Original Article

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Association between Specific Single Nucleotide Polymorphisms, Clinical Features and Severe Hepatitis in Dengue Patients

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Abstract:

Objective: This study aimed to identify predictors associated with severe hepatitis, especially genetic features, among dengue patients.

Material and Methods: One hundred seventy nine dengue patients from the years 2009-2014 were identified from the hospital inpatient database. Their baseline characteristics, laboratory data and hospital course were recorded and analyzed. Seventy five patients had been tested for specific single nucleotide polymorphisms either the Janus kinase 1 (*Jak1*) or the cluster of differentiation 209 genes.

Results: Most of the identified study patients were female (64.8%), 74.3% had elevated liver enzymes, 48.6% of whom showed mild hepatitis. Serum aspartate aminotransferase was predominate over serum alanine aminotransferase (ALT) in most patients. Myalgia was the most common initial presentation (65.4%). Severe hepatitis (ALT ≥10 times the upper limit of normal) was associated with a higher intensive care unit (ICU) admission rate (26.3%) and complications (36.8%). There was significant elevation of liver enzymes among patients with initial platelets <50,000/microliter and albumin <3.5 gram (g)%. An initial serum albumin <3.5 g% had an odds ratio=4.16, 95% confidence interval 1.41-12.27 in association with severe hepatitis. No difference in specific single nucleotide polymorphisms was found between the severe liver involvement group and the mild group.

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Conclusion: Liver involvement among dengue infection is frequent, mostly manifesting as mild transaminitis. Patients with severe hepatitis usually have poorer clinical outcomes due to complications and often require ICU admission. Initial serum albumin <3.5 g% appears to be a predictor associated with severe hepatitis and bad outcomes. The genetic susceptibility needs more study.

Keywords: dengue fever, dengue hemorrhagic fever, genetic susceptibility, hepatitis

Introduction

Dengue infection is an important problem worldwide. There are 50–100 million dengue-infected patients each year with a mortality of approximately 24,000–25,000 patients per year. Most cases are from tropical areas, especially Southeast Asia.[1]

In Thailand, there have been many dengue outbreaks including 1987, when 174,285 persons were infected with dengue and 1,007 patients died, and 2007 when 58,836 patients were infected and more recently in 2013, when 124,225 patients were infected with 114 fatalities.[2] The highest incidence is found during the rainy season between June and August, with the most common serotypes being type 1 and 2.[3]

The diagnosis of dengue infection is mainly based on clinical symptoms and basic laboratory investigations, especially in developing countries in which the dengue serology test is often not available. Systematic reviews have demonstrated a high yield and accuracy of dengue diagnosis and other acute febrile illnesses using clinical symptoms and basic laboratory investigations in endemic areas. The basic laboratory investigations are thrombocytopenia, leucopenia, especially neutrophils, and evidence of elevated liver enzymes.[4,5] Symptomatic dengue virus infections can present with a wide range of clinical manifestations, from a mild febrile illness to a life-threatening shock syndrome or organ dysfunction.[6]

One important characteristic found in dengue patients is liver involvement, expressed as an elevation of transaminase enzymes from acute hepatitis. In various studies this situation was found in 60.0–80.0% of dengue patients.[7–11] The mechanism causing the elevation of transaminase enzymes is thought to be direct hepatocyte injury from the dengue virus, leading to inflammation and release of these enzymes into the blood circulation.[12] Usually, mild hepatitis cases display liver enzymes 2–5 times above the normal limit which can be detected at 3 days after the appearance of clinical symptoms. Severe cases of elevated liver enzymes are frequently found in dengue hemorrhagic fever, which is a severe syndrome of dengue infection.[7,13] Severe hepatitis patients often experience acute liver failure and high mortality rates.[14–17]

Previous studies have demonstrated high mortality and morbidity among patients with severe hepatitis, and more hospital stays than patients with mild transaminitis. Bleeding disorders and acute renal failure are the most common complications.[11,18] Some studies have tried to identify factors related to severe manifestations in dengue patients and found that severe hepatitis was an important factor.[18,19] However, no previous studies have identified specific factors related to severe hepatitis in dengue patients, which would help clinicians employ appropriate care and strategies to reduce morbidity and mortality.

With recent advances in genetics and medicine, many close relationships between illnesses and specific genetic markers have been identified.[20] A genome-wide association study was conducted to identify associations between single nucleotide polymorphisms (SNPs) and various diseases. To date there have been few studies examining potential associations between specific host factors and dengue-infected patients with severe forms of dengue hemorrhagic fever. In an earlier study, we found

interferon type I was responsible for the natural course of diseases involving the *Jak1* gene that is essential for signaling for this cytokine.[21] We also found that single nucleotide polymorphisms in the *Jak1* gene were associated with dengue hemorrhagic fever [odds ratio (OR)=4.2].[22] In Thailand, another study in children found an association between specific single nucleotide polymorphisms in the cluster of differentiation 209 (*CD209)* gene and severe forms of dengue infection (OR=5.8).[23] The *CD209* gene encodes a protein called Dendritic Cell-Specific Inter-cellular adhesion molecule-3-Grabbing Non-integrin which is a receptor present on the surface of both macrophages and dendritic cells. It recognizes and binds to mannose-type carbo-hydrates commonly found on viruses.[24] All of these studies indicate a link between more severe hepatitis and severe forms of dengue. We hypothesized an association between the mentioned genes and severe hepatitis.

The aim of this study was to examine possible associations between the host genome, especially the genes mentioned above, clinical factors and severe hepatitis in dengue patients. It was hoped this work would help to minimize dengue morbidity and mortality, and contribute basic scientific knowledge for further research.

