces a wide spectrum of conditions ranging from undifferentiated dengue fever, dengue without/with warning signs to severe dengue and sometimes dengue related death. Dengue illness is divided into 3 phases: the febrile phase, critical or defervescence, and recovery (resorption), or convalescent phase. The febrile phase occurred from day one to day three, while the critical phase started from day three to five henceforth from day five onwards (Simmons et al. 2012).

Malaysia is a dengue hyperendemic country, with the number of dengue cases continuing to increase every year. The first case of dengue fever in Malaysia was documented in 1902, and the first laboratory-confirmed case of dengue haemorrhagic fever was reported in 1965 (Skae 1902; Rudnick et al. 1965). Malaysia recorded a four-year cycle of high numbers of DEN cases followed by a two-year cycle of low numbers of DEN cases (Mudin 2015). All four serotypes of DENV co-circulate in Malaysia with serotype displacement occurring every 2 or 3 years. In 2013, DENV2 was the dominant serotype in Malaysia before DENV1 displacement in 2014 to be the dominant serotype. Serotype shift usually occurs before major outbreaks, in which the previously predominantly dengue serotype before the outbreak was replaced by another serotype that will remain until the end of the outbreak season (Suppiah et al. 2018).

As a dengue hyperendemic country with all four serotypes circulating, effective patient management is important to ensure the patient was diagnosed and treated accurately. Previously, all four DENV serotypes were thought to induce similar clinical manifestations, nonetheless, recent studies reported that each serotype of DENV was associated with distinct clinical manifestations (Suppiah et al. 2018; Vicente et al. 2016; Rocha et al. 2017). Studies conducted in local hospitals have reported that dengue infection without warning signs were observed more frequently in patients who were infected with DENV1 and DENV3 while DENV-2 infected patients commonly progressed to severe dengue (Suppiah et al. 2018; Bujang et al. 2017). Patients diagnosed as severe dengue cases were patients with severe organ impairment, severe plasma leakage, and severe haemorrhage (Ministry of Health 2015).

However, since the declaration of COVID-19 pandemic and the movement control order that was imposed by the Malaysia government from March 2020, mosquito-borne diseases were predicted to be significantly higher during lockdown and partial lockdown (Jindal and Rao 2020). Thus, this study aims to determine the current situation of dengue incidence during the COVID-19 pandemic in dengue hotspot areas in Kuala Lumpur, Malaysia. In addition to that, prevalence of circulating serotypes and the association between dengue serotype with clinical manifestations were also determined in the subset of dengue patients that were admitted to a teaching hospital located in Kuala Lumpur, Malaysia.

MATERIALS AND METHODS

STUDY POPULATION

This study was conducted in a teaching hospital (Hospital Canselor Tuanku Muhriz) which is located in dengue hotspot area - Cheras, Kuala Lumpur. Patient information details were retrieved from the Communicable Disease Control Information System (CDCIS) dengue e-notifikasi from two duration; 1) prior to the COVID-19 pandemic (January 2019 until December 2019) and during the COVID-19 pandemic (January 2020 until December 2021). A subset of dengue patients admitted to the hospital during the COVID-19 pandemic from January 2021 until January 2022 were recruited for serotyping and characterization of clinical presentation. The patient inclusion criteria for this study were 1) admitted patients with a positive rapid NS1 antigen test; and 2) presented with fever and two of the following clinical symptoms such as nausea and vomiting, rashes, arthralgia, myalgia, leukopenia, and having warning signs. The patient exclusion criteria for this study were 1) not admitted to the ward and 2) negative for the NS1 antigen test. A designated questionnaire was used to collect patient demographic details and clinical symptoms.

SAMPLE COLLECTION

A total of 5 ml of whole blood was collected from the patients in EDTA tubes. The blood samples were

centrifuged at 3,000 rpm for 10 min at 4°C. The plasma sample was stored at -80°C before further processing.

