ORIGINAL ARTICLE

Association of dengue serotypes and its complications: a retrospective cohort study

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ABSTRACT

Introduction: Dengue fever is an arthropod-borne disease and has a wide clinical spectrum. It is hypothesised that dengue serotypes could be a possible factor for such phenomena and therefore be a possible predictor for the development of severe dengue.

Method: A retrospective cohort study was done to explore the association between dengue serotypes and the various complications. All patients who underwent dengue serotyping from 1st January to 31st December 2018 in Tengku Ampuan Rahimah Hospital were selected. Serotypes were randomly done for admitted dengue patients. Notes were then retrieved for data collection. Secondary outcomes like length of stay and highest lactate level were also studied. Data analysis was done using SPSS version 20.

Result: A total of 193 patient records were included in the analysis. Chi-square test for independence indicated that the proportion of dengue complications between male and female were significantly different ($\chi^2(1) = 11.37$, $p = 0.001$). Dengue serotype was not associated with the development of dengue complications, total number of dengue complications, length of admission, lactate level and survival among the serotypes. Results of the binary logistic regression showed that men have thrice the odds (AOR = 3.3, 95% CI: 1.6 6.7) for developing dengue complications. One patient was found to be co-infected with serotype 2 and 3.

Conclusion: Our study did not reveal any association between the different dengue virus serotypes and its complications. Therefore, all dengue infection should be approached with equal meticulousness. There are possibilities that apart from serotype, dengue genotype and lineage would determine clinical outcome. However, more studies are required to study such associations.

KEYWORDS:
Dengue, dengue complications, serotypes

INTRODUCTION

Dengue is an arthropod-borne infectious disease caused by the dengue virus. The virus consists of four serotypes (DENV-1, DENV-2, DENV-3, DENV-4) and is transmitted through bites of infected Aedes mosquitoes.

Being endemic in tropical and sub-tropical countries, dengue is a major public health issue in Malaysia with an incidence 245 per 1000 population in the 2018.[1] The incidence of dengue has alarmingly risen seven-fold from the year 2000 to 2010 and peaked in 2019 with an incidence of 399 cases per 10,000 population.[1,2] It was estimated that Malaysia spent around USD175.7 million annually on the treatment and prevention measures of dengue.[3]

The dengue virus is from the genus *Flavivirus* which falls under the family of *Flaviviridae*. Dengue virus was first identified and isolated in 1943 in Japan. That specific isolated virus is now referred to as the dengue virus 1 or DEN 1. Since then, dengue virus has been divided into four serotypes based on the interaction with the antibodies in the human blood serum.[4] Such discovery is followed by the question of clinical outcomes among patients who are infected with different serotypes. Different dengue serotypes had been observed to be associated with different complications as reported by Vincente CR et al.[5] It is noteworthy that patients with DENV-2 appeared to have a higher tendency of progression to severe dengue than among those of DENV-1 and DENV-4.[5] A retrospective study conducted in Thailand also revealed that DENV2 was the most frequent serotype isolated from patients suffering from haemorrhagic fever and dengue shock syndrome.[6]

Hospital Tengku Ampuan Rahimah (HTAR) is considered to be one of the busiest tertiary hospitals in the country and has a high number of dengue fever admissions. In this retrospective cohort study, we explore the association between different dengue serotypes and dengue complications. In addition, we examined the type complications encountered and length of hospital stay among patients of different dengue serotypes in HTAR.

MATERIALS AND METHODS

Study Design and Population

In HTAR, Real-Time Quantitative Reverse Transcriptase Polymerase Chain Reaction (RT-qPCR) serotype identifications were done for dengue patients who were admitted to the intensive care unit (ICU) and randomly selected dengue patients from the dengue wards. In this retrospective cohort study, we compiled a list of all 230 patients with dengue serotypes record from microbiology laboratory spanning from 1 January 2018 till 31 December

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2018. Patients under the age of 18 years with missing case notes were excluded from the study. From this list of 193 patients, we retrieved patients' case notes and retrospectively analysed the associated dengue complications, highest lactate level, length of stay (LOS) in hospital and outcome (alive/dead).