Material and Methods

Enrollment

A 6-years retrospective electronic search was conducted on inpatient medical records from January 2009 to December 2014 for the target populations. Patient records were included in the study if they were diagnosed as dengue fever (A90) or dengue hemorrhagic fever (A91) according to the 10th revision of the International Statistical Classification of Disease and Related Health Problems (ICD-10), and had compatible clinical and laboratory results according to the dengue guidelines for dengue diagnosis, World Health Organization 2009, with or without definite serological testing.[4-6]

Diagnosis dengue infection definitions

Probable dengue: was diagnosed if the patient lived in or had travelled to a dengue endemic area and had fever and any 2 of the following criteria: nausea, vomiting; rash; aches and pains; tourniquet test positive; leucopenia.

Serological testing: was diagnosed for patients with positive dengue ribonucleic acid or non-structural protein 1 antigen or dengue immunoglobulin M; enzyme-linked immunosorbent assay or equivalent or a 4-fold rise of immunoglobulin after the convalescent phase (at least 10 to 14 days after the acute phase serum).

Target populations: inpatients from January 2009-December 2014 were included in the study if they met the following criteria: more than 18 years old at the time of enrollment, diagnosed as dengue infection with at least one transaminase enzymes test during their hospital course, and no other conditions or known preexisting liver diseases that could explain their illness. Patients who had a previous abnormal liver function test before admission were excluded to minimize confounding factors.

Demographic data, comorbidities, body mass index, clinical presentation, initial and serial laboratory results, serologic testing, and clinical outcomes were reviewed from the hospital records and further analyzed.

Case and control definitions

We designed the study as a nested case-control study with a 1:3 ratio between the case and control groups. The case group included patients who had severe hepatitis [defined as serum alanine aminotransferase (ALT) ≥10 times normal values or ALT ≥5 times normal value] coexisting with abnormal bilirubin levels; total bilirubin (TB) ≥1.5 milligram (mg)% or coagulopathy; prothrombin time (PT) prolonged more than 4 seconds or activated partial thromboplastin time prolonged more than 10 seconds. The controls were patients who had normal or

mild hepatitis, defined as ALT <5 times normal value. The upper limit of normal ALT was set at 40 units per litre (U/L) according to our hospital laboratory system.

The study involved two steps first identifying the appropriate patient records, and secondly contacting those patients for genetic testing by phone. The patients who agreed to participate in the study received some financial compensation. Initially, we used a case-control formula to calculate the sample size with target cases of 21 (based on the ratio of positive *CD209*=5.84 in DHF compared with DF[23]). We tried to enroll all possible cases at that time. Finally, 75 individuals (including 19 cases and 56 controls) who were identified as having had dengue during the study period agreed to participate in SNP genetic testing.

Blood sampling and genetic testing

All 75 patients had the study explained to them again on the day of their appointment, and informed consent was then given before the blood sample was taken. The test needed only 6 mL of blood in an ethylene-diaminetetraacetic tube. The samples were cold centrifuged at a rate of 4,000 rpm for 5 min to separate the blood into the buffy coat, serum and red cell components. The separated tubes were frozen at -70 °C, and transported to the Department of Biochemistry, Chulalongkorn University for deoxyribonucleic acid (DNA) extraction and real time polymerase chain reaction (PCR) for the specific SNPs *CD209* (rs4804803), *Jak1* (rs11208534, and rs2780831).

Statistical analysis

Data are expressed as mean±standard deviation for all variables with normal distribution and median and interquartile range for variables with non-normal distribution. Multiple group comparisons were performed using one-way analysis of variance followed by Tukey's test for normally distributed data and the the Kruskall-Wallis Test with Dunns comparison for non-normally distributed data. Associations between baseline characteristics, initial laboratory predictors and severe hepatitis (ALT ≥400 U/L) are expressed as an odds ratio with 95% confidence interval. A probability value (p-value) of <0.05 was considered significant. Data were analyzed using the R statistical program.

Results

One hundred seventy nine inpatients were included in the study. The mean age of the patients was 33.7±11.3 years. Among these patients, 64.8% were female, and 77.7% were previously healthy without any underlying diseases. 11.2% were pregnant (Table 1). After categorization of the patients according to maximum ALT, the largest group (48.6%) had mild hepatitis [ALT 1-5 times the upper limit of normal (ULN)], 25.0% had normal ALT (<40 U/L), 15.1% had moderate hepatitis (ALT 5-10 times ULN) and 10.6% had severe hepatitis (ALT ≥10 times ULN) (Figure 1). The average body mass index (BMI) was 22.6±4.2 kg/m^2, and no significant differences in BMI were observed between the severity groups. Most patients were admitted on the 4th day of fever.

After high grade fever, myalgia and nausea or vomiting were the most common clinical presentations, at 65.4 and 44.7% respectively. We also found diarrhea (13.4%) and petechiae (6.1%) as less common presentations.

At initial admission, the median white blood cell count was 3,120/μL, and was highest in the severe hepatitis group (4,170/μL), while lowest in the mild hepatitis group (2,860/μL). Absolute lymphocyte count was highest in the severe hepatitis group (1,487/μL). Atypical lymphocytes were highest in the severe hepatitis group but the difference was not statistically significant. The median platelet count was less than 100,000/μL (75,000/μL) after initial admission and lowest among the moderate hepatitis group (34,000/μL). The median initial hematocrit was highest in the moderate hepatitis group (42.4%) and lowest in the severe hepatitis group (39.5%).