DETERMINATION OF DENGUE VIRUS SEROTYPE

The viral RNA of the dengue virus was isolated from plasma using a QIAamp viral RNA kit (QIAGEN, Germany). The serotype of DENV was determined using TaqMan real-time reverse transcriptase-polymerase chain reaction as described previously (Ito et al. 2004). The cut-off of positive samples was below the Cycle Threshold (CT) of 40.

DETERMINATION OF DENGUE NS1 ANTIGEN LEVEL USING ELISA

The levels of dengue NS1 antigen were determined using Panbio Dengue Early ELISA (Abbott, United States of America) which quantifies the levels of NS1 antigen in the plasma or serum. A Panbio Index Unit (PBU) with more than 10.0 was interpreted as positive for dengue NS1 antigen according to the manufacturer's instructions. The overall sensitivity and specificity of the PanBio Dengue Early ELISA kit was 72.3% and 100.0%, respectively (Lima et al. 2010).

DATA ANALYSIS

Data was analyzed using Statistical Package for the Social Science for Windows (SPSS) version 28.0 (IBM, Armonk, NY, USA). Fisher-Exact test, independent t-test and one-way ANOVA test were used for categorical variables and the determination of the association between categorical and numerical variables, respectively. P-values <0.05 were considered to indicate statistical significance.

ETHICS STATEMENT

Ethics approval was obtained from the institutional review and ethics board committee of Universiti Kebangsaan Malaysia (JEP-2020-279), in accordance with the Declaration of Helsinki. All participants gave either written or oral consent either before being recruited for this study.

RESULTS

DENGUE CASES DURING THE PRE- AND COVID-19 PANDEMICS IN HCTM

Prior to the COVID-19 pandemic, there were a total of 616 laboratory-confirmed dengue cases in HCTM (Figure 1). However, since the start of COVID-19 in 2020 and 2021, the number of laboratory-confirmed dengue was 496 cases and 138 cases, respectively. There was a significant decrease in the number of laboratory-confirmed dengue cases in 2020 (19.4% decreased, p=0.0064) and 2021 (77.6% decreased, p<0.001) when compared with the number of laboratory-confirmed dengue cases in 2019. The number of DEN NS1-positive patients in HCTM also showed a decline from 81 cases (January – August 2019) to 35 cases (June 2021- Jan 2022).

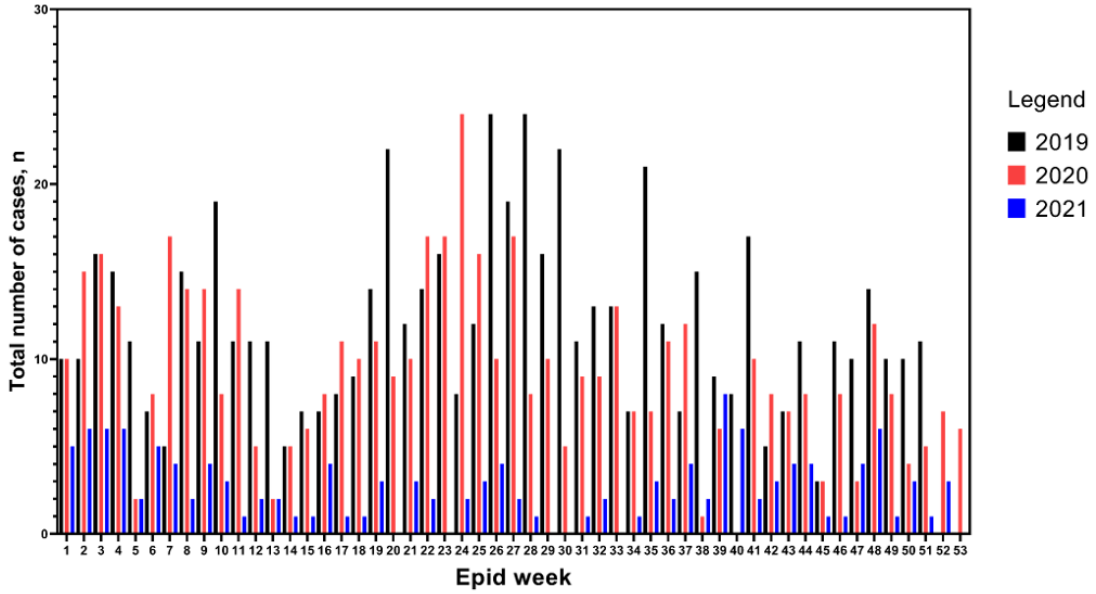

FIGURE 1. Number of laboratory-confirmed dengue cases in Hospital Cancelor Tuanku Muhriz based on the Epid week

