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Role of Soluble Heat Shock Proteins 70 in Severity of Dengue Infection

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Conflicts of Interest: Nil.

Abstract

Objective: to prove the role of soluble HSP 70 in the severity of dengue infection by comparing the level of soluble HSP 70 in patients who diagnosed as dengue fever (DF), dengue hemorrhagic fever (DHF) and in healthy people.

Methods: This was a prospective and longitudinal study, samples were taken by using consecutive sampling with a sample size were 22 DF patients, 20 DHF patients and 20 healthy people as a control group. Soluble HSP 70 in serum was measured by an enzyme-linked immunosorbent assay (ELISA).

Results: The soluble HSP 70 was significantly increased (p=0.003) in DHF patients (0.33 ± 0.17 ng/ml) compared to in DF patients (0.149 ± 0.19 ng/ml) and control group (0.147 ± 0.37 ng/ml), but there was not significantly increased (p=0.241) in DF patients compare within control group.

Conclusion: This result suggesting that soluble HSP 70 may play important roles in the severity of dengue infection.

Keywords: Soluble HSP70, Dengue fever, Dengue hemmorhagic fever

Introduction

Dengue infection is a serious health problem in tropical and subtropical regions of the world. This disease is caused by DENV transmitted by Aedes mosquitoes, primarily Aedes aegypti. Two-fifths of the world's population at risk of dengue infection, estimated approximately 50 million cases occur annually about 2.5% of those affected die.[1] Dengue has a wide spectrum of clinical presentations, often with unpredictable clinical evolution and outcome. Dengue infection can result in wide clinical symptoms, ranging from asymptomatic, undifferentiated febrile illness (viral syndrome), dengue fever (DF) to the most severe form, which is characteristic by plasma leakage : dengue haemorrhagic fever (DHF) and may progress to dengue shock syndrome (DSS) that can cause death.[2,3] Although much research has been done, pathogenesis which can explain the severity of dengue still cannot be explained certainty.[4,5] DHF is the result of a complex interaction between the virus and the immune response evoked by the host with secondary infection. Recent studies showed that cytokines may induce plasma leakage such as interferon, interleukin(IL)2, IL6, Il8, IL10 and tumour necrosis factor(TNF) α are increased in DHF cases and estimated has been associated with the pathogenesis of DHF.[5]

Heat shock proteins (HSP) 70 are a group of heat shock proteins with a molecular weight of 68-73 kDa which in physiological conditions serves to protect cells from various kinds of stress. HSP 70 plays an important role in human health. HSP 70 function as molecular chaperones in regulating cellular homeostasis and prolong cell survival.[6] Although HSPs are intracellular, they can be released from the cells and become detectable in the blood

of healthy individuals as soluble HSP.[7] The release of HSP 70 to the extracellular environment can occur via two ways: active and passive release. HSP 70 released passively from the damaged, necrotic or infected cell by viral[8], while HSP 70 can be released actively from living cells through exosome.[9] HSP 70 which is released into the systemic circulation will produce an immune response and release of cytokines.[7] Many studies showed soluble HSP 70 activated cells in the immune system, including monocytes main venue of replication of dengue viral.[9] Soluble HSP 70 induced cytokine responses were broad includes TNF - α, IL - 12 and IL - 1β is released from macrophages, TNF- α, IL- 6 and IL - 1β from monocytes[10] and the release of IL - 12 and IL - α on dendritic cells.[11] Soluble HSP is a danger signal because it can induce high levels of cytokines.[12]

This study investigated role soluble HSP 70 in manifestation severity of dengue infection by comparing the level of soluble HSP 70 in dengue fever patient, dengue hemorrhagic fever patients and healthy people.

Materials and Methods

This was a prospective and longitudinal study, the sample of blood for examination of soluble HSP 70 level was conducted on less than 3 days of fever, before severe manifestation, and then patients were followed up daily to capture clinical manifestation and severity of the disease. Base on the severity of the disease, dengue infection patient was divided into DHF and DF group, based on WHO criteria 2011. Enrollment criteria for case group were age ≥15 years, history of fever for 1- 2 days and positive results of serological tests anti-dengue IgG and/or anti-dengue IgM and/or NS-1 antigen and willing to participate in this study. While the control group is healthy people which have criteria like the case group. This study was conducted after obtaining approval from the Health Research Ethics Committee of the Faculty of Medicine, University of Sumatera Utara, Indonesia

Soluble HSP 70 was measured by using Assay Designs™ Hsp70 High Sensitivity Enzyme Immunometric Assay (EIA) kit. The whole blood sample collected in EDTA tubes and then centrifuged at 1000 x g for 15 minutes at 4°C. The supernatant may be stored at or below -20°C or used immediately in the assay. The wells were washed by adding 400 µL of wash buffer to every well. Washing was repeated 3 times. After the final wash, the wells drained by the paper towel to remove any remaining wash buffer. By using the pippete, add 100 µL of the solution of the polyclonal antibody specific for HSP 70 into each well, and incubate for 1 hour at room temperature. Then 100 µL of a solution of HRP conjugate was added into each well and shake for 1 hour at room temperature. Put 100 µL of substrate solution into each well and incubate a for 30 minutes at room temperature. Then 100 µL of stop solution was added into each well and read with Optical density at 450 nm.