Outcome Measures

Our primary goal is to determine any correlation between serotypes and each dengue complication. In addition, we also investigated for possible linkage between average number of complications for each serotype, the highest lactate levels, average LOS and the number of mortalities according to serotype. Patients' clinical classification and complications were clinically diagnosed by the physicians according to the 2012 World Health Organisation (WHO) guideline for dengue. Based on WHO and local guidelines, multiple complications are decided as the study outcome. For haemophagocytic lymphohistiocytosis (HLH), patients with serum ferritin ≥ 20,000 µg/L or ≥ 10,000 µg/L plus organ impairment were included. For carditis, patients with CK ≥ 1000U/L, raised troponin or evidence of carditis via echocardiographic imaging were included. To fulfil the complication of hepatitis, patients will have ALT or AST ≥ 1000U/L. In the case for polyuria, patients with urine output of more than 3 L/day were included. Lastly, for complications such as encephalitis, acute kidney injury (AKI), bleeding (occult/overt) or plasma leakage (ascites/pleural effusion), we included patients who were clinically diagnosed as such by attending clinicians.

Statistical Analysis

Statistical analysis was performed using the Statistical Analysis Software Version 20 (SPSS 20).

RESULTS AND ANALYSIS

A total of 193 patient records were included in the analysis, one dengue patient was found to be co-infected with serotype 2 and 3. The age of the subjects varied from 18 to 85 years, with an average age of 39.5 years (SD 16.0). Majority of the subjects were female (55.4%), native (51.8%), were infected with dengue serotype 3 (40.4%) and developed dengue related complications (71.5%).

Among the complications studied, it was found that a third of subjects developed plasma leakage (34.2%). The second most common complication encountered was bleeding (24.9%), followed closely by carditis (21.2%), HLH (16.1%), AKI (14%), polyuria (10.9%), hepatitis (9.8%) and encephalitis (9.3%). Complications developed were not mutually exclusive as many subjects developed more than one complication during their hospitalisation. Figure 1 shows the number of complications developed by subjects in this study. The sociodemographic details of the study population and complications are presented in Table 1.

Chi-square test for independence indicated that the proportion of dengue complications between male and female were significantly different ($\chi2(1) = 11.37$, $p = 0.001$). Lactate levels were significantly associated with dengue complication. The length of stay was also prolonged when dengue complication occurs. Age, ethnicity and dengue serotype did not influence this outcome.

Age, ethnicity and dengue serotype did not affect the development of complications for both univariable and multivariable analysis. Interaction between lactate levels and length of stay was noted, thus length of stay was not included in the multiple logistic regression analysis. Male gender and higher lactate levels were found to be significant predictors for dengue related complications after adjusting for age, ethnicity and dengue serotype.

(Male adjusted odds ratio [AOR]: 3.07 [1.43, 6.56] when compared to female; lactate levels 2.1-3.0 AOR: 4.15 [1.86, 9.24]; lactate levels >3.0 AOR: 11.25 [4.17, 30.36] when compared to lactate levels of 2.0 and below)

Further analysis showed that men had 2.65 times risk of bleeding (95% CI: 1.35, 5.19), 3.51 times risk of acute kidney injury (95% CI: 1.45, 8.48) and 4.68 times risk of developing carditis (95% CI: 2.18, 10.05) when compared to women in this study. Other complications studied which were plasma leakage, HLH, polyuria, encephalitis and hepatitis were found to be not significantly affected by gender.

Dengue serotype was not associated with neither development of AKI, carditis, HLH, bleeding, encephalitis, hepatitis, nor plasma leakage using Pearson Chi-square test (Figure 2).

Chi-square test for independence indicated that there were no significant associations between dengue serotype with lactate levels and number of dengue complications, whereas Fischer exact test analysis found that this variable was not associated with the overall survival of dengue patients. As the distribution of length of stay among the serotypes was skewed, Kruskal-Wallis test was used to show no significant difference for duration of hospitalisation between the different serotypes ($\chi^2(2) = 1.45$, $p = 0.483$).