CHARACTERISTICS OF DENGUE PATIENTS ADMITTED TO THE HCTM DURING THE COVID-19 PANDEMIC

A total of 35 positive-dengue NS1 antigen patients from June 2021 to January 2022 were recruited in this study (Table 1). This sample size was calculated based on single proportion formula for estimation of DEN NS1 positive cases in HCTM in 2021 (n=81) with a 95% confidence level and margin error of 0.05. Majority of the patients were aged less than 60 years old (87.5%), with a median age of 29 years, and male (58.8%). The plasma samples were collected at different times of admission ranging from day 0 (62.9%), day 2 (25.7%), and day 4 (11.4%). A total of 15 patients (42.9%) were identified as having primary dengue infection while a total of 20 patients (57.1%) were identified as having secondary dengue infection (Table 1). Most of the patients were in the critical phase (n=21, 60%). The mean day of ward admission was 5.23 days. There was one death (n=1 (2.85%)) with the others being discharged well (97.14 %). All patients had no overseas or interstate traveling history, and none were vaccinated against the dengue virus, the Japanese encephalitis (JE) virus, or yellow fever. Among the patients, three of them (8.6 %) had a history of previous dengue infection. The presence of warning signs did not differ between patients diagnosed as primary dengue infection or secondary dengue infection (Table 2).

TABLE 1 Characteristics and clinical manifestation of dengue patients that were recruited in this study (n=35)

Characteristics/Clinical Manifestations	Total, n (%)
Age group, years old	
<60	30 (85.7)
>60	5 (14.3)
Median Age, y (Range)	29 (13-70)
Sampling Time (Days post-admission)	
0	22 (62.9)
2	9 (25.7)
4	4 (11.4)
Type of dengue infection	
Primary	15 (42.9)
Secondary	20 (57.1)
Mean duration of hospital stays, day (range)	5.25 (2-10)
Stages of dengue	
Convalescent	3 (8.6)
Critical	21 (60.0)
Febrile	11 (31.4)
Patient status	
Dead	1 (2.9)
Alive	34 (97.1)
History of the previous infection	
Yes	3 (8.6)
No	32 (91.4)
Vaccination history (JE/Yellow fever)	
Yes	0 (0.0)
No	35 (100.0)
Travel history	
Yes	0 (0.0)
No	35 (100.0)
Median temperature, Celsius (range)	37.8 (35.6-40.4)
Median heart rate, bpm (range)	95 (60-150)
Median number of white cells, x10,000 (range)	4 (1.7-11.2)
Median platelet count, x10,000 (range)	122 (12-329)
Median haematocrit value, % (range)	43.6 (19.52.5)
Median lactate value, mmol/L (range)	1.2 (0.5-2.8)
Median bicarbonate value, mEq/L (range)	24.2 (12.7-27.0)
Median liver function, mmol/L (range)	51 (101-1294)
Median urea level, mmol/L(range)	3.4 (1.3-50)