Table 1 Baseline characteristics

Patient characteristics	Total n=179	Normal n=46 (25.7)	Mild hepatitis n=87 (48.6)	Moderate hepatitis n=27 (15.1)	Severe hepatitis n=19 (10.6)	p-value[#]
Sex (%)						
Male	63 (35.2)	12 (26.1)	31 (35.6)	13 (48.1)	7 (36.8)	0.297
Female	116 (64.8)	34 (73.9)	56 (64.4)	14 (51.9)	12 (63.2)	
Age (years)	33.7±11.3	30 (24.2, 36.8)	33 (25.5, 44.0)	28 (26.5, 33.0)	29 (24.0, 38.0)	0.224
BMI (kg/m^2)	22.6±4.2	20.9 (19.5, 22.8)	23.2 (20.5, 25.7)	18.7 (16.6, 26.1)	21.0 (19.6, 23.5)	0.054
Comorbidity (%)						
None	139 (77.7)	31 (67.4)	69 (79.3)	23 (85.2)	16 (84.2)	0.266
DM	1 (0.6)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)	
HT	2 (1.1)	1 (2.2)	1 (1.1)	0 (0.0)	0 (0.0)	
Dyslipidemia	2 (1.1)	0 (0.0)	0 (0.0)	1 (3.7)	1 (5.3)	
Pregnancy	20 (11.2)	9 (19.6)	8 (9.2)	2 (7.4)	1 (5.3)	
Others	15 (8.4)	5 (10.9)	8 (9.2)	1 (3.7)	1 (5.3)	
Medications (%)						
None	166 (92.7)	42 (91.3)	81 (93.1)	26 (96.3)	17 (89.5)	0.329
DM-drugs	1 (0.6)	0 (0.0)	1 (1.1)	0 (0.0)	0 (0.0)	
HT-drugs	1 (0.6)	1 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	
DLP-drugs	2 (1.1)	0 (0.0)	0 (0.0)	1 (3.7)	1 (5.3)	
Others	9 (5.0)	3 (6.5)	5 (5.7)	0 (0.0)	1 (5.3)	
Days of admission	4 (1-8)	4 (1-7)	4 (1-7)	5 (3-8)	5 (3-8)	
Symptoms (%)						
Abdominal pain	7 (3.9)	1 (2.2)	3 (3.4)	2 (7.4)	1 (5.3)	0.566
Myalgia	117 (65.4)	30 (65.2)	59 (67.8)	15 (55.6)	13 (68.4)	0.692
Diarrhea	24 (13.4)	8 (17.4)	11 (12.6)	4 (14.8)	1 (5.3)	0.635
Rash	3 (1.7)	1 (2.2)	2 (2.3)	0 (0.0)	0 (0.0)	1.000
Petechiae	11 (6.1)	4 (8.7)	3 (3.4)	2 (7.4)	2 (10.5)	0.366
Nausea/vomiting	80 (44.7)	18 (39.1)	38 (43.7)	16 (59.3)	8 (42.1)	0.395

*BMI=body mass index, DM=diabetes mellitus, HT=hypertension, DLP=dyslipidemia, kg=kilograms, m^2=meter squared

[#]Analyzing differences across all groups

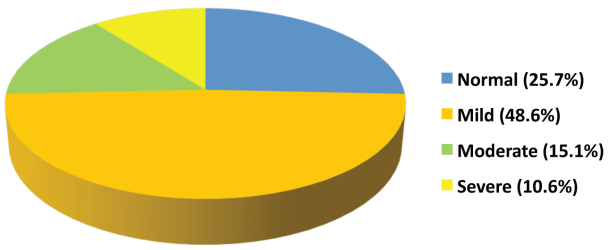

Figure 1 Categorization of hepatitis severity

The initial liver function tests were mostly performed on the 5th day of fever (2nd day of admission). The aspartate aminotransferase (AST) was predominately higher than ALT for all patient categories, and albumin was lowest among the severe hepatitis group (3.7 g%) (Table 2).

Table 2 Laboratory investigations

Laboratory values	Total n=179	Normal n=46 (25.7)	Mild hepatitis n=87 (48.6)	Moderate hepatitis n=27 (15.1)	Severe hepatitis n=19 (10.6)	p-value#
Admission labs						
WBC (/μL)	3,120 (2,235, 4,815)	3,410 (2,312, 6,035)	2,860 (2,135, 4,385)	3,070 (2,370, 3,945)	4,170 (2,425, 5,325)	0.211
Absolute lymph (/μL)	714 (428, 1,125)	629 (462, 1,075)	640 (411, 962)	936 (425, 1,134)	1,487 (444, 2,341)	0.128
Atypical lymph (%)	4 (0.0, 9.0)	2 (0.0, 8.0)	2 (0.0, 8.0)	6 (2.0, 11.5)	8 (4.0, 10.0)	0.021
Band (%)	2.0 (0.0, 7.0)	2.5 (0.0, 5.8)	2.0 (0.0, 6.5)	4.0 (2.0, 9.0)	1.0 (0.5, 10.5)	0.373
Platelets (/μL)	75,000 (35,500, 118,000)	113,500 (61,500, 134,000)	71,000 (35,000, 108,000)	34,000 (18,000, 90,000)	64,000 (36,500, 11,7000)	<0.001
Hct (%)	40.8 (37.0, 44.0)	40.9 (38.0, 43.3)	40.4 (36.4, 44.5)	42.4 (39.8, 45.2)	39.5 (34.9, 42.9)	0.217
Minimum WBC	2,360 (1,795, 3,400)	2,470 (1,812, 3,510)	2,280 (1,780, 3,140)	2,360 (1,800, 3,390)	2,600 (2,000, 4,050)	0.680
Minimum platelets	37,000 (19,000, 67,000)	62,500 (29,500, 87,500)	35,000 (20,000, 61,000)	24,000 (14,000, 47,000)	35,000 (17,500, 55,500)	0.002
Day of 1st LFT	5 (3.5, 5.0)	4 (3.0, 5.0)	4 (3.0, 5.0)	5 (4.0, 6.0)	5 (5.0, 6.5)	0.002
1st LFT						
TB (mg%)	0.4 (0.3, 0.7)	0.4 (0.3, 0.6)	0.5 (0.3, 0.6)	0.5 (0.3, 0.6)	0.7 (0.4, 1.6)	0.010
DB (mg%)	0.2 (0.1, 0.3)	0.1 (0.1, 0.2)	0.2 (0.1, 0.3)	0.2 (0.1, 0.5)	0.4 (0.2, 1.1)	<0.001
AST (U/L)	105 (58, 226)	54 (34, 69)	112 (66, 177)	313 (146, 460)	959 (608, 2,636)	<0.001
ALT (U/L)	65 (35, 148)	26 (19, 35)	73 (51, 93)	216 (142, 246)	588 (446, 1,078)	0.041
ALP (U/L)	64 (51, 102)	56 (47, 78)	63 (50, 106)	69 (54, 96)	79 (54, 113)	0.102
TP (g%)	6.7±0.7	6.8±0.7	6.7±0.6	6.6±0.7	6.3±0.9	0.022
Alb (g%)	3.9 (3.6, 4.2)	4.1 (3.7, 4.3)	4.0 (3.7, 4.3)	3.9 (3.4, 4.2)	3.7 (3.0, 4.2)	0.073