DISCUSSION

In Malaysia, although all four serotypes can be isolated from the community acquired infection, DENV1 and DENV2 are the predominant serotypes seen. The number of patients with DENV3 and DENV4 infection has reduced since 2013. Notably, the number of DENV4 serotypes infection was not captured from 1996 to 2000.[7] The absence of DENV4 infection is consistent in our study also. However, our cohort showed that DENV3 was the most prevalent serotype to infect our cohort.

The current study focuses on the isolated serotypes and its complications. Based on our analysis of these 193 dengue patients in HTAR from the year 2018, there had been no or negligible association between the serotypes and types of complications encountered. In terms of disease severity, there seemed to be no marked difference in the highest lactate levels recorded between each serotype.

There are myriad of studies studying DENV serotypes and dengue severity but their discoveries were not consistent. A

Table I: Sociodemographic characteristics of dengue patients and dengue related complications

Variable	Complications		*p* value
	No	Yes	
No. of persons	55 (28.5%)	138 (71.5%)	
Age (years)			0.236[a]
median (IQR)	34.0 (27.0)	38.0 (25.0)	
Gender			0.001[b]
Male	14 (25.5%)	72 (52.2%)	
Female	41 (74.5%)	66 (47.8%)	
Ethnicity			0.936[b]
Native	30 (54.5%)	70 (50.7%)	
Chinese	6 (10.9%)	14 (10.1%)	
Indian	14 (25.5%)	38 (27.5%)	
Others	5 (9.1%)	16 (11.6%)	
Dengue serotype			0.488[b]
1	16 (29.1%)	34 (24.6%)	
2	15 (27.3%)	50 (36.2%)	
3	24 (43.6%)	54 (39.1%)	
Lactate levels (mmol/L)			<0.001[b]
2.0 and below	30 (54.5%)	22 (15.9%)	
2.1-3.0	18 (32.7%)	56 (40.6%)	
3.1-4.0	4 (7.3%)	48 (34.8%)	
4.1 and higher	3 (5.5%)	12 (8.7%)	
Outcome			0.559[c]
Survived	55 (100.0%)	135 (97.8%)	
Died	0 (0.0%)	3 (2.2%)	
Length of Stay (days)			0.007[a]
median (IQR)	4.0 (2.0)	5.0 (3.0)	

IQR: interquartile range
[a]: Mann-Whitney U test
[b]: Pearson chi-square test
[c]: Fischer exact test

Table II: Results of univariable and multivariable analysis of study variables with complications

Variable	Crude OR (95% CI)	*p* value
Age (years)	1.01 (0.99, 1.03)	0.354
Gender		0.001
Female	1.00 (Ref.)	
Male	3.20 (1.60, 6.39)	
Ethnicity		0.936
Malay	1.00 (Ref.)	
Chinese	1.00 (0.35, 2.85)	1.000
Indian	1.16 (0.55, 2.46)	0.692
Others	1.37 (0.46, 4.09)	0.571
Dengue serotype		0.490
3	1.00 (Ref.)	
1	0.94 (0.44, 0.23)	0.883
2	1.48 (0.70, 3.14)	0.305
Lactate levels (m mol/L)		<0.001
2.0 and below	1.00 (Ref.)	
2.1 to 3.0	4.24 (1.98, 9.11)	<0.001
3.1 and higher	11.69 (4.49, 30.43)	<0.001
LOS (days)	1.17 (1.01, 1.37)	0.043

cross-sectional study by Vincente CR et al. in Brazil of 485 dengue cases revealed that patients with Serotype 2 had a higher proportion of severe dengue as compared to those of Serotype 1 and 4.[5] This result was corroborated by other previous studies.[8-13]

