Serum Lactate and Lactate Dehydrogenase as Parameters for the Prediction of Dengue Severity

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Background: *Lactate and lactate dehydrogenase (LDH) have been found to be elevated in cardiopulmonary failure, sepsis, shock, and hepatic injury. Severe dengue hemorrhagic fever (DHF) patients also develop shock and experience a certain degree of hepatic injury, implicating that serum lactate and LDH may be elevated in Dengue shock syndrome (DSS).*
Objective: *To determine serum lactate and LDH levels in dengue patients to see whether they can be used as predictors of severe dengue cases.*
Material and Method: *A cross sectional study was conducted on suspected dengue patients admitted to the dengue ward, Queen Sirikit National Institute of Child Health (QSNICH), between May 2011and February 2012. Laboratory tests were used to confirm dengue cases in the enrolled patients. Blood for serum lactate was drawn in patients every day after enrollment. Blood for LDH and liver function test (LFT) were drawn 3 times: enrollment day, day of leakage, and discharge day. Lactate and LDH levels are compared among dengue and non-dengue patients. Dengue fever (DF), DHF and DSS patients were classified according to the WHO 1997 dengue classification.*
Results: *253 patients were enrolled, comprising of 120 DF, 75 DH, 30 DSS, and 28 non-dengue patients. The majority of dengue patients had liver impairment, demonstrated by elevated aspartate aminotransferase (AST) (94.9%) and alanine aminotransferase (ALT) levels (68.6%) while non-dengue patients have minimal elevation. Serum lactate levels were not elevated in the early stages in dengue patients, but were elevated in non-dengue patients. The mean serum lactate levels in DSS patients increased towards the end of febrile phase and reached maximum values on Day 0 (2.2 U/L). On the other hand, serum lactate levels were found to be decreasing in the non-dengue group. The mean serum lactate levels on Day 0 was found to be different in DSS patients (2.26 U/L) compared to DF 1.63 U/L), DHF (1.79 U/L) and non-dengue patients (1.68 U/L) ($p<0.05$).*

Mean serum LDH levels were elevated in the early stages of the disease in all groups of patients, but with different levels. Mean serum LDH levels was 709.2 in DF, 1,873 in DHF, 654.5 in DSS, and 434 IU in non-dengue patients. The mean LDH levels in dengue patients were >500 IU, while it was <500 IU in non-dengue patients. The increasing mean levels of LDH towards the end of febrile phase were only seen in DHF and DSS patients, but not in DF and non-dengue patients. The mean levels of LDH on Day 0 in DHF, DSS, DF and non-dengue patients are 1,060.7, 1,180.7, 787.2, and 423.8 IU, respectively.
Conclusion: *Serum lactate and LDH was found to be elevated in DHF and/or DSS patients. Lactate may be used as a predictor of DSS if the level is >2 U/L on Day 0. LDH can be used to differentiate patients with or without dengue in the early febrile phase, if the level is >500 IU. If the level of LDH is increased to approximately 1,000 IU on Day 0, it may be a predictor of severe dengue infection or DHF and DSS with plasma leakage.*

Keywords: *Lactate, Lactate dehydrogenase, Dengue, DF, DHF, DSS, Predictors*

J Med Assoc Thai 2014; 97 (Suppl. 6): S220-S231
Full text. e-Journal: *http://www.jmatonline.com*

Dengue infection is one of the most common viral diseases that leads to death in children and adults. In Thailand, the incidence of dengue infection ranges from 52,261-150,174 cases per year, from 2009 to 2013, with the case fatality rate (CFR) rate between 0.09-0.12%[2,3]. Dengue infections present with a wide spectrum of clinical presentations and can be classified as dengue fever (DF), dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS), and expanded dengue syndrome (EDS), which is also known as unusual manifestations[4].

DF is considered the milder form of the disease

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because it rarely leads to mortality. DHF and DSS are considered more severe because they may lead to death. DHF and DSS have selective plasma leakage, into pleural and peritoneal spaces, during the critical period, which is usually co-incident with defervescence. In cases with massive plasma leakage, patients will develop shock, which results in death if there is no proper management. EDS is the most severe form of dengue infections and it is usually difficult to make a diagnosis at the first presentation. EDS is not so common. It usually occurs due to prolonged shock in DHF and DSS and patients present with organ(s) failure, co-infections, or other co-morbidities, especially in adults. Some cases may be due to virulence of certain types of dengue viruses[4].

During the first few days, or febrile phase, of all dengue patients, the clinical presentations of DF, DHF, DSS, and EDS are the same[4]. Even if physicians know that these are dengue patients, they have to follow every patient until the defervescence stage to make a diagnosis for DHF/DSS. DHF and DSS patients will have plasma leakage with 20% rising hematocrit (Hct), pleural effusion, or ascites, which is detected by physical examination, chest film right lateral decubitus, ultrasound, or hypoalbuminemia, which is defined as serum albumin $\leq$3.5 gm% in normal nutritional patients. Frequent complete blood count (CBC) must be performed to look for leukopenia, which is defined as white blood cells (WBC) $\leq$5,000 cells/cumm. This may suggest dengue infections and the onset of defervescence. Thrombocytopenia, which is defined as platelet count $\leq$100,000 cells/cumm, may indicate the critical period in DHF and DSS; however, it can also be seen in DF patients[4].

