

Dengue hemorrhagic fever: The role of Soluble E-Selektin, Soluble Intra Cellular Adhesion Molecule-1 (Sicam-1) and Soluble Vascular Cellular Adhesion Molecule -1 (Svcam-1) in overweight children



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ABSTRACT

Introduction: Adhesion molecule (E-selectin, ICAM-1 and VCAM-1) have a crucial role in neutrophil extravasation caused by endothelial cell activation in viral Dengue infection. In overnutritious state, the serum adiponectin level is tend to be lower which limit its ability to inhibit the expression of pro inflammatory cytokines (TNF α , IL-6 and NF- κ B) and adhesion molecules. There is also increased level of TNF α , IL-1 β , IL-6, IL-8 which increase the expression of adhesion molecules even further. However, the role of sE-selectin, sICAM-1 and sVCAM-1 in pediatric DHF patient with overweight is need to be evaluated.

Methods: An analytic observational nested case-control study was conducted in Paediatric Division Sanglah General Hospital, Bali,

Indonesia from January 2015 to October 2016 which DHF as a cases and DD as a control. Diagnosis of dengue infection was according to WHO 1997.

Result: This study showed that overweight increased the risk of DSS although it was not statistically significant (OR: 2.67; 95%CI 0.72-9.95). The level of sE-selectin, sICAM-1, and sVCAM-1 yield no significant differences in all categories except for the level of sVCAM-1 in DF group.

Conclusion: Role of sICAM-1 and sVCAM-1 except sE-selektin showed that the means level of sICAM-1 and sVCAM-1 in DHF, DF, DSS and non-DSS group with overweight was found to be consistently higher than normal weight but it was not statistically significant.

Keywords: Dengue Haemorrhagic Fever, sE-selectin, sICAM-1, sVCAM-1, Children, Overweight.

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INTRODUCTION

The interaction between dengue hemorrhagic fever (DHF) and over-nutrition status and how they affect each other are still unclear. In DHF, plasma leakage occurred due to increased capillary permeability. Various pathogenic mechanisms have been described regarding the phenomenon but not all of them are fully explained yet and one of them is endothelial dysfunction. In this case, the endothelium is stimulated by inflammatory cytokines such as TNF α , IL-1 β , IL-8, and IFN γ produced by infected target cells and directly influenced the endothelial functions.¹⁻³ As a result from such stimulation, the endothelial cells increase the expression of adhesion molecules such as ICAM-1, VCAM-1, E-selektin, P-selektin and vWF which lead to local inflammation, endothelial damage, and plasma leakage.⁴⁻⁷ In the secondary infection, higher amount of TNF α , IL-1 β , IL-8, and IFN γ are produced which lead to higher expression of adhesion molecules (ICAM-1, VCAM-1, E-selektin, P-selektin and vWF).^{1,2,8-10} The result is increased degree of plasma leakage and more severe symptoms.

In physiological condition, the expression of adhesion molecules is regulated by adiponectin which has an immunomodulatory property. However, in over-nutritional state, the levels of pro-inflammatory adipokines are increased while the anti-inflammatory properties of adiponectin decreased.¹¹⁻¹⁵ Thus, this study aimed to reveal the role of sE-selectin, sICAM-1, and sVCAM-1 in DHF children with over-nutrition compared to normal one.

METHOD

This study was an analytic observational with *nested case control study* design. The case group included pediatric patients with DHF which diagnosed according to 1997 *World Health Organization* (WHO) criteria: 6 months to 12 years old children with established DHF diagnosis while those which were not allowed to participate by their parents and acquiring another infectious disease were excluded. The control group consist of 1) all children with Dengue Fever in the same period with the case group and selected by consecutive sampling according to the inclusion and exclusion

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criteria. The sample size was determined by using the formula for case control study with RO (3), $Z\alpha = 1.96$, and $Z\beta = 80\%$ with a minimum of 57 samples required for this study.

This study was conducted in Pediatric Department of Sanglah General Hospital, Denpasar, Bali from January 2015 to October 2016 and had approved by the ethic committee and had permitted by the Research and Development Department of Sanglah General Hospital. sE-selectin, sICAM-1 and sVCAM-1 assessments were conducted in Molecular Biology Department of Eijkmann Institute by professional laboratory officers. The technique used was ELISA using R & B system kit with all procedures were following the manufacturer instruction.