However, there were also studies with conflicting results. In 2011, a study done on hospitalised cases in Vietnam revealed that Serotype 1 and 2 had similar chances of progressing to dengue haemorrhagic fever.[14] Fox et al suggest that patients with DENV 1 had a higher probability of developing dengue haemorrhagic fever as compared to those with Serotype 2.[15] Suppiah, et al showed that patients infected with DENV2 would developed severe dengue more frequently but also discussed about the role of genotypes affecting clinical manifestations of dengue infection.[16] Yung et al. discussed about the possibility that the same serotype may have different genotype which results in a different clinical outcome.[17] E-envelope sequencing were done to identify genotypes of specific serotypes. The author showed that the DENV 2 serotype of a cosmopolitan genotype circulating in Singapore and Malaysia was different from the

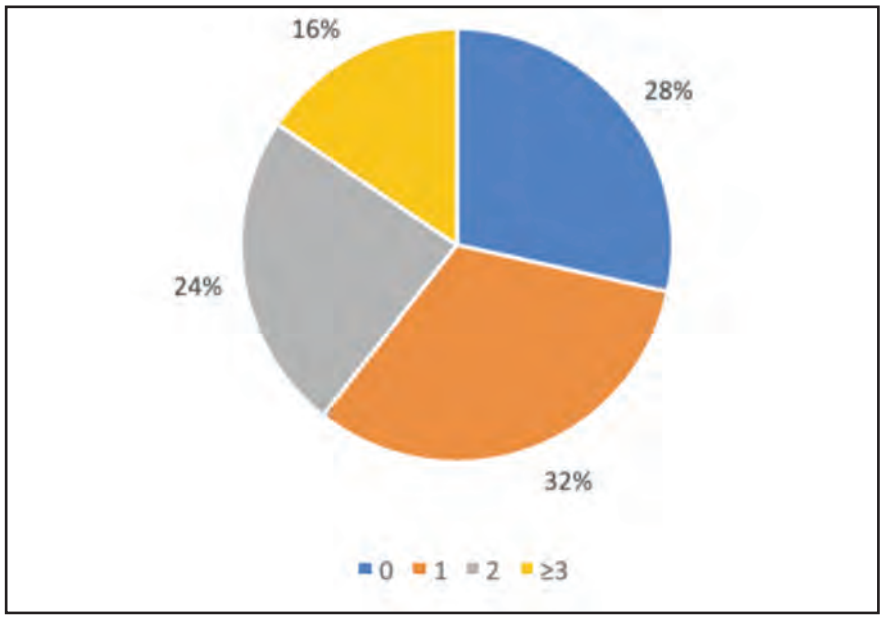

Fig. 1: Number of dengue related complications by subjects

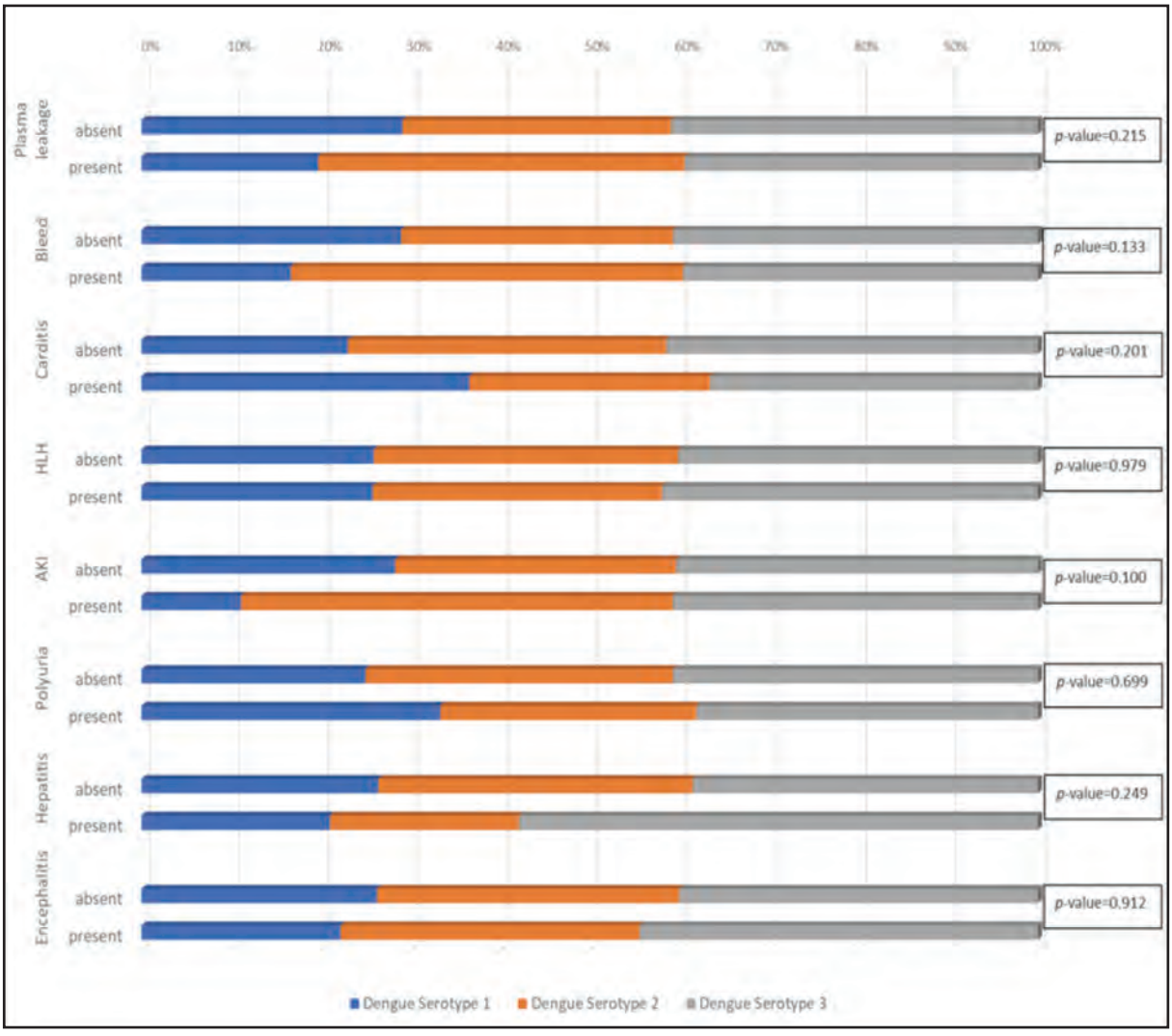

Fig. 2: Association of dengue complications with dengue serotype

American/Asian genotype in Brazil and can be the reason for the reduced severity observed.[17] Jiang et al further studies the lineage of DENV2 genotype and the risk of developing haemorrhage and shock. They concluded that Malaysia/Indian subcontinent genotype for DENV2 may be less virulent.[18] It is possible that due to the genotypic variability of the DENV in our population, the association of DENV serotype and severity or complication is less straightforward.[16] More studies need to be done to identify DENV serotypes, genotypes and lineage in our population.