Physicians have to observe and admit suspected dengue patients to the hospital when they have low platelet count with warning signs of clinical deterioration or no clinical improvements. Signs include no fever, abdominal pain, vomiting, bleeding, irritability or restlessness, behavioral changes, change of consciousness. It is also recommended to admit high risk patients, including patients who are infants, elderly, pregnant, obese, bleeding, experiencing prolonged shock, change of consciousness, or patients with underlying diseases. These patients have to be closely monitored by doctors, nurses and medical teams every 1-3 hours during the critical period. This can be labor intensive. Physicians have to monitor about 1,000 dengue patients in order to save one life. In order to reduce the workload of close monitoring on all dengue patients, there is a need to find any predictors for severe dengue cases, which may help to reduce the workload and lower CFR.

Lactate is used as one of the parameters to predict severity of sepsis. Glycolysis produces the metabolite pyruvate, which is converted to acetyl CoA, and enters the Kreb's cycle under aerobic conditions. Under anaerobic conditions, pyruvate is converted to lactic acid. Hyperlactemia in sepsis results from tissue hypoxia under anaerobic conditions and impaired lactate clearance by the liver[5]. In severe DHF cases, plasma leakage induces inadequate intravascular volume that causes tissue hypoxia and multi-organ failure. Hypoxic tissue and other complications may induce hyperlactaemia in DHF, similar to sepsis.

Lactate Dehydrogenase (LDH) isoenzyme is divided into 5 subgroups. LDH-5 is found in the liver and striated muscles. LDH elevates in tissues containing this enzyme breakdown such as liver, heart, and striated muscle. In patients with hypoxic liver, LDH and transaminase values increases 12 to 48 hours after the initial event[6]. About 60-90% of dengue hemorrhagic fever have elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, so LDH may be elevated and may be used to detect hypoxic tissue in DHF and DSS cases[7].

Early detection of leakage or critical phase in DHF can prevent shock and severe complications, therefore, this study hypothesizes that serum lactate and LDH levels may serve as parameters to predict the severe dengue cases.

Objective

The objective of the present study is to determine serum lactate and LDH levels in dengue patients to be used as predictors of severe dengue cases.

Material and Method

A cross sectional study was conducted on suspected dengue patients admitted to the dengue ward, Queen Sirikit National Institute of Child Health (QSNICH), between May 2011and February 2012. The enrolled cases are classified into to four groups, according to the World Health Organization (WHO) classification[1]. Laboratory tests, PCR or MAG/GAG ELISA, were used to confirm dengue cases in the enrolled patients. The research project was approved by the Ethics Committee of QSNICH.

Case definitions

Non-dengue patients were defined as cases

that present with acute febrile illness and suspected to have dengue infection, but PCR and serology results are negative.

Dengue infection patients were defined as cases with positive PCR for dengue and/or positive serology for dengue. The patients were classified into DF, DHF, and DSS, according to WHO Regional Office for South-East Asia (SEARO) 2011, Revised and Expanded editions[4].

DF was defined as dengue cases without signs of plasma leakage, stable Hct of less than 10%, no pleural effusion, and no ascites.

DHF was defined as dengue cases with signs of plasma leakage, including 20% hemoconcentration, pleural effusion, ascites, hypoalbuminemia, and without shock.

DSS was defined as DHF with narrowing pulse pressure, pulse pressure ≤20 mmHg, and/or hypotension.

Definitions for the grading of DHF severity

DHF grade 1: Fever and hemorrhagic manifestation, from the positive tourniquet test, and evidence of plasma leakage.

DHF grade 2: As in grade 1, plus spontaneous bleeding.

DHF grade 3: As in grade 1 or 2, plus circulatory failure, including weak pulse, narrow pulse pressure (≤20 mmHg), hypotension, and restlessness.

DHF grade 4: As in grade 3, plus profound shock with undetectable blood pressure (BP) and pulse.

Date of illness

Day 0 was defined as the 1st day without fever or the 1st day of critical phase or shock.

Day 2 and Day 1 were 2 days and 1 day before defervescence.

Day 1 and Day 2 were 1 day and 2 days after defervescence.

Blood tests

Confirmation of dengue infections was done at Armed Forces Research Institute of Medical Sciences (AFRIMS). PCR to confirm dengue infection was performed on blood samples collected on admission or enrollment. Serology of dengue IgM and IgG ELISA were collected for 2 times: on admission and 2 days after defervescence.

Serum lactate levels were measured daily after enrollment. Lactate levels were measured by the enzymatic colorimetic assay. Blood samples for LDH levels were collected on day of enrollment, Day 0 or Day 1, and day of discharge. LDH levels were measured according to the methods by Deutschen Gesellschaft fur Klinische Chemie (DGKC). Both lactate and LDH levels were analyzed by COBA Integra 400 Plus (Roche Diagnostics, Basel, Switzerland) at the Biochemical Laboratory of QSNICH. Lactate and LDH levels were compared among groups of patients on Day 2, Day 1, Day 0, Day 1 and Day 2.