Table 2 (continued)

Laboratory values	Total n=179	Normal n=46 (25.7)	Mild hepatitis n=87 (48.6)	Moderate hepatitis n=27 (15.1)	Severe hepatitis n=19 (10.6)	p-value[#]
PT (sec)	11.7	12.5	11.2	11.7	13.8	
	(11.1, 14.5)	(11.8, 13.1)	(11.0, 11.3)	(10.8, 11.7)	(12.1, 15.1)	
PTT (sec)	42.1±7.5	38.5±12.7	38.9±8.0	42.7±6.3	43.9±7.5	
Day of max LFT	5	4	5	6	6	<0.001
	(4.0, 6.0)	(3.0, 5.0)	(4.0, 6.5)	(5.0, 7.0)	(5.0, 7.0)	
Max LFT						
TB (mg%)	0.4	0.4	0.5	0.5	0.8	0.001
	(0.3, 0.7)	(0.3, 0.6)	(0.3, 0.6)	(0.4, 0.8)	(0.4, 1.6)	
DB (mg%)	0.2	0.1	0.2	0.2	0.4	<0.001
	(0.1, 0.3)	(0.1, 0.2)	(0.1, 0.3)	(0.1, 0.5)	(0.2, 1.0)	
AST (U/L)	133	54	129	403	1,050	<0.001
	(69, 316)	(34, 69)	(86, 192)	(311, 662)	(666, 2,608)	
ALT (U/L)	82	26	82	242	692	<0.001
	(38, 202)	(19, 36)	(62, 120)	(220, 298)	(538, 1,078)	
ALP (U/L)	64	56	61	80	90	0.013
	(50, 99)	(46, 78)	(49, 84)	(60, 140)	(59, 123)	
TP (g%)	6.6±0.8	6.7±0.7	6.6±0.7	6.4±0.9	6.4±1.1	0.205
Alb (g%)	3.9	3.9	3.9	3.5	3.7	0.009
	(3.5, 4.2)	(3.7, 4.3)	(3.7, 4.2)	(3.2, 3.9)	(3.0, 4.2)	
PT (sec)	11.7	12.5	11.2	11.7	14.5	
	(11.0, 14.3)	(11.8, 13.1)	(11.0, 11.4)	(10.3, 12.5)	(12.8, 16.6)	
PTT (sec)	42.4±7.8	38.5±12.7	38.9±8.0	43.7±7.7	43.9±7.5	
Serological test						
performed	144 (80.4)	39 (84.8)	64 (73.6)	24 (88.9)	17 (89.5)	0.146
not performed	35 (19.6)	7 (15.2)	23 (26.4)	3 (11.1)	2 (10.5)	
Serological result						
Positive	81 (56.0)	14 (35.9)	40 (62.5)	13 (54.2)	14 (82.4)	0.039
Negative	14 (10.0)	5 (12.8)	6 (9.3)	3 (12.5)	0 (0.0)	
Inconclusive	49 (34.0)	20 (51.3)	18 (28.2)	8 (33.3)	3 (17.6)	

*WBC=white blood cells, Hct=hematocrit, LFT=liver function test, TB=total bilirubin, DB=direct bilirubin, AST=aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase, TP=total protein, Alb=albumin, PT=prothrombin time, PTT=partial thromboplastin time

[#]Analyzing differences across all groups

The maximum TB was observed in the severe hepatitis group (0.8 mg%). The maximum AST and ALT values were 133 U/L (69, 316) and 82 U/L (38, 302), respectively. Both were recorded on the 5th day of fever. Partial thromboplastin time (PTT) ≥35 sec was observed among the tested patients conversely to PT. Serological tests for dengue were administered for most patients (80.4%), and 56.0% were positive.

No statistically significant differences in duration of admission were seen between the severity groups. Intensive care unit admission rate (26.3%) and complications (36.8%) were highest among the severe hepatitis group. Renal failure was the most common complication (15.8%), followed by significant bleeding (10.5%) and acute respiratory failure (10.5%) (Table 3).

After analysis according to baseline characteristics and initial laboratories, AST and ALT were significantly elevated among patients with an initial platelet count of <100,000/μL and the significance was even greater when platelets were <50,000/μL. The AST and ALT were also significantly elevated among patients with serum albumin less than 3.5 g%. We also noticed significant AST elevation among patients with initial prothrombin time prolongation (PT ≥16 sec) or who presented with atypical lymphocytes. The hepatitis was less severe in pregnant patients (Table 4).