The DHF status was established using 1997 WHO criteria. According to the criteria, the grade 3 and 4 were classified as Dengue Shock Syndrome (DSS) while grade 1 and 2 were considered as non-SSD group. The nutritional status was classified into over-nutrition and normal. The body weight and body length/height recorded was plotted to WHO curve (for children aged <2 years old) and CDC 2000 curve (for children aged >2 years old) so the ideal body weight (IBW) can be calculated. The IBW then plotted using Waterlow so the percentage would be obtained. The over-nutrition status was established when the percentage > 110% while IBW within 90-110% considered as normal nutritional status.

Chi-square or its alternative was used to analyze categorical data while the numeric data were analyzed by using unpaired t-test or its alternative. The mean differences between the two study groups were also analyzed by t-test or its alternative. The risk estimate was also calculated and expressed as odds ratio.

RESULT

40 subjects were enrolled for case and control group. Initially, the baseline characteristics of both groups were analyzed. No significant differences were found in age, sex, body weight, body length/height, and nutritional status as well as the type of infection between the two study groups.

The mean concentration of sICAM-1 and sVCAM-1 were found to be consistently higher in all groups with overnutrition compared to the normal ones. However, the sE-selectin level show inconsistent pattern. Previous study by Shen *et al.* (1997) about the level and kinetic of E-selectin, ICAM-1 and VCAM-1 in cell infected with WNV (flavivirus West Nile virus) accompanied by TNF α , IFN γ , IL-1 α and IL-1 β induction, explain this phenomenon.¹⁶ The study revealed that the expression of sE-selectin was faster and reach peak concentration after 2 hours of induction compared to dengan sICAM-1 and sVCAM-1. The inconsistency of the mean level of sE-selectin might

Table 1 The differences of sE-selectin, sICAM-1, and sVCAM-1 in DHF group with overnutrition and normal nutritional status

Variables	DHF Overnutrition n=18	DHF normal n=22	p	DF Overnutrition n=11	DF normal n=29	p
sE-selectin (ng/mL) Mean(SD)	44.39 (14.55)	53.76 (22.24)	0.118	57,18 (23.86)	48.95 (24.79)	0.350
sICAM-1 (ng/mL) Mean(SD)	407.25 (137.54)	354.42 (87.49)	0.148	363.65 (182.09)	359.71(146.88)	0.944
sVCAM-1 (ng/mL) Mean(SD)	5333.86 (1048.18)	4703.90 (956.64)	0.054	4666.44 (950.74)	4549.40 (1027.11)	0.745

Table 2 The differences of sE-selectin, sICAM-1, and sVCAM-1 in DSS group with overnutrition and normal nutritional status

Variables	DSS Overnutrition n=9	DSS normal n=6	p	Non-DSS Overnutrition n=9	Non-DSS normal n=16	p
sE-selektin (ng/mL) Mean(SD)	42.44 (9.44)	40.93 (14.56)	0.810	46.35 (18.76)	58.58 (23.05)	0.188
sICAM-1 (ng/mL) Mean(SD)	367.25 (105.86)	313.34 (68.65)	0.293	447.25 (159.36)	369.83 (90.64)	0.132
sVCAM-1 (ng/mL) Mean(SD)	4638.64 (934.99)	4358.37 (1350.35)	0.641	5680.80 (601.77)	5029.37 (999.75))	0.090

Table 3 The differences of the level of sE-selectin, sICAM-1 and sVCAM-1 in the DF with overnutrition, Non-DSS with overnutrition, DHF with overnutrition, and DSS with overnutrition

Variables	DF Overnutrition n=11	Non-DSS Overnutrition (DHF grade1&2) n=9	DHF Overnutrition (DHF grade 1to4) n=18	DSS Overnutrition (DHF Grade 3&4) n=9	p
sE-selectin (ng/mL) Mean(SD)	57.18 (23.86)	46.35 (18.76)	44.39 (14.55)	42.44 (9.44)	0.199
sICAM-1 (ng/mL) Mean(SD)	363.65 (182.09)	447.25 (159.36)	407.25 (137.54)	367.25 (105.86)	0.570
sVCAM-1 (ng/mL) Mean(SD)	4666.44 (950.74)	5680.80 (601.77)	5333.86 (1048.18)	4830.62 (1146.38)	0.049

be related with the kinetic of E-selectin expression studied by Shen *et al.* (1997) which was affected the sample collection. In this study, the blood samples for sE-selectin, sICAM-1 and sVCAM-1 analysis were taken at the same time.