Normal levels of AST and ALT (reference from biochemistry laboratory QSNICH)

AST <51 U/L, ALT <39 U/L.

Statistical analysis

Demographic data are presented as percentages and mean ± SD. The Chi-square statistic or Fisher's exact test was used for categorical analysis. The Mann-Whitney U test was used for multiple comparisons. All statistical analyses were performed using SPSS software version 16. The $p<0.05$ was considered statistically significant.

Results

Demographic data

Two hundred fifty-five cases were enrolled in the present study. Two cases that were diagnosed with Systemic Lupus Erythematosus (SLE) and sepsis were excluded. The remaining 253 suspected dengue cases were classified to 120 DF, 75 DHF, 30 DSS and 28 non-dengue cases. The mean ages for DF, DHF, DSS patients were 8.81, 9.6 and 10.32 years old, respectively. This was higher than the non-dengue group, which was 7.17 years old. The male to female ratio was not different. Mean length of stay in the hospital of non-dengue patients and DF groups was 3 days, while in DHF and DSS group was 4 days. Demographic data are shown in Table 1.

Serology by MAG and GAG ELISA

Serology confirmed dengue infection in 90% of suspected patients. Primary dengue infections were found in 13.9 and 8.7% of DF and DHF patients, respectively. None was found in DSS patients. Secondary dengue infections were found in 86.1, 91.3% and 100% of DF, DHF, and DSS, respectively (Table 1).

PCR

Dengue viruses were identified in 79.1% of dengue patients. All four dengue serotypes were found in the present study: DEN 1 (25.3%), DEN 2 (44.9%),

DEN 3 (26.4%), DEN 4 (3.4%). The most common serotype was type 2 (Table 1).

Levels of AST and ALT in no dengue group, DF, DHF and DSS patients

Abnormal elevations of AST/ALT were found in 86.2/71.1% of dengue patients, while it was only found in 32.1/14.3% of non-dengue patients. The percentage of abnormal elevation of AST/ALT was found in 100/ 90% of DSS, 93.3/77.3% of DHF, and 78.3/ 62.5% of DF patients (Table 2).

AST elevation was found early in the majority of dengue patients. On Day 2 or Day 1; the percentage of AST elevation in DF, DHF and DSS was 60.6%, 87.5% and 100%, respectively, while 20% of non-dengue patients had AST elevation. During critical phase (Day 0 or Day 1), the percentage of AST elevation was found in more patients. Results showed that the percentage of AST elevation was 72.7% in DF, 94.9% in DHF, and 100% in DSS patients. On the other hand, 21% of non-dengue patients had AST elevation, which is similar to Day -2 or Day -1. In the convalescence phase (Day +2), all DSS patients still had elevated AST, while in DHF patients, the percentage of AST elevation was reduced. In contrast, DF and non-dengue patients have higher percentages of AST elevation. The mean AST levels on Day -2, Day -1, Day 0, Day +1 and Day +2 in DF, DHF, DSS and non-dengue patients patients were 64.6,

Table 1. Demographic data, serology and PCR results among non-dengue and dengue groups

	Non-dengue group	DF	DHF	DSS	Total dengue	*p*-value
n	28	120	75	30	225	
Age, years (mean ± SD)	7.17±2.99	9.60±3.78	10.32±3.26	8.81±3.65		0.010
Male, n (%)	15 (53.6)	63 (52.5)	36 (48)	13 (43.3)		0.779
Length of stay, day (mean ± SD)	2.75±1.65	2.67±1.05	3.48±1.44	3.87±1.22		0.000
Serology (%)						
Primary	0	15 (13.9)	6 (8.7)	0 (0)	21	NA
Secondary	0	93 (86.1)	63 (91.3)	27 (100)	183	
Total		108	69	27	204	
Serotype, No. (%)						
Dengue 1	0	26 (28.6)	11 (18)	8 (30.8)	45	
Dengue 2	0	30 (32.9)	37 (60.7)	13 (50)	80	
Dengue 3	0	29 (31.9)	13 (21.3)	5 (19.2)	47	
Dengue 4	0	6 (6.6)	0	0	61	
Total negative		91	61	26	78	
	28 (100)	29 (24.1)	14 (18.7)	4 (13.3)	75	NA

n = numbers of patients whose blood was sent on the individual day

Table 2. Abnormal AST/ALT levels among non-dengue group, DF, DHF and DSS patients

	AST			ALT		
	AST >51 U/L	n	%	ALT >39 U/L	n	%
Non-dengue group	9	28	32.1	4	28	14.3
DF	94	120	78.3	75	120	62.5
DHF	70	75	93.3	58	75	77.3
DSS	30	30	100	27	30	90
Total dengue	194	225	86.2	160	225	64.8

n = numbers of patients

101.4, 107.5, 109.5, 100.7; 338.6, 216.8, 242.6, 197.9, 129.9; 117.6, 271.9, 235.0, 246.9, 232.1 and 46.4, 38.1, 40.7, 36.2, 36.6 U/L, respectively. The mean levels of non-dengue patients remained normal throughout the clinical illness. The levels of AST in DSS patients remained high at least 2 days after the critical period. However, in DF and DHF patients, there was a tendency to be lower. Maximum AST levels in most dengue patients were found on Day 0 or Day 1 (Table 3 and 4).