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Median sodium level, mmol/L (range)	135.42 (131-139)
Had headache	
Yes	7 (20.0)
No	28 (80.0)
Myalgia	
Yes	20 (57.1)
No	15 (42.9)
Rash	
Yes	2 (5.7)
No	33 (94.3)
Haemorrhagic manifestation	
Yes	3 (8.6)
No	32 (91.4)
Abdominal pain	
Yes	8 (22.9)
No	27 (77.1)
Persistent vomit	
Yes	12 (34.3)
No	23 (65.7)
Persistent Diarrhoea	
Yes	11 (31.4)
No	24 (68.6)
Third space loss	
Yes	2 (5.7)
No	33 (94.3)
Spontaneous Bleeding	
Yes	3 (8.6)
No	32 (91.4)
Confuse/Lethargic/Restlessness	
Yes	7 (20)
No	28 (80)
Liver Tenderness	
Yes	4 (11.4)
No	31 (88.6)
High Haematocrit	
Yes	16 (45.7)
No	19 (54.3)
Leukopenia	
Yes	18 (51.4)
No	17 (48.6)
Platelet Count	
Platelet count between 50,000-100,000 mmol/L	8 (22.9)
Platelet count less than 50,000 mmol/L	5 (14.3)
Platelet count of more than 100,000 mmol/L	22 (62.9)
High Lactate or metabolic acidosis	
Yes	5 (14.3)
No	30 (85.7)
Transaminitis	
Yes	16 (45.7)
No	19 (54.3)
Renal Impairment	
Yes	6 (17.1)
No	29 (82.9)
Low Sodium Level	
Yes	10 (28.6)
No	25 (71.4)

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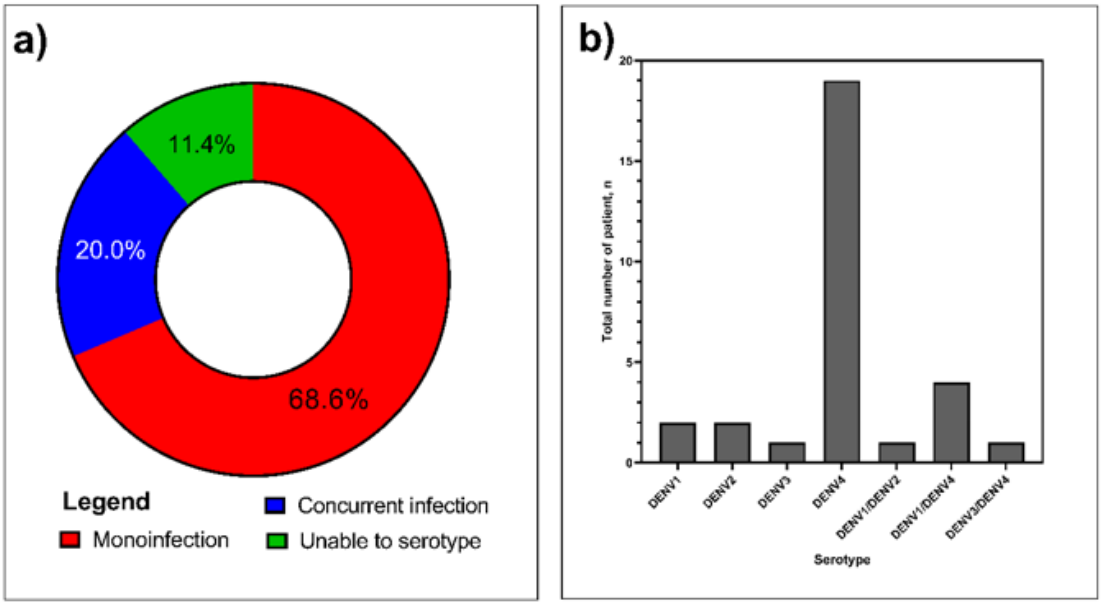
Dengue IgM	
Non-reactive	29 (82.9)
Reactive	6 (17.10)
Dengue IgG	
Non-reactive	17 (48.6)
Reactive	18 (51.4)