Our study showed that there is a higher proportion of female who has been admitted for dengue infection. This could be due difference in health care-seeking behaviour. Female have been reported to visit healthcare provider more and would translate to a higher pick-up rate.[19-21] The increased dengue cases for female is reflected by a studies in Puerto Rico and Mexico which showed that female made up a slight majority of adult dengue cases.[22,23] However, this is in contrast with Singapore, which had a larger proportion of men among those who had dengue infection reported from year 2000 to 2005.[24] A local study by Shekhar et al also observed a higher dengue infection rate in males.[24] It is important to note that the studies above are epidemiological studies and therefore may reflect differently from our study which is hospital based. Despite the increase in female cases, our study revealed that men are at higher risk of having dengue complication regardless of serotypes. Higher incidence of dengue haemorrhagic fever and mortality among males has been reported by Shekhar et al.[24] Md-Sani et al studied 199 patients with severe dengue patients and found that two-third of his cohort was noted to be male.[25] Fatal dengue cases were also observed more frequently in male as noted by Dakeek et al in Yemen.[26] It is likely that such findings may be due to the fact that men are likely to "underreact" to illness leading to late diagnosis and a more severe presentation.[27]

One patient in our study had coinfection with Serotype 2 and 3. She was a 17-year-old female who suffered from plasma leakage as a result of a complication from dengue. However, her highest lactate level was 1.5 mmol/L and she was discharged well after spending 4 days in the hospital.