Similar to AST levels, the percentage of DHS/DSS patients with abnormal ALT levels was significantly more than DF patients were in each period. The mean

Table 3. Abnormal AST/ALT levels among non-dengue, DF, DHF, and DSS patients on each period of dengue infection

	Abnormal AST , n (% of cases)			Abnormal ALT, n (% of cases)		
	Day -2 or Day -1	Day 0 or Day 1	Day 2	Day -2 or Day -1	Day 0 or Day 1	Day 2
Non-dengue group	3 (20%)	4 (21.1%)	3 (25%)	1 (7.7%)	1 (5%)	2 (16.7%)
DF	40 (60%)	64 (72.7%)	42 (79.2%)	17 (30.9%)	43 (44.3%)	37 (67.3%)
DHF	28 (87.5%)	56 (94.9%)	35 (85.4%)	15 (60%)	45 (68.2%)	28 (68.3%)
DSS	13 (100%)	29 (100%)	14 (100%)	11 (84.6%)	23 (79.3%)	12 (92.3%)
Total dengue	81 (73%)	149(84.7%)	91 (84.3%)	43 (46.2%)	111 (57.8%)	77 (70.6%)
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.001

n = numbers of patients

Table 4. Mean AST levels among non-dengue and dengue groups

	AST level (U/L)				
	Day-2	Day-1	Day 0	Day +1	Day +2
Non-dengue group					
n	5	10	9	10	12
Mean	46.40	38.10	40.67	36.20	36.58
SD	25.71	12.33	15.32	13.50	15.78
Minimum	32	20	24	21	20
Maximum	92	58	64	66	73
DF					
n	25	47	55	45	53
Mean	64.56	101.43	107.47	109.49	100.72
SD	53.02	91.95	99.64	99.33	65.07
Minimum	17	22	28	22	29
Maximum	231	392	469	490	322
DHF					
n	5	31	41	29	41
Mean	338.60	216.77	242.59	197.90	129.90
SD	475.78	267.84	410.56	198.52	103.62
Minimum	46	24	27	25	26
Maximum	1,168	1,068	2,624	1,087	518
DSS					
n	5	11	24	16	14
Mean	117.60	271.91	235.04	246.88	232.14
SD	46.80	273.06	178.11	249.66	194.64
Minimum	62	107	44	52	86
Maximum	168	855	831	1,053	773

n = numbers of patients whose blood was sent on the individual day

level of ALT in DSS/DHF patients was higher than DF patients. On the other hand, almost all of non-dengue patients had normal ALT level for each period (Table 3 and 5).

Lactate levels in non-dengue, DF, DHF and DSS patients

During the febrile phase (Day -2), the mean lactate level was higher in the non-dengue group compared to DF, DHF, and DSS group ($p>0.05$). On Day -1, the mean lactate levels among the four groups of patients were not statistically different ($p>0.05$). Results are demonstrated in Table 6.

In DF, the mean lactate levels in Day -2, Day -1 and D 0 are 1.22, 1.43 and 1.63 U/L, respectively, while in DSS they were 1.07, 1.68 and 2.26 U/L, respectively. In DHF patients, the mean lactate levels did not change in Day -2, Day -1 and Day 0. Results are shown in Table 6.

In non-dengue patients, the mean lactate levels decreased from Day -2, Day-1 and Day 0. On Day 0, the mean lactate level of DSS group is highest (2.26 U/L) and it was statistically different from DF group (1.63 U/L) ($p<0.05$), but not statistically different from non-dengue patients (1.68 U/L) and DHF group (1.79 U/L).

After defervescence, the mean lactate levels remained high in all dengue groups for at least 2 days in the present study. The mean levels of lactate on Day 0, Day 1 and Day 2 in DF, DHF, and DSS are shown in Table 6.

There were no significant changes in the mean lactate levels in non-dengue patients. The mean levels of lactate on Day 0, Day 1 and Day 2 are 1.68, 1.55 and 1.61 U/L, respectively.

LDH levels in non-dengue, DF, DHF and DSS patients

The mean levels of LDH during the febrile phase (Day -2 and Day -1) in all dengue groups were higher than non-dengue patients, but the significant difference is detected only on Day -1 ($p<0.05$). The mean levels of LDH in Day -2, Day -1, and Day 0 in DF,

Table 5. Mean ALT levels among non-dengue and dengue groups

	ALT level (U/L)				
	Day -2	Day -1	Day 0	Day +1	Day +2
Non-dengue group					
n	4	9	11	11	12
Mean	14.25	23.00	17.55	21.18	25.17
SD	4.27	20.08	8.63	12.54	16.71
Minimum	11	6	9	6	11
Maximum	20	72	34	54	72
DF					
n	21	40	62	49	55
Mean	30.95	47.92	50.06	63.10	67.84
SD	33.11	48.12	54.14	62.77	53.77
Minimum	9	10	9	11	12
Maximum	162	201	265	296	301
DHF					
n	5	24	44	34	41
Mean	127.80	129.75	98.00	114.56	85.51
SD.	191.28	169.83	168.70	134.21	87.66
Minimum	14	11	11	10	13
Maximum	459	717	1046	706	429
DSS					
n	5	11	21	19	13
Mean	57.60	116.36	118.14	101.95	126.69
SD	33.31	104.43	107.36	118.09	109.72
Minimum	16	29	9	10	26
Maximum	102	342	410	425	368

n = numbers of patients whose blood was sent on the individual day

DHF and DSS patients are shown in Table 7.