From the 35 plasma samples, 24 patients were monoinfection (68.6%), 7 patients had concurrent infections, and there were four patients who had not available for serotyping due to RNA degradation (Figure 2a). For monoinfection, DENV4 was the most common dengue serotype (n=19, 61.3%), followed by DENV1 (n=2, 6.5%), DENV2 (n=2, 6.5%) and DENV3 (n=1, 3.2%) while for the concurrent infection, infection with DENV1/DENV4 was the most common (n=4, 12.9%), followed by DENV3/DENV4 (n=2, 6.5%), DENV1/DENV2 (n=1, 3.2%) (Figure 2b). The majority of patients infected with DENV1 and DENV3 had primary dengue infection while the majority of the patients infected with DENV2 and DENV4 had secondary dengue infection case (Figure 2c). The mean CT value varied between serotypes, types of dengue infection and dengue phases. The mean CT values of patients infected with DENV4 were the lowest ($Mean_{ctvalue}$=26.45 ± 5.58), followed by DENV1 ($Mean_{ctvalue}$=30.75 ± 5.34), DENV3 ($Mean_{ctvalue}$=33.99 ± 3.53), and DENV2 ($Mean_{ctvalue}$=35.68 ± 3.46) (Figure 2d). We found significant differences between CT values between patients infected with DENV4 and patients infected with DENV2 (p=0.028) as well as patients infected with DENV3 (p=0.031). The mean CT value was also varied between dengue phases (Figure 2e). We found significant differences between CT values between febrile phases with critical phases (p=0.039) and convalescent phases (p=0.035), respectively. The mean CT values for primary infection were 28.38 ± 5.95, while for secondary infection was 29.01 ± 6.17, but there was no significant differences between the mean CT values between primary and secondary infection, p=0.77 (Figure 2f).

Levels of DEN NS1 antigen in the plasma samples ranged from 2.1 PBU to 100.27 PBU (Figure 3a). A total of 17 samples were negative for the presence of DEN NS1 antigen because the level of NS1 antigen was less than 10 PBU, while another 17 samples were positive for the presence of DEN NS1 ELISA during the time of sample collection. We found there are no significant differences between the time interval between patients' diagnoses and the time of sample collection, p=0.056 (Figure 3b).