The data was analysed using the Statistical Package for Social Science (SPSS). A non-parametric Kruskal Wallis tests were used to compare the level of soluble HSP 70 between the groups. And then the Mann Whitney U test was used for paired comparison with the Bonferroni correction was performed. The significant level was set at P value < 0.05.

Results

This study has 62 samples consisting of 42 dengue patients, of which 22 DF patients (53 %), and 20 DHF patients (47%), and 20 healthy people as control group. Demographic and clinical data for the samples are summarized in Table 1.

Table 1 : Demographics and clinical data of samples (n = 62)

Variable		Control (n=20) n (%)	DF (n = 22) n (%)	DHF (n = 20) n (%)	Total n (%)
Age (years)*		24 (±1.39)	26 (±13.16)	23 (±9.96)	23 (±9.89)
Gender	Male	10 (50.0)	15 (68.2)	7 (35.0)	32 (51.6)
	Female	10 (50.0)	7 (31.8)	13 (65.0)	30 (48.3)
Haemorrhagic manifestation	Spontan bleeding		1 (4.5)	2 (10.0)	3 (7.1)
	Petechiae		0 (0.0)	3 (15.0)	4 (9.5)
Symptom	Headache		22 (100.0)	20 (100.0)	42 (100.0)
	Arthralgia		22 (100.0)	20 (100-0)	42 (100.0)
	Nausea and vomiting		7 (31,8)	10 (50)	17 (41,5)
	Conjunctival injection		1 (4.5)	0 (0.0)	1 (2,4)
	Rash		1 (4.5)	3 (15.0)	4 (9.5)
	Sore throat		1 (4.5)	0 (0.0)	1 (2.4)
Serological assays	IgM (+)		4 (18.2)	2 (10.0)	6 (14.3)
	IgG (+)		20 (90.9)	19 (95.0)	39 (92.9)
	NS1 (+)		18 (81.8)	19 (95.0)	37 (88.1)

*Mean (SD)

Based on the gender of the dengue patients as many as 20 people (47 %) were female and 22 (53 %) were male. The mean age of DF patients was 26 ± 13.16 years and the mean age of DHF patients was 23 ± 9.96 years, there was no significant difference in age between DF and DHF patients ($p \geq 0.05$). The control group consisted of 20 healthy people, 10 people (50 %) were female and 10 people (50 %) were male. The mean age of the control group was 24 ± 1.39 years, there was no significant difference in age between the case group and the control group ($p \geq 0.05$).

Hemorrhagic manifestations, spontaneous bleeding such as epistaxis and gum bleeding was found in 5 % of DF patients and 10 % in DHF patients. Petechiae was found 15 % of DHF patients and not found in DF patients. Headaches and joint pain were found in all DF and DHF patients. Nausea was found in 32 % of DF patients and 50 % of DHF patient. Conjunctival injection and sore throat 0.5 % respectively in DF patients and are not found in DHF patients. Rash mainly on the face and extremities was found in 0.5 % of DF patients and 15 % of DHF patients.

The median soluble HSP 70 level in the control group, DF patients, and DHF patients was 0.190 (0.07), 0.150 (0.02), and 0.225 (0,17) ng/mL, respectively (Figure 1). The DHF patients showed elevated median soluble HSP 70 levels compared to the DF patients and the control group. The Kruskal–Wallis test showed significant differences in the mean soluble HSP70 levels between the three groups ($P < 0.001$).