On Day 0, both DHF and DSS patients have high mean LDH levels of 1,060.74 and 1,180.73 IU, respectively, when compared to DF and non-dengue patients, which were 787.21 IU and 423.82 IU, respectively (p<0.05). The mean levels of LDH during the febrile phase in non-dengue patients were 434.00 and 417.13 IU, respectively.

After defervescence, the mean levels of LDH in dengue patients groups remained high, while increasing in Day 0, Day 1, and Day 2, in non-dengue patients (Table 6).

Discussion

In the present study, 253 suspected dengue cases were admitted to QSNICH. Dengue infections were confirmed in 88.93% of the patients, while 11.07% were non-dengue patients. The mean age of dengue infections was 9.6 years old, which is higher than non-dengue patients, which was 7.17 years old according to a previous study[8]. The mean age is different among each dengue group.

This difference in age among dengue groups is similar to a previous study[8]. DF patients are likely to be younger than DHF/DSS patients because DF is usually the result of primary or first infection. However, in this study, only 12.5% of DF patients have primary infections. This may be the reason for the similarity in the mean age of DF and DHF patients. DHF/DSS are usually the result of repeated or secondary dengue infection[4]. In the present study, we found that 85.7% of DHF/DSS patients have secondary dengue infections.

The percentage of DSS patients in the present study is 11.9%, which is rather low compared to other studies[9]. This may be because dengue infections are common in Thailand, and much effort is spent in making early clinical diagnoses by using simple techniques, laboratory tests, including the tourniquet test and CBC, follow-ups, early admissions especially in high risk

Table 6. Mean lactate levels among non-dengue and dengue groups

	Lactate level (U/L)				
	Day -2	Day -1	Day 0	Day 1	Day 2
Non-dengue group					
n	5	12	19	25	14
Mean	2.29	1.55	1.68	1.55	1.61
SD	1.63	0.48	0.65	0.48	0.68
Minimum	1.29	0.63	0.96	0.93	0.91
Maximum	5.15	2.44	2.73	2.75	2.76
DF					
n	18	48	102	112	65
Mean	1.22	1.43	1.63	1.71	1.88
SD	0.35	0.54	0.65	0.63	0.62
Minimum	0.66	0.67	0.72	0.64	0.94
Maximum	2.12	2.97	3.68	3.84	3.88
DHF					
n	12	26	60	71	59
Mean	1.756	1.64	1.79	1.90	1.92
SD	0.94	0.67	0.60	0.62	0.67
Minimum	0.89	0.87	0.77	0.88	0.97
Maximum	4.05	3.70	3.91	3.83	3.55
DSS					
n	4	7	19	27	28
Mean	1.07	1.68	2.26	1.98	2.01
SD	0.21	0.21	0.93	0.38	0.77
Minimum	0.95	1.52	1.00	1.21	1.21
Maximum	1.38	2.10	5.34	2.71	4.66

n = numbers of patients whose blood was sent on the individual day. * Significant comparison of lactate levels between severity type (p-value <0.05). On Day 0 is DF and DSS; On Day 1 is non-dengue and DF, non-dengue and DSS.

Table 7. Mean LDH levels among non-dengue and dengue groups

	LDH level (IU)				
	Day -2	Day -1	Day 0	Day 1	Day 2
Non-dengue group					
n	5	8	11	12	11
Mean	434.00	417.13	423.82	488.92	524.45
SD	109.25	67.32	64.78	141.22	168.40
Minimum	318.00	314.00	327.00	353.00	368.00
Maximum	614.00	541.00	520.00	845.00	974.00
DF					
n	15	34	57	40	52
Mean	709.20	632.74	787.21	730.55	858.29
SD	361.79	235.41	395.71	179.33	310.64
Minimum	392.00	332.00	394.00	382.00	416.00
Maximum	1,679.00	1,478.00	2,353.00	1,078.00	2,046.00
DHF					
n	3	14	35	25	33
Mean	1,873.67	868.00	1,060.74	1,031.96	836.55
SD	1,435.59	443.22	615.26	441.05	278.28
Minimum	585.00	321.00	383.00	472.00	427.00
Maximum	3,421.00	1,614.00	3,722.00	2,225.00	1,574.00
DSS					
n	4	3	15	12	7
Mean	654.50	1,398.33	1,180.73	856.83	1,446.86
SD	214.29	904.04	545.31	372.33	840.56
Minimum	449.00	807.00	586.00	369.00	664.00
Maximum	956.00	2,439.00	2,605.00	1,817.00	2,486.00

* Significant comparison of LDH levels between severity type (*p*-value <0.05). On Day -1 is non-dengue and DF, and non-dengue and DHF; On Day 0 is non-dengue and DF, non-dengue and DHF, non-dengue and DSS, DF and DHF and DF and DSS; On Day 1 is non-dengue and DF, non-dengue and DHF, non-dengue and DSS, and DF and DHF; On Day 2 is non-dengue and DF, non-dengue and DHF, and non-dengue and DSS.

groups, and intensive monitoring with proper IV fluid management in the hospital to prevent shock.