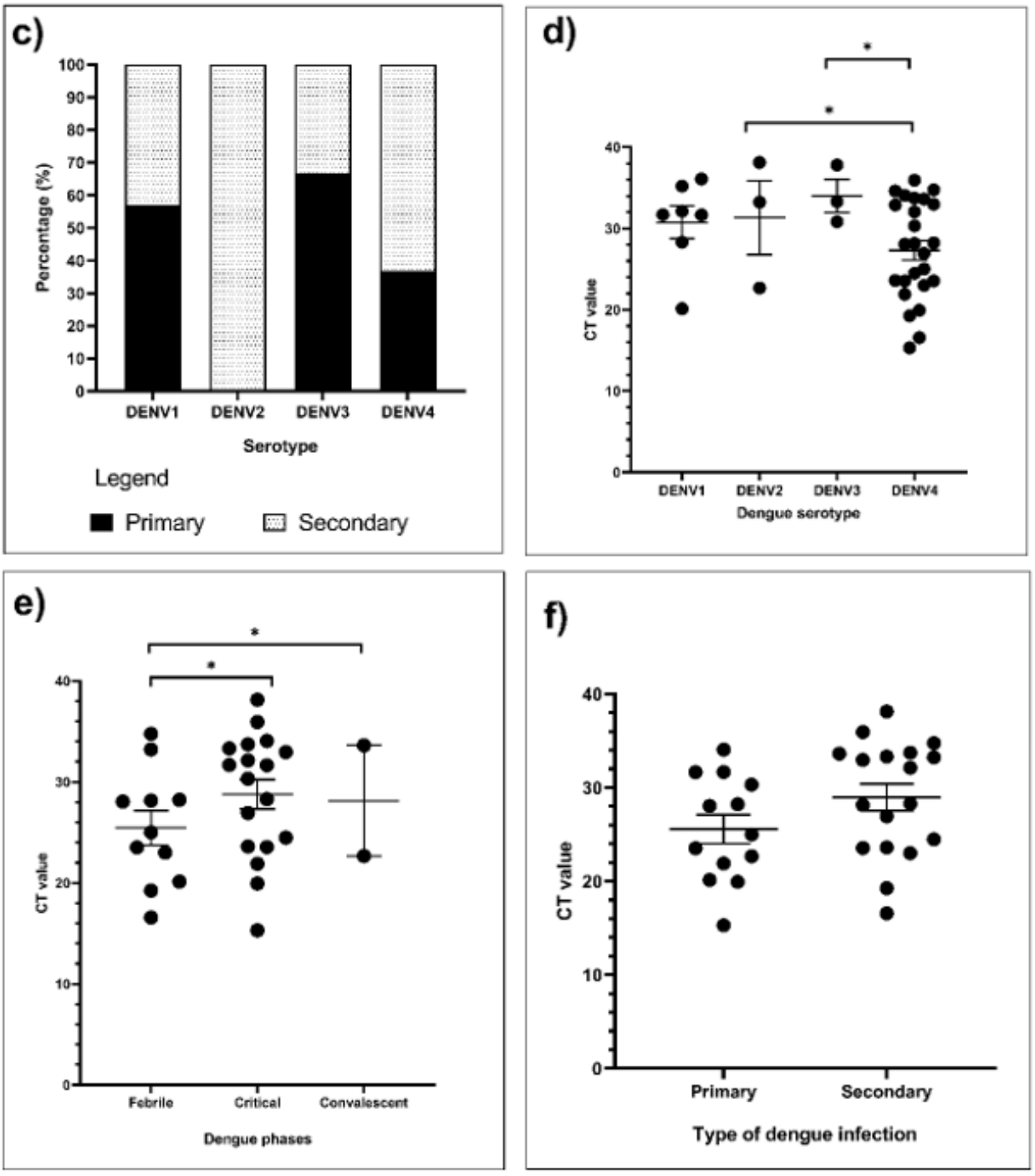


FIGURE 2. The serotype of dengue virus and viremia level of dengue NS1-positive patients admitted to HCTM; (a) The prevalence of monoinfection, concurrent infection in the patient; (b) The prevalence of dengue serotype in the patient; (c) Proportions of Dengue Serotypes in UKMMC based on primary or secondary dengue; (d) The viremia load based on the CT values categorized by the dengue serotype; (e) The viremia load based CT values categorized by the dengue phases ; (f) The viremia load based on CT values categorized by the type of dengue infection. Asterisk refers to statistically significant differences, $p<0.05$.