In the association study between baseline characteristics, initial laboratory tests and severe hepatitis (ALT ≥400 U/L), we found that initial albumin was the only predictor associated with severe hepatitis, OR=4.16 (1.41–12.27) (Table 5).

Table 3 Hospital course

Patient characteristics	Total n=179	Normal n=46 (25.7)	Mild hepatitis n=87 (48.6)	Moderate hepatitis n=27 (15.1)	Severe hepatitis n=19 (10.6)	p-value[#]
Admission duration (days)	3 (2, 4)	3 (2, 4)	4 (3, 4)	3 (2, 5)	3 (3, 5)	0.671
ICU admission	9 (5.0)	0 (0.0)	2 (2.3)	2 (7.4)	5 (26.3)	<0.001
Complications						
None	163 (91.1)	46 (100.0)	82 (94.3)	23 (85.2)	12 (63.2)	<0.001
Renal failure	3 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	3 (15.8)	
Significant bleeding	8 (4.5)	0 (0.0)	2 (2.3)	4 (14.8)	2 (10.5)	
Acute respiratory failure	3 (1.7)	0 (0.0)	1 (1.1)	0 (0.0)	2 (10.5)	
Superimposed infection	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Other	2 (1.1)	0 (0.0)	2 (2.3)	0 (0.0)	0 (0.0)	

ICU=intensive care unit

[#]Analyzing differences across all groups

Table 4 Maximum aspartate aminotransferase and alanine aminotransferase in relation to baseline characteristics and initial laboratory tests

Patient characteristics and laboratory values	Maximum AST (U/L)	p-value	Maximum ALT (U/L)	p-value
Sex				
Male (63)	139 (77, 363)		97 (52, 230)	
Female (116)	128 (65, 284)	0.477	76 (36, 187)	0.119
BMI				
≤23 (61)	106 (60, 294)		66 (36, 191)	
>23 (41)	139 (82, 207)	0.230	82 (57, 164)	0.158
Age				
<40 (133)	149 (74, 349)		88 (38, 218)	
≥40 (46)	95 (66, 183)	0.100	73 (48, 151)	0.521
Comorbidity				
None (139)	140 (69, 334)		88 (46, 220)	
Present (40)	121 (61, 235)	0.376	75 (33, 147)	0.114
Pregnancy				
No (159)	135 (71, 321)		88 (44, 207)	
Pregnant (20)	123 (52, 230)	0.279	64 (25, 119)	0.049
WBCs				
≥5,000 (40)	156 (59, 379)		75 (33, 189)	
<5,000 (139)	127 (69, 308)	0.710	83 (46, 202)	0.449
≥3,000 (94)	136 (66, 356)		75 (38, 204)	
<3,000 (85)	129 (75, 309)	0.941	94 (51, 198)	0.447
Platelets				
≥100,000 (63)	83 (52, 171)		62 (30, 167)	
<100,000 (116)	156 (84, 347)	0.003	91 (53, 207)	0.041
≥50,000 (113)	100 (58, 220)		74 (36, 152)	
<50,000 (66)	210 (105, 438)	<0.001	106 (57, 238)	0.006
Atypical lymphocytes				
Absent (54)	97 (64, 175)		71 (37, 144)	
Present (125)	145 (74, 403)	0.011	88 (48, 238)	0.060
Band				
Absent (54)	119 (64, 226)		80 (38, 175)	
Present (125)	140 (74, 355)	0.182	82 (43, 218)	0.828
Initial PT				
<16 (18)	629 (259, 1,839)		381 (95, 754)	
≥16 (3)	7,668 (4,409, 10,772)	0.039	2,884 (1,581, 3,168)	0.097
Initial PTT				
<35 (4)	358 (100, 610)		233 (53, 446)	
≥35 (16)	1,054 (398, 2,815)	0.080	516 (191, 1,107)	0.171
Alb				
≥3.5 (123)	119 (65, 255)		74 (38, 188)	
<3.5 (26)	358 (149, 1,102)	<0.001	177 (76, 601)	0.002

BMI=body mass index, AST=aspartate aminotransferase, ALT=alanine aminotransferase, WBC=white blood cells, PT=prothrombin time, PTT=partial thromboplastin time, Alb=albumin, U/L=international units per liter

Table 5 Association between baseline characteristics, initial laboratory tests and severe hepatitis (ALT >400 U/L)

Patient characteristics and laboratory values	Odd	95% CI
Sex		
Male	–	–
Female	1.08	(0.40–2.91)
BMI		
≤23	–	–
>23	1.40	(0.39–4.98)
Age		
<40	–	–
≥40	0.96	(0.33–2.84)
Comorbidity		
None	–	–
Present	1.60	(0.44–5.81)
Pregnancy		
No	–	–
Pregnant	2.43	(0.31–19.22)
WBCs		
≥5,000	–	–
<5,000	0.58	(0.21–1.65)
≥3,000	–	–
<3,000	0.78	(0.30–2.05)
Platelets		
≥100,000	–	–
<100,000	0.57	(0.22–1.48)
≥50,000	–	–
<50,000	1.00	(0.37–2.68)
Atypical lymphocytes		
Absent	–	–
Present	0.40	(0.11–1.44)
Band		
Absent	–	–
Present	0.81	(0.28–2.37)
Initial PT		
<16	–	–
≥16	0.40	(0.03–5.25)
Initial PTT		
<35	–	–
≥35	0.33	(0.23–3.93)
Alb		
≥3.5	–	–
<3.5	4.16	(1.41–12.27)

CI=confidence interval, BMI=body mass index, WBC=white blood cells, PT=prothrombin time, PTT=partial thromboplastin time

Genetic testing

As with the whole cohort, most patients were female (66.7%). The median age was lower in the case group (27 years vs. 36.5 years). No significant differences were found in BMI, comorbidities and initial clinical presentation between the case and control groups (Supplementary Table 1).