In the present study, the majority of dengue patients have liver impairment, demonstrated by elevation of AST/ALT (86.2/ 71.1%). This is consistent with previous studies[10]. On the other hand, the majority of non-dengue patients did not have elevated AST/ ALT levels. The mean of AST and ALT of DHS and DFS, or leakage groups, are higher than DF and non-dengue, or non-leakage groups. This is most apparent during the period of leakage of Day 0 and Day 1. Severe leakage in DHF or DSS, leading to poor hepatic perfusion, can induce elevation of AST and ALT. This result is consistent with the study by ET Ooi et al, that demonstrated higher ALT levels in DHF/DSS group than DF group[11]. Consistent with the study by S. Kalayanarooj et al the elevation of AST and ALT to >200 U was more in the DHF/DSS group than DF group[9]. DSS cases, with prolonged shock experience liver failure and very high AST/ALT levels from at least 1,000 U/L up to 20,000 U/L, signifies poor prognosis.

Results from the present study showed that the lactate levels were significantly increased in DSS, and is more than DF and non-dengue patients are, especially during the critical phase. According to the pathophysiology of DHF and DSS, hyperlactemia in DSS patients is likely to be the result of severe plasma leakage, inducing contracted intravascular perfusion, tissue hypoxia, and multi-organs failure[4]. This result is consistent with the study from Indonesia[13] that showed significant increase in lactate levels in DSS when compared to DHF. The present study also showed a negative correlation between lactate levels, pO_2, and oxygen saturation. Decrease in oxygen saturation or

tissue hypoxia was followed by an increase in blood lactate level[12].

Hyperlactemia is a result of increased lactate production and decreased clearance. One of the causes of increased lactate production is tissue hypoxia or cellular stress[5]. Under aerobic conditions, glucose is converted to pyruvate, which is then fully oxidized to CO_2 and generates 36 ATP in the Kreb's cycle. Large amounts of energy are required under cellular stress, which accelerates glycolyisis to generate much more energy. Excess glycolysis results in pyruvate accumulation, which is diverted to lactate in order for glycolysis to proceed[13].

Another cause of hyperlactemia in DSS may be a result of decreased hepatic lactate clearance. The liver is the main organ to remove lactate (70%). When the liver blood flow is reduced to 25%, lactate clearance decreases[5]. Furthermore, under anaerobic conditions, glycolysis is the main process of hepatic energy production. The consequence of this process leads to over production of lactate[13]. Inadequate intravascular volume in DSS leads to a decrease in hepatic circulation, which in turn decreases hepatic lactate clearance, resulting in hyperlactemia.

Evidence from this study suggests that serum lactate levels may be used as a parameter for early detection of the critical phase of DHF. From a study by Santosa et al serum lactate was used as a marker of plasma leakage in children with dengue infections using a cutoff point of >2.4 U/L, and sensitivity and specificity to predict plasma leakage were 79.31% and 77.42%, respectively[14]. Serum lactate in DSS patients at the critical period was 2.26 U/L, which is near to their cutoff point. The slight difference may be due to different laboratory techniques of lactate detection.

Results from the present study showed no significant difference in lactate levels in DHF compared to DSS. This is because DHF patients have a lesser extent of plasma leakage than DSS, resulting in less tissue hypoxia. Moreover, in DHF patients, the clearance of lactate by the liver may not be impaired as the blood flow to the liver was still >75%. Results showed that serum lactate levels were higher in DHF patients than in DF and non-dengue patients, though they were not statistically significant. This could be due to the lesser degree of tissue hypoxia and less impaired clearance of lactate by the liver in DHF patients. Lactate levels in DHF patients appeared to be higher than DF and non-dengue patients were, but it was also not statistically significant.

Elevation of lactate levels in DSS patients may be used to predict which dengue cases are likely to develop shock. The present study by Rungteeranon et al found that normal lactate levels in dengue patients within 6 hours after defervescence is associated with mild dengue diseases. Dengue cases with high lactate levels need more intravenous fluid and/or have more pleural effusion than dengue patients with normal lactate levels[15]. In addition, lactate levels can be used to predict the outcome of sepsis cases, as intermediate and high serum lactate levels are associated with a 28-day mortality in both non-shock and shock patients[16]. The authors may apply this finding to our DHF/DSS patients. Those who have sustained intermediate or high levels of lactate may result in sepsis, which can lead to death, if there is no early detection and proper management.

Since hyperlactemia in the present study was found in DSS and reflects low oxygenation in tissue level, the authors may use lactate levels to evaluate their management. If the lactate level remains high, the authors have to review and reassess their treatment. This finding is supported by the study on the association between lactate clearance and central venous oxygen saturation ($ScvO_2$) in septic shock patients. Those patients with normal lactate clearance have better outcomes compared to those with normal $ScvO_2$[17]. Another study, by H. Bryant Nguyen about the association between lactate clearance and outcome in severe sepsis and septic shock, showed that patients with higher lactate clearance after 6 hours of emergency intervention have better outcomes compared to those with lower lactate clearance[18].