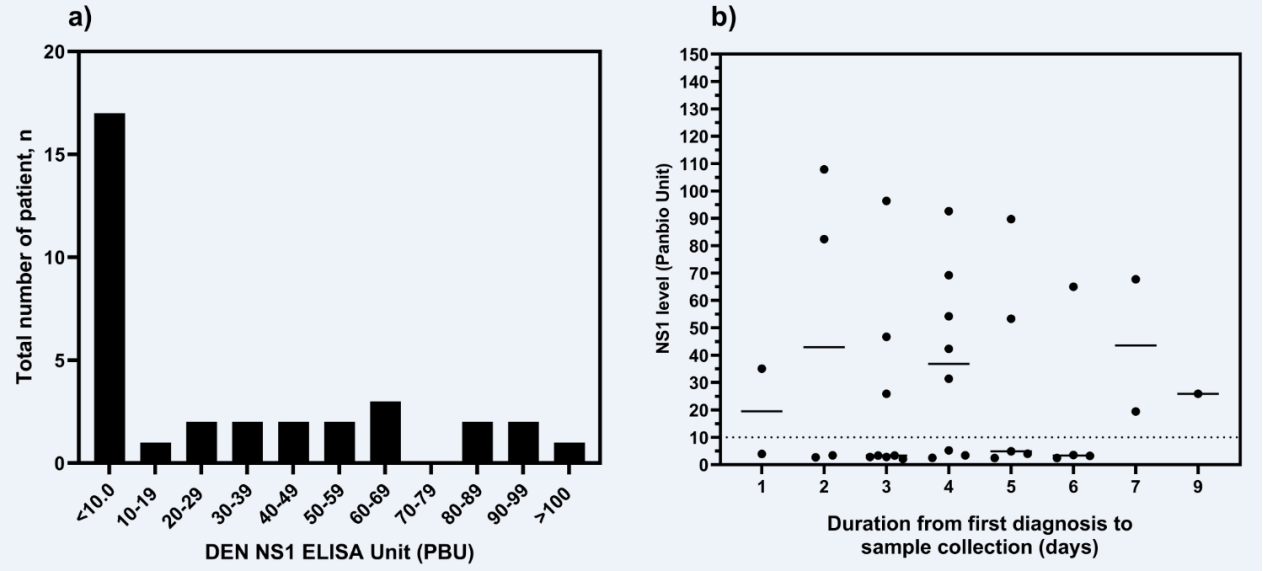


FIGURE 3 Levels of NS1 DEN antigen in dengue patients admitted to the HCTM. (a) The levels of NS1 DEN antigen in the plasma samples; (b) The levels of NS1 DEN antigen categorized by the duration (time interval) from the first diagnosis to sample collection.

ASSOCIATION BETWEEN SEROTYPE/ VIREMIA LEVEL WITH CLINICAL MANIFESTATION

In this present study, we found no significant association between the DENV serotypes and the dengue fever clinical manifestations and warning signs (Table 3). In dengue fever, there was no significant association between serotypes with headache (p=1.00), retro-orbital pain (p= 1.00), myalgia/arthralgia (p= 0.57), leukopenia (p=0.52), and rashes (p=0.632) (Table 3). In addition to that, no significant association was found between the serotypes with any warning signs such as haemorrhagic manifestations (p=0.518), abdominal pain (p=0.649), persistent vomiting (p= 0.787), persistent diarrhoea (p= 0.851), third space loss (0.483), spontaneous bleeding (p=0.404), restlessness (p=0.327) or liver tenderness (p= 0.518) and with any particular blood parameters such as level of thrombocytopenia (p=0.315), haemoconcentration (p=0.062), high lactate with/without acidosis(p=0.121), transaminitis (p=1.00), renal function impairment (p= 0.303) or hyponatremia (0.365). We also did not find any significant association between viremia levels (based on CT values) with dengue with warning signs (Table 3). Nonetheless, we found a significant correlation between the temperature of the patients with CT values (p=0.022) based on the spearman correlation test. Those patients with higher temperature were significantly correlated with low CT values.

DISCUSSION

Due to COVID-19, the Malaysian government imposed a movement control order (MCO) from 18 March 2020 to halt the transmission of COVID-19 in local communities. A study using an agent-based model and simulation predicted that the risk and severity of the mosquito borne diseases would increase during lockdown, and it was discovered that MCO and limited human movement contributed to the low number of dengue cases during the lockdown phase (Jindal and Rao 2020). We found a significant decrease in laboratory-confirmed dengue cases admitted to our teaching hospital during the COVID-19 pandemic as compared to the pre-COVID-19 year. According to the Institute for Clinical Research Malaysia, dengue incidence decreased from 90,304 in 2019 to 26, 365 in 2020 with a decrease rate of 70.8%. A previous study reported the reduction of new dengue cases ranging from 1.14% in southern peninsular Malaysia to 52.62% in central peninsular Malaysia (Rahim et al. 2021). This scenario was also seen across many dengue-endemic regions beginning in March 2020 with 2.28 million cases in 2020 as compared to 4.08 million cases in 2019 with a 44.1% decreased (Chen et al. 2022; Rahim et al. 2021). We postulated that due to restrictions in human movement and large gathering has resulted in limiting the transmission of DEN in the human transmission cycle and reduced the vector-host interaction. During the pre-covid era, in a highly mobile human population, the ease of transportation had facilitated viral transmission in viremia people and the maintenance of transmission cycles in urban areas.

In this study, we found that DENV4 was the

predominant serotype during the COVID-19 in 2021. A similar result was reported by the Institute for Clinical Research Malaysia where there was a surge of DENV4 serotype from January 2021 to November 2021 (Mokhtar 2022). This observation is interesting because it is uncommon for DENV4 to be the predominant serotype as the predominant serotype is usually DENV1, DENV2 and DENV3. In 2013, DENV2 was the dominant serotype in Malaysia, but in 2014-2015, DENV-1 surpassed DENV-2 and became the dominant serotype in Malaysia (Hii et al. 2016; Mohd-Zaki et al. 2014). The emergence of DENV4 will contribute to the surge in the DENV4 outbreak as previous studies on neutralizing antibodies on the Malaysian adult population reported that only 70% of the population possessed neutralizing antibodies to DENV4 (Azami et al. 2020). This points to the need for the health authority to prepare for a new wave of DENV4 outbreaks.