The absolute lymphocyte count and atypical lymphocytes were higher in the case group. The platelet count was lower in the case group, as was the albumin level (Supplementary Table 2).

The duration of admission was not different between the case and control groups, but the intensive care unit (ICU) admission rate (31.6% vs. 3.6%, respectively, p-value=0.003) and complication rate (26.3% vs. 5.4%, respectively, p-value=0.020) were both higher in the case group. Renal failure and acute respiratory failure were the most common complications in the case group (Supplementary Table 3).

Table 6 Specific single nucleotide polymorphisms testing

SNPs	Controls n*=56 Number (%)	Cases n=19 Number (%)	p-value
rs11208534			1.000
GG	8 (14.5)	3 (15.8)	
AG	47 (85.5)	16 (84.2)	
rs2780831			0.429
CT	12 (21.4)	7 (36.8)	
CC	9 (16.1)	3 (15.8)	
AA	35 (62.5)	9 (47.4)	
rs4804803			0.436
AG	10 (18.2)	5 (26.3)	
GG	1 (1.8)	1 (5.3)	
AA	44 (80.0)	13 (68.4)	

*SNPs in rs11208534 and rs4804803 of one patient in the control group could not be identified

SNP=single nucleotide polymorphism, nucleotide bases: GG=guanine-guanine, AG=adenine-guanine, CT=cytosine-thymine, CC=cytosine-cytosine, AA=adenine-adenine

For the genetic testing, there were no differences of SNPs among the patients in the case and control groups. Adenine–guanine (AG) was the predominant allele of the rs11208534 allele, adenine–adenine (AA) was predominant in rs2780831 and rs4804803 had the AA allele predominate (Table 6 and Figure 2).

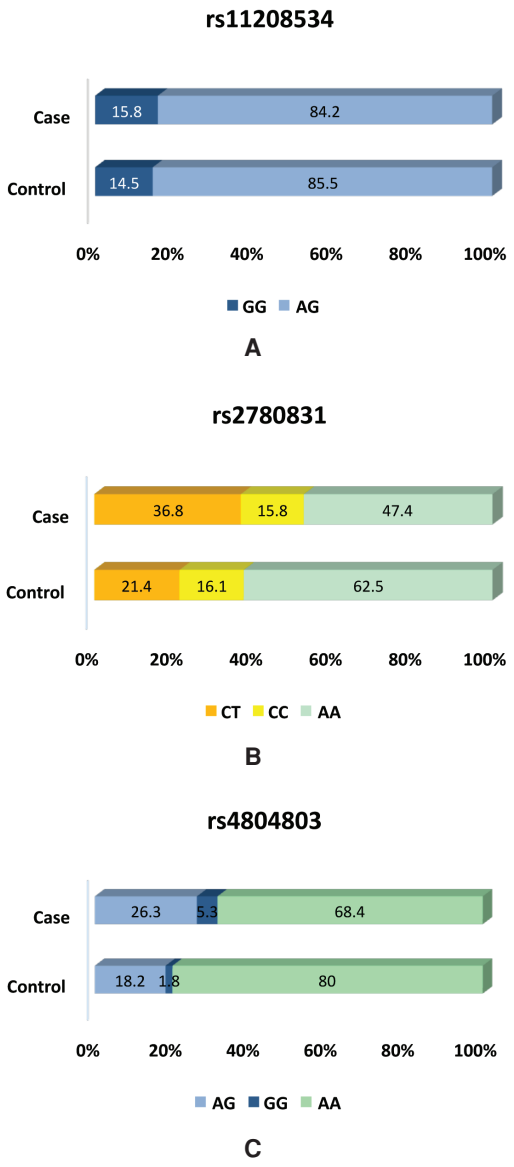

Figure 2 A, B and C Comparing specific single nucleotide polymorphisms between case and control groups

Discussion

Many studies and case reports have previously demonstrated an association between severe hepatitis and poor outcomes in dengue.[11,14–18] The current study was conducted to search for predictors associated with severe hepatitis, including genetic testing for specific SNPs. Most study patients were female and had mild hepatitis (74.3%), similar to a previous large study from Brazil.[7] However, this study had more cases of severe hepatitis (10.6% vs. 3.8%), likely due to the tertiary care center status of our institute as a referral center which receives more severe case than other hospitals.

Apart from fever, myalgia was the most common initial clinical presentation (65.4%). This is the same as the study of Thomas[19], but we found fewer initial presentations of abdominal pain (3.9% vs 42.7%) than they reported. We found that nausea and vomiting was also a common presenting symptom (44.7%), and a few with diarrhea (13.4%). In this study we could not differentiate between dengue fever or dengue hemorrhagic fever due to the retrospective study design and incomplete available laboratory data to confirm the required criteria. Thus, we assumed mainly dengue hemorrhagic fever when the initial platelet count was below 100,000/μL and the patient was admitted (more severe).