LDH is an enzyme, which catalyzes the reversible oxidation of lactate to pyruvate. There are 5 LDH isoenzymes. The types of isoenzymes found in liver and skeletal muscle are LDH type 4 and type 5. Other types are found in myocardium, brain, kidney and red blood cell (RBC). The mechanisms of elevated LDH enzyme include increased release from necrotic tissue or inflammatory tissue and increase of tissue sources of enzymes[19].

In the present study, LDH levels were significantly increased in the leakage group (DSS and DHF) when compared with the non-leakage group from 1 day before the critical phase. Levels of LDH in DHF/DSS are still high after the critical period of plasma leakage. According to pathophysiology of dengue infection, the main reason of elevated LDH level in DHF and DSS may be due to hypoxic tissue or liver injury.

The study by ET Ooi et al, found that more

liver enzymes abnormalities were found in DHF/DSS cases[11]. S. Kalayanarooj reported that elevation of AST was correlated with dengue severity. The elevation of AST was found in 43.1% of DF, 54.4% in DHF, and 33.3% in DHF grade 3 and 100% in DHF grade 4. The percentage of ALT elevation is less than AST, but it is also correlated with disease severity. The elevation of ALT was found in 1.1% of DF, 10.6% of DHF, 50% of DHF grade 3, and 66.7% in DHF grade 4. The same study also revealed that the percentage of AST elevation in viral infection patients was only 9.5 with no elevation of ALT[20]. The authors study was consistent with S. Kalayanarooj in that the majority of dengue patients had elevation of both AST and ALT, especially in DHF and DSS. Patients in the non-dengue group of this study also had minimal percentage of AST/ALT elevation[11].

After cells are infected by the dengue virus, the infected cells are attacked by activated T lymphocytes, with the subsequent release of vasoactive cytokines and rapid activation of complement systems. Cytokines and activated complement systems induce an increase in vascular permeability in DHF. Capillary damage allows fluid and electrolytes to leak into pleural and peritoneal spaces[21]. Severe plasma leakage leads to shock, hypoxia, tissue injury, impaired liver, and other organs, causing elevation of LDH levels, especially in DSS and severe complicated DHF cases.

Results from the present study on elevated LDH levels in DSS and severe complicated DHF cases is consistent with a study that showed that DHF cases have higher levels of LDH, CK, and AST in DHF cases when compared to DF cases and other acute febrile illnesses[22]. Elevated LDH levels can occur in other diseases such as malarial infection. It is believed that the mechanism of elevated LDH in DHF/DSS may be similar to malaria infection. Acute hepatocellular injury is found in both malaria infection and the majority of dengue patients. Red cell hemolysis induced by invading merozoites is another factor that causes LDH elevation in malaria. In a group of dengue patients, acute hemolysis of RBC due to other underlying diseases, such as hemoglobinopathy and G-6-PD deficiency, may be another cause of LDH elevation. Studies showed that the mean serum LDH level in malaria cases is significantly higher than the mean of the control group[23]. In the present study, the degree of LDH elevation in DHF/DSS patients is much higher than the malaria patients from the Khosya S study are. LDH in DHF patients ranges from 836.5-1,873.7 IU while in DSS patients ranges from 654.5-1,446.9 IU.

Conclusion

In conclusion, serum lactate and LDH were found to be elevated in severe dengue patients. Lactate levels may be used as a predictor of DSS if the level is >2 U/L on Day 0. LDH levels can be used to differentiate dengue and non-dengue patients in the early febrile phase, if the level is >500 IU. If the level increases to 1,000 IU on Day 0, it may be a good predictor of severe dengue infection.

Acknowledgement

The authors wish to thank all patients who enrolled in this project, all staff members of the Dengue Ward, QSNICH, and AFRIMS nurses who helped in coordinating this project. Thank you to Dr. Pra-on Supradish for her suggestions and Ms. Somporn Krasasab for her help in statistic analysis. Thank you to Ms. Jutaporn Akatot who had set up LDH and lactate laboratory tests. Also, thank you to the Armed Forces Research Institute of Medical Sciences Armed Forces Research Institute of Medical Sciences AFRIMS for supporting all the dengue laboratory confirmations (PCR and MAG and GAG ELISA).

Potential conflict of interest

The present study was supported by WHO SEARO grant: Project No. SECDS1003954; AMS code: Award 56521; Allotment No: Task 7.1.