DENV coinfection is an interesting phenomenon. The very first reported case of coinfection with different dengue serotypes was in Puerto Rico in the year 1982.[28] Although there had been reports from other countries, at the time of this writing, this is the only the second known published report of concurrent infections in Malaysia. Suppiah et al described one case of DENV1 and DENV2 coinfection in 2015. The study however did not describe any association between such occurrence and dengue complications.[16] An analysis of the different dengue serotypes coinfection during an outbreak in India identified nine patients with DENV co-infection. It showed that six out of the nine subjects had dengue haemorrhagic fever but did not conclude any statistical significance.[29] In contrast, a study in 2011 by Martin et al involving an outbreak in in Brazil revealed that patients with co-infection tend to develop severe dengue or minor haemorrhagic phenomena. The report also concluded that there is an increase of alarm sign in those with two serotype co-infections.[30]

We postulate that the patients with DENV co-infection may have been exposed to a single mosquito which was heteroserotypic. A study in Southern Thailand which involved the capture of Aedes sp. mosquito in the fields was done in 2005. Viral RNA was extracted from all the captured mosquitoes. The researchers revealed three mosquitoes which were infected with two different serotypes. It is postulated that such phenomena could have occurred when the vector feed on dengue patients, each with different serotype thus exposing it to different serotypes or vector feeding on patients with existing heteroserotypic infection.[31] Nevertheless, we believe such occurrence would only be likely in a community deemed to be hyperendemic with dengue virus as in our population.[32]

In our study, patients with different serotypes appeared to have similar average length of stay LOS. A longer LOS result in higher healthcare cost incurred for food, bed occupancy and treatment. This corresponds to our finding of a similar degree of severities across all serotypes. A multivariate analysis by Mallhi et al. of dengue patients attending tertiary care centres revealed that dengue haemorrhagic fever, coagulopathy and multiple organ dysfunctions were independently associated with prolonged hospitalisation.[33] Such finding is consistent with our study.

Serum lactate has long been used to be a marker of end-organ hypoperfusion or shock. Due to the fact that dengue shock syndrome is a well-known complication of severe dengue, lactate can be a good predictor to indicate severity.[34] M Gupta et al while studying a few prognostic markers went as far as to report that lactate was found to be the best prognostic marker for mortality for severe dengue.[36] Our study reaffirms this notion by revealing the association between high lactate level and dengue complications.[35-37]

CONCLUSION

Dengue virus infection, regardless of the serotypes, should be approached identically and treated with an equal level of meticulousness as all of them appeared to have similar severity. More studies can be done to explore the inherent pathologic nature of the dengue serotype, such as dengue genotypes and lineage. Further studies in regard to dengue serotype co-infection and complications should be pursued due to our hyperendemic circumstances.

STUDY LIMITATION

This was a retrospective cohort study of dengue patients in HTAR in the year 2018 and the authors are aware of the inherent limitation of a small sample size and low mortality rate to draw any consequential conclusion from this study. Not all dengue patients who were admitted in 2018 were included in this study as PCR serotypes identification was done only for dengue patients who were admitted to the intensive care unit (ICU) and randomly selected dengue patients from the dengue wards.

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DECLARATION

This study has no conflict of interest and is not funded by any organisation.

ETHICAL APPROVAL

This study was registered with National Medical Research Register (NMRR) and approved by the Medical Research and Ethics Committee (MREC) and the Ministry of Health (MOH). MREC Approval Letter 22-01488-KLB (1) dated 26 September 2022. NMRR ID 22-01488-KLB

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