The initial liver function tests showed AST as the more predominant transaminase enzyme over ALT for most patients in all categories, as was also found in previous studies.[7–11] This is different from other viral hepatitis that usually have ALT predominant over AST. The true mechanism is still unknown, but the direct attack of the virus itself causing an inflammatory response is the most commonly proposed mechanism. Our study is the first to identify low initial serum albumin in more severe hepatitis cases and those with poorer outcomes. We also found predominately abnormal PTT but not PT in the more severe hepatitis cases. Serological testing was performed in

80.4% of all patients, due to accurate probable dengue diagnoses in endemic areas with typical clinical and laboratory methods.[4,5] There was no difference in the length of hospital stay in the severe hepatitis group, possibly related to their referral status and this was not their 1st hospital admission, and in this study we did not attempt to include hospital course from referral hospitals. However, the ICU admission rate was higher in the severe group, which also had more complications. Acute kidney injury was the most significant complication, as in a study of Parkash et al.[11] from Pakistan, followed by significant bleeding and acute respiratory failure. These findings strongly emphasize the correlation between severe hepatitis and poor clinical outcomes.[11,18]

This study examined genetic differences among mild (controls) and severe (cases) hepatitis groups by looking at specific SNP tests as explained in the introduction. We categorized these patients with more specific criteria to confirm separate severity entities. From 179 target populations and a match case-control ratio of 1:3, finally we got 19 cases and 56 controls due to difficulties in recruitment and finding patients willing to participate in the 2nd phase of the study. The median age was lower in the case group (27 years vs. 36.5 years), but there were no differences in other baseline characteristics. The absolute lymphocyte count and atypical lymphocytes, which are always found in typical viral infections, were higher in the case group, while initial albumin levels were lower in the case group. In the overall analysis, ICU admission rate and complications were higher in the case group.

After analyzing transaminitis according to baseline characteristics and initial laboratory factors, we found significant AST and ALT elevations among patients with initial platelet levels <50,000/μL and initial serum albumin <3.5 g%. Interestingly, AST and ALT levels were not significantly elevated in overweight patients (BMI ≥23 kg/m^2). We also found that dengue infection during pregnancy and among female patients in general was not associated with hepatitis severity. There was also an indication that these factors were associated with conversely less severe transaminitis, but this difference was not statistically significant. Of these factors, we found that initial albumin <3.5 g% was the only predictor significantly associated with severe hepatitis. This result emphasizes the importance of initial albumin as a predictor of severe hepatitis in dengue infection. Albumin tests are available at basic laboratories in all the hospitals of Thailand, so initial albumin levels in patients with dengue infection should be checked to help predict the likely severity and outcome of dengue patients to optimize care and management.

As discussed in the introduction, previous reviews have demonstrated that some specific SNPs in the *Jak1* and *CD209* genes were associated with severe dengue (DHF), and more transaminitis in DHF than DF. Because of this, we hypothesized that these SNPs are important candidate genes for susceptibility to severe hepatitis. We did not perform a genome wide association study (GWAS) due to limitations in resources and budget. The results showed no significant difference of specific SNPs between the two severity groups. This can be explained by the specific genetic testing without previous strong evidence of genetic susceptibility, not performing GWASs, and the uncertainty of whether or not many patients had or did not have DHF (platelets <100,000/μL). Further studies with a wide range of SNPs or GWASs should be conducted to accurately identify genetic susceptibility among dengue patients with severe hepatitis.

This study investigated dengue infection, which has high morbidity and mortality in some areas of Thailand each year. We used many baseline characteristics and initial and other available laboratory results to identify significant predictors associated with severe hepatitis, and then poor outcomes. We also demonstrated the natural

course of the disease with initial clinical presentation, laboratory changes and outcomes to be helpful in dengue patient care.

The limitations of this study were a small sample size, and only looking at the natural disease course during admission in one hospital. Many patients were referred from smaller hospitals, and some patients were health care workers in this hospital, so it was not a true representation of the overall Thai population.

Conclusion

Liver involvement in dengue infection is frequent, mostly manifesting as mild transaminitis. Patients with severe hepatitis usually have poorer clinical outcomes, more complications and need ICU admission. An initial serum albumin level of <3.5 g% appears to be an accurate laboratory predictor associated with severe hepatitis and worse clinical outcomes. We found no associations between body weight, sex, age or pregnancy status on hepatitis severity. Genetic susceptibility needs further investigation.

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Conflict of interest

All authors in this study declare no conflicts of interest

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Supplementary Table 1 Baseline characteristics among genetics testing patients

Patient characteristics	Total n=75	Control n=56 (74.7)	Case n=19 (25.3)	p–value
Sex (%)				
Male	25 (33.3)	17 (30.4)	8 (42.1)	
Female	50 (66.7)	39 (69.6)	11 (57.9)	0.511
Age (years)	35.2±10.6	36.5 (27.8, 47)	27 (22.5, 34)	0.005
BMI (kg/m^2)	22.8±4.2	22.1 (20.4, 24.3)	20.5 (19.3, 22.4)	0.189
Comorbidity (%)				
None	58 (77.3)	41 (73.2)	17 (89.5)	0.330
DM	0 (0.0)	0 (0.0)	0 (0.0)	
HT	2 (2.7)	2 (3.6)	0 (0.0)	
Dyslipidemia	2 (2.7)	1 (1.8)	1 (5.3)	
Pregnancy	6 (8.0)	5 (8.9)	1 (5.3)	
Others	7 (9.3)	7 (12.5)	0 (0.0)	
Medication (%)				
None	69 (92.0)	51 (91.1)	18 (94.7)	0.534
DM–drug	0 (0.0)	0 (0.0)	0 (0.0)	
HT–drug	1 (1.3)	1 (1.8)	0 (0.0)	
DLP–drug	2 (2.7)	0 (1.8)	1 (5.3)	
Others	3 (4.0)	3 (5.4)	0 (0.0)	
Day of admission	5 (1–8)	5 (1–7)	5 (4–8)	

Supplementary Table 1 (continued)

Patient characteristics	Total n=75	Control n=56 (74.7)	Case n=19 (25.3)	p-value
Symptoms (%)				
Abdominal pain	3 (4.0)	1 (1.8)	2 (10.5)	0.156
Myalgia	51 (68.0)	41 (73.2)	10 (52.6)	0.168
Diarrhea	8 (10.7)	5 (8.9)	3 (15.8)	0.410
Rash	1 (1.3)	1 (1.8)	0 (0.0)	1.000
Petechiae	3 (4.0)	1 (1.8)	2 (10.5)	0.156
Nausea/vomiting	37 (49.3)	25 (44.6)	12 (63.2)	0.259