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ระดับ Lactate และ lactate dehydrogenase เป็นตัวแปรที่สำคัญในการพยากรณ์ความรุนแรงของการติดเชื้อไวรัสเดงกี

พักต์เพ็ญ สิริคุตต์, ศิริเพ็ญ กัลยาณรุจ

ภูมิหลัง: ค่า Lactate และ lactate dehydrogenase (LDH) สูงขึ้นในผู้ป่วยหัวใจล้มเหลว ภาวะช็อกจากการติดเชื้อในกระแสโลหิต และภาวะตับวาย เนื่องจากผู้ป่วยไข้เลือดออก (dengue hemorrhagic fever (DHF)) ที่มีอาการรุนแรง จะก่อให้เกิดภาวะช็อก และ ตับขาดเลือดได้ ดังนั้นค่า lactate และ LDH อาจมีค่าสูงขึ้นได้ในผู้ป่วยไข้เลือดออกช็อก (dengue shock syndrome (DSS))

วัตถุประสงค์: เพื่อหาระดับ Lactate และ LDH ในผู้ป่วยติดเชื้อไวรัสเดงกีว่าสามารถใช้เป็นตัวแปรที่ใช้พยากรณ์ความรุนแรงของโรคได้หรือไม่

วัสดุและวิธีการ: การศึกษานี้เป็นการศึกษาแบบตัดขวางในผู้ป่วยที่สงสัยการติดเชื้อไข้เลือดออกที่รับเข้ารักษาตัวในตึกไข้เลือดออก สถาบันสุขภาพเด็กแห่งชาติมหาราชินี ในช่วงเดือนพฤษภาคม พ.ศ. 2554 ถึง เดือนกุมภาพันธ์ พ.ศ. 2555 ผู้ป่วยที่เข้าร่วมการศึกษาจะได้รับการตรวจยืนยันการติดเชื้อไข้เลือดออกทุกราย ส่งการตรวจหาค่า lactate ทุกวันหลังเข้าสู่การศึกษาและตรวจหาค่า LDH และ ค่าเอมไซม์ในตับทั้งหมด 3 วัน คือวันแรกที่เข้าสู่การศึกษา วันที่เริ่มมีการรั่วของพลาสมา และ วันที่จำหน่ายค่า lactate และ LDH จะนำมาเปรียบเทียบกันระหว่างผู้ป่วย dengue และ ผู้ป่วย non-dengue ซึ่งคำจำกัดความของ Dengue fever (DF), DHF และ DSS อ้างอิงตามคำจำกัดความจาก WHO 1997

ผลการศึกษา: ผู้เข้าร่วมโครงการทั้งหมด 253 รายแบ่งออกเป็น กลุ่ม DF 120 ราย DHF 75 ราย DSS 30 ราย และ non-dengue 28 ราย ผลการศึกษาพบว่า ผู้ป่วยไข้เลือดออกส่วนใหญ่มีภาวะที่เซลตับถูกทำลาย ซึ่งแสดงให้เห็นจากระดับ AST/ALT ที่เพิ่มขึ้น ในขณะที่กลุ่ม non-dengue มีระดับ AST/ALT เพิ่มขึ้นน้อยมาก ในกลุ่มผู้ป่วย dengue ค่า lactate มักไม่สูงในช่วงต้น แต่ มีสูงขึ้นได้ในกลุ่ม non - dengue ในกลุ่ม DSS พบว่า ค่าเฉลี่ยของ lactate จะเริ่มสูงขึ้น ตอนช่วงปลายของระยะไข้และ สูงสุดในวันที่ 0 คือ 2.2 U/L ในขณะที่กลุ่ม non - dengue ค่า lactate กำลังลดต่ำลง ในช่วงนี้ ค่าเฉลี่ยของ lactate ในวันที่ 0 พบว่ามีความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่างกลุ่ม DSS และ กลุ่มอื่นๆ (ค่าเฉลี่ยของ lactate ในกลุ่ม DSS คือ 2.26 U/L DF คือ 1.63 U/L, DHF คือ 1.79 U/L และกลุ่ม non - dengue ซือ 1.68 U/L) ($p < 0.05$)

ค่าเฉลี่ยของ LDH สูงขึ้นในช่วงต้นของโรคในทุกกลุ่มการศึกษาซึ่งมีระดับที่แตกต่างกันคือ DF 709.2, DHF 1,873, DSS 654.5 และ กลุ่ม non - dengue 434 IU และในกลุ่ม dengue มีค่า LDH มากกว่า 500 IU ในขณะที่กลุ่ม non - dengue มีค่า LDH น้อยกว่า 500 IU การเพิ่มขึ้นของ LDH ในช่วงท้ายของระยะไข้พบเฉพาะในกลุ่ม DHF และ DSS แต่ไม่พบในกลุ่ม DF และ non-dengue ค่าเฉลี่ยของ LDH ของกลุ่ม DHF, DSS, DF และ non-dengueเท่ากับ 1060.7, 1,180.7, 787.2 และ 423.8 IU ตามลำดับ

สรุป: ค่า lactate และ LDH สูงขึ้นในผู้ป่วย dengue ที่มีอาการรุนแรงได้แก่ DHF และ DSS ซึ่งถ้ามีค่า lactate มากกว่า 2 U/L ในวันที่ 0 อาจพยากรณ์การเกิด DSS ได้ ค่า LDH สามารถใช้แยกความแตกต่างระหว่างผู้ป่วยติดเชื้อ ไข้เลือดออกและ ผู้ป่วย non - dengue ได้ตั้งแต่ระยะไข้ ถ้ามีค่า LDH มากกว่า 500 IU มีแนวโน้มจะติดเชื้อเดงกี และถ้ามีค่าสูงขึ้นจนถึง 1,000 IU ก็อาจจะสามารถพยากรณ์ว่าอาจเกิดภาวะไข้เลือดออกที่รุนแรงหรือ DHF/DSS ได้