We characterized our dengue NS1 positive patients to determine the association between serotype, viremia levels and clinical manifestations for dengue fever and dengue fever with warning signs. We found patients infected with DENV4 had a higher viral load as compared to patients infected with DENV2 and DENV3. However, a study in Brazil reported that patients infected with DENV2 had a higher viral load as compared to other serotypes (Vicente et al. 2016). A study reported that infection with DENV4 was associated with haemoconcentration and plasma leakage, but we did not observe similar results in our patients (Vicente et al. 2016). We found that a low CT value correlated with a high viral load and viremia level, however we were able to demonstrate a significant difference in viremia levels between the febrile phase, critical phase and convalescent phase. Previous studies have reported that during the febrile phase (day 1 to 5 from onset), high viremia was more likely to be observed than during the critical and convalescent phases (Jang et al. 2019; M. C. Guzman et al. 2010). In addition to that, we found that high temperature was associated with higher viral load because during febrile phases, patients with fever will have high levels of viremia and as the fever subsided, the viremia level will wane (Vaughn et al. 1997).

We found sampling time and the time interval from the onset/diagnosis influenced the diagnosis of viremia level (levels of NS1 and viral load), however the differences were not significant. As the time interval increased, the level of viremia decreased. A previous study reported that NS1 antigenemic was influenced by the collection time and that samples were recommended to be collected two days prior to defervescence instead of after defervescence (Vaughn et al. 1997). Interestingly, the majority of those negative for DEN NS1 ELISA was patients that was infected with DENV4, despite the fact that all the patients who were recruited are positive for NS1 using the rapid dengue testing kit. There is a possibility of false-negative results as another study in Brazil also reported that during the surge of DENV4, the PanBio Dengue Early had reported a false negative result for DEN NS1 antigen in the majority of DENV4 infected cases (Colombo et al. 2013). Although there is possibility of the false negative result due to the kit performance, additional factors such as the sample condition during the sampling and time sampling could possibly contribute to the negative result.

Due to the small sample size, we could not find any association between serotype/viremia level and clinical manifestation in dengue patients because of the low number of the laboratory-confirmed dengue patients who was admitted to the hospital. Although the movement control order was beneficial in limiting the dengue virus transmission, there is a possibility that the number of dengue incidences was higher than reported. All dengue cases in Malaysia are reported and monitored using a passive dengue surveillance system, which requires the doctor to report the suspected symptomatic dengue cases within 24 hours of detection. Nonetheless, due to the COVID-19 pandemic and hospitals being designated as COVID-19 hospitals to treat the patients infected by the disease, there is a possibility that the decreased number of dengue patients was due to patients' reluctance to visit the hospital even though they are symptomatic because hospitals were seen as infectious reservoirs (WongLaura, HawkinsJessica, and MurrellKaren 2020). Thus, in-depth studies and seroprevalence studies are needed to investigate the real magnitude of dengue incidence during the lockdown phase and the COVID-19 pandemic.

CONCLUSION

This present study demonstrated a decrease in the number of laboratory-confirmed dengue cases and DENV4 was the predominant circulating serotype during the COVID-19 pandemic in our hospital. Patients infected with DENV4 had a higher viral load compared to other serotypes. Due to the small sample, we did not find any significant association between serotype/viremia level and clinical manifestations for dengue fever and dengue fever with warning signs. However, this study point to the need to better characterize the clinical manifestations of dengue patients and sampling time in order to help predict and identify severe cases for early intervention and patient management.

CONFLICTS OF INTERESTS

All the authors declare that they have no conflicts of interest concerning the work reported in this paper.

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