BMI=body mass index, DM=diabetes mellitus, HT=hypertension, DLP=dyslipidemia, kg=kilograms, m^2=meter squared

Supplementary Table 2 Laboratory tests

Laboratory values	Total n=75	Control n=56 (74.7)	Case n=19 (25.3)	p-value
Admission labs				
WBC (/μL)	3,160 (2,230, 4,705)	3,060 (2,200, 4,702)	3,460 (2,425, 4,785)	0.596
Absolute lymph (/μL)	680.0 (434, 1,043)	598.5 (436, 904)	967.0 (461, 1,535)	0.087
Atypical lymph (%)	4 (0.5, 11.5)	2 (0.0, 8.2)	10 (6.0, 12.0)	0.002
Band (%)	2 (0.0, 4.5)	2 (1.0, 7.5)	2 (0.0, 4.0)	0.616
Platelets (/μL)	86,000 (39,000, 121,500)	93,000 (48,500, 126,250)	35,000 (16,500, 108,500)	0.009
Hct (%)	40.5±5.0	40.8±5.0	39.7±5.1	0.411
Minimum WBC	2,360 (1,905, 3,445)	2,345 (1,895, 3,257)	2,440 (2,030, 3,530)	0.665
Minimum platelets	43,000 (22,500, 71,000)	46,000 (28,000, 77,250)	35,000 (14,500, 55,500)	0.074
Day of 1st LFT	5 (4.0, 5.0)	4 (3.0, 5.0)	5 (4.5, 6.0)	0.044
1st LFT				
TB (mg%)	0.4 (0.3, 0.7)	0.4 (0.3, 0.6)	0.6 (0.4, 1.6)	0.016
DB (mg%)	0.2 (0.1, 0.3)	0.1 (0.1, 0.2)	0.6 (0.2, 1.1)	<0.001
AST (U/L)	96 58, 226)	78 (48, 135)	733 (497, 1,976)	<0.001
ALT (U/L)	67 (36, 167)	52 (24, 80)	451 (300 ,890)	<0.001
ALP (U/L)	59 (49, 84)	56 (46, 72)	84 (57, 148)	0.006

Supplementary Table 2 (continued)

Laboratory values	Total n=75	Control n=56 (74.7)	Case n=19 (25.3)	p-value
TP (g%)	6.9 (6.2, 7.1)	7.0 (6.5, 7.2)	6.6 (5.4, 6.9)	0.005
Alb (g%)	3.9±0.5	4.0±0.4	3.5±0.6	0.001
PT (sec)	14.6±3.9	12.0±0.7	15.0±4.2	0.452
PTT (sec)	42.6±8.7	34.9±7.6	44.3±8.4	0.180
Max LFT day	5 (4.0, 6.0)	5 (4.0, 6.0)	5 (5.0, 6.5)	0.057
Max LFT				
TB (mg%)	0.4 (0.3, 0.7)	0.4 (0.3, 0.6)	0.8 (0.4, 1.5)	0.002
DB (mg%)	0.2 (0.1, 0.4)	0.2 (0.1, 0.2)	0.5 (0.3, 1.0)	<0.001
AST (U/L)	133 (65, 426)	86 (52, 165)	763 (585, 2,188)	<0.001
ALT (U/L)	78 (38, 202)	55 (29, 98)	588 (393, 1,063)	<0.001
ALP (U/L)	57 (45, 85)	55 (42, 68)	105 (59, 161)	<0.001
TP (g%)	6.8 (6.2, 7.0)	6.9 (6.5, 7.1)	6.2 (5.4, 6.9)	0.028
Alb (g%)	3.9 (3.4, 4.1)	4.0 (3.7, 4.2)	3.4 (3.0, 3.9)	0.010
PT (sec)	14.5±4.0	12.6±1.6	14.8±4.3	0.505
PTT (sec)	43.1±9.0	34.9±7.6	44.6±8.6	0.166
Serological test				
Performed	59 (78.7)	42 (75.0)	17 (89.5)	0.330
Not performed	16 (21.3)	14 (25.0)	2 (10.5)	
Serological result				
Positive	41 (69.5)	27 (64.3)	14 (82.4)	0.300
Negative	3 (5.1)	2 (4.8)	1 (5.9)	
Inconclusive	15 (25.4)	13 (30.9)	2 (11.7)	

WBC=white blood cells, Hct=hematocrit, LFT=liver function test, TB=total bilirubin, DB=direct bilirubin, AST=aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase, TP=total protein, Alb=albumin, PT=prothrombin time, PTT=partial thromboplastin time, g=gram, mg=milligram

Supplementary Table 3 Hospital course

Patient characteristics	Total n=75	Control n=56 (74.7)	Case n=19 (25.3)	p-value
Admission duration				
Median (IQR)	3 (2.0, 4.5)	3 (2.0–6.0)	3 (2.0–4.0)	0.906
ICU admission	8 (10.7)	2 (3.6)	6 (31.6)	0.003
Complications				
None	67 (89.3)	53 (94.6)	14 (73.7)	0.020
Renal failure	2 (2.7)	0 (0.0)	2 (10.5)	
Significant bleeding	2 (2.7)	1 (1.8)	1 (5.3)	
Acute respiratory failure	3 (4.0)	1 (1.8)	2 (10.5)	
Superimposed infection	0 (0.0)	0 (0.0)	0 (0.0)	
Other	1 (1.3)	1 (1.8)	0 (0.0)	

IQR=interquartile range, ICU=intensive care unit