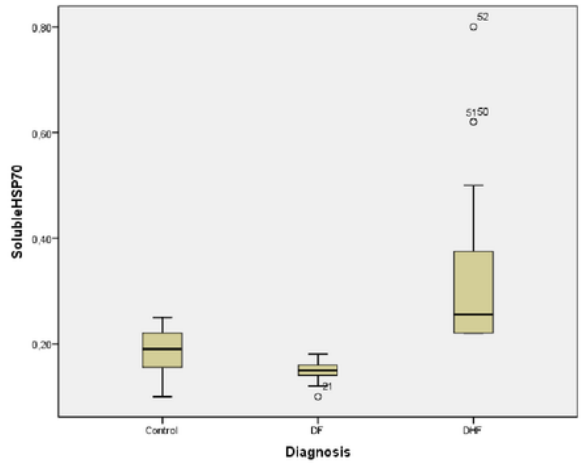

Figure 1. Box plot comparing level of soluble HSP 70

Furthermore, the different soluble HSP70 levels between two groups were conducted with a Mann–Whitney U test (Table 2). Bonferroni correction was performed by dividing the P-value of the Mann–Whitney test by 3 (number of pairs). The significance level was set at $P < 0.05$. The median soluble HSP 70 levels between the DHF patients and DF patients and between the DF patients and the control group showed significant differences ($P < 0.017$).

Table 2. Comparison of soluble HSP 70 levels in control group, DF (dengue fever) and DHF (dengue haemorragic fever) patients Level of HSP 70

*Kruskal Wallis test, $P < 0.05$ is significant. IQR, interquartile range

Pos Hoc Mann-Withney test with Bonferroni corection, $P < 0.017$ is significant

DF patients versus controls, $P = 0.003$; DF patients vs DHF patients, $P < 0.001$;

DHF patients versus controls, $P < 0.001$

Discussion

DENV is transmitted through mosquito bites into the human body and undergo replication in dendritic cells. Infected dendritic cells migrate to draining lymph nodes, where infection spreads to monocytes and macrophages.[13]

Monocytes and macrophages are responsible for the dissemination of the virus after its entrance into the dermis by mosquito bite.[14]

DENV-infected cells can lead cell death by apoptosis and necrosis. HSP 70 are released from cells undergoing death and leads increased levels of soluble HSP. The soluble HSP activate many cells of the immune system including monocytes, macrophages, dendritic cells, neutrophils and mast cells. HSP induce a range of cytokine responses in vitro including TNF-, IL-12, and IL-1 release from macrophages.[15] Soluble HSP70 has effects both on cytokine production and maturation of dendritic cells and can bind to human monocytes, resulting in the upregulation of TNF-, IL-6, and IL-1.[16]

Released HSP70 shows danger signal by upregulating CD83 expression and IL-12 release from naïve dendritic cells and inducing many cytokines.[9,12] HSP110, proteins constitute a subfamily of the HSP70, induces increased expression of MHC class II, CD40, and CD86 on dendritic cells, and also their secretion of the cytokines IL-6, IL-12, and TNF-α.[12]

Soluble HSP can bind to cell surfaces and enter cells. Soluble HSP is important in the protection of cells,

Groups	n	Levels of sHSP 70 (ng/ml) Median (IQR)	P value
Control	20	0.190 (0.07)	
DF	22	0.150 (0.02)	0.000
DHF	20	0.255 (0.17)	

especially cells that produce low levels of HSP and that these cells depend on the uptake of extracellular HSP for their survival. Neuroblastoma cells can take up HSP70 from media, utilizing this to improve their tolerance to heat shock. Monocytes are also able to take up HSP70 and become be more resistant to apoptosis and necrosis.[7]

DENV are detected by Immune cells through its binding to receptors and initiate an inflammatory response by the activation of signaling pathways and transcription factors including NFκB. Inactive NFκB is normally found in the cytoplasm bound to its inhibitory protein, IkB. The recent study showed there are interactions between HSP70 and IkB-α, allowing NFκB to translocate into the nucleus and bind to its target gene that activate inflammatory cytokines.[17]The studies reported that there are increased cytokine levels in serum of dengue patients and the markers level cytokin were higher in DHF/DSS patients than in DF patients.The cytokin mediated the increase of vascular permeability and caused plasma leakage. Cytokines that may induce plasma leakage such as interferon, interleukin (IL)2, IL-6, IL-8, IL-10 and tumour necrosis factor (TNF) α are increased in DHF cases. Also, interferon γ enhances uptake of dengue particles by target cells through increasing Fc cel lreceptors.[18]

This study is showing soluble HSP70 level to be affected by severity dengue infection, that they may be useful clinical markers. As well as being simply elevated in patients compared to controls, they are also affected by the extent of disease. A problem with using soluble HSP as clinical markers is their upregulation by a range of stresses, including non-disease related physiological and psychological.[19]

Limitation of this study are not measure level soluble HSP70 in blood samples at the same day of fever, and not evaluate the time course of the levels of soluble HSP70.

Conclusions

This study compares the levels of soluble HSP70 in the sera of patients with dengue infection and the control group. The level of soluble HSP70 in DHF patients are higher than the level soluble HSP70 in DF patient and control. This result suggesting that soluble HSP 70 may play important roles in the severity of dengue infection.

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