The result of odds ratio (OR) analysis according to the nutritional status in DSS cases and non-DSS cases revealed that the patients with overnutrition had 2.67 times higher risk of shock compared with non-DSS. Although the differences were not statistically significant, the result is still clinically meaningful because the value is more than 2.

DISCUSSION

Obesity has been proved to relate with subclinical inflammation state because of increased amount of cytokines expressed by the extended adipocytes. The typical change in cytokines profile of the extended adipocyte include increased expression of TNF- α , IL-6, resistin, and angiotensin accompanied by decreased production of adiponectin, resulting in increased tendency of inflammatory state while lowering the physiological immune-modulation properties. According to this fact, it is appear that obesity could have wider impact than just metabolic disease. The cytokines change could potentially affect the natural response toward infectious disease in which in this case is DHF.

In accordance to our result, several study showed that obesity could increase the severity of DHF in pediatric population. Pichainarong *et al.* (2006) found that patients with obesity had higher risk of experiencing more severe degree of DHF compared to normal nutritional status with OR: 2.77; p=0.001; IK 95% 1.20-7.48.¹⁷ Then, the meta-analysis conducted by Trang *et al.* also reported an increased risk of DHF but the result is not statistically significant.¹⁸ Recent meta-analysis using larger data conducted by Zulkipli *et al.* (2018) also found the same result despite in lower magnitude than Pichainarong *et al.* at OR = 1.38 (95% CI: 1.10-1.73).¹⁹

Shifting inflammatory response observed in obesity, result in higher possibility of inducing more severe inflammatory response in the case of infection. The higher level of inflammatory cytokines provides the basis for more severe acute response when the infection occurred. Higher concentration of TNF- α and IL-6 induce typical inflammatory response such as leukocytes migration, vasodilation and enhance the expression of adhesion molecules. The adhesion molecules facilitates further leukocytes migration and, thus, enhancing the acute inflammatory response.¹⁹

The adhesion molecules expressed by the endothelium could induce local inflammation, endothelial damage, and plasma leakage in the presence of specific signal for wound response or inflammation and activated by TNF- α and IL-1. The inflammation triggers the adhesion between endothelial cell and leukocytes and further enhances by adhesion molecules and their ligands. This event leads to the diversion of blood flow, margination, and migration of neutrophil, monocyte, and eosinophil to the center of inflammation site. The neutrophil is the first leukocyte that adheres to endothelium and move to the extravascular compartment. The extravasation of neutrophil is divided into four steps namely: Rolling, activation by chemo-attractant, adhesion, and trans-endothelial migration. The adhesion molecules play great role during extravasation process.^{20,21}

The adhesion between leukocyte and endothelium is initiated by the expression of L-Selectin on the surface of leukocyte while P- and E-selectin are expressed on the endothelial surface which leads to the margination of leukocyte along the inflamed vascular wall. E-selectin is usually not expressed in normal condition and synthesize only if needed, usually as a result of stimulation by TNF α and IL-1 β . The selectin function in the rolling process of leukocyte onto the endothelial surface. The other chemokines/chemo-attractants bind to specific receptor on the leukocyte surface, leading

to the activation of the leukocytes and subsequent changes in integrin. Next, the integrin bind tightly to the Ig superfamily such as ICAM-1 and VCAM-1 which lead to the tight adhesion of leukocyte to the endothelial surface. Both ICAM-1 and VCAM-1 play crucial role in the process by stabilizing the extravasation process of leukocytes and during trans-endothelial migration of leukocytes from the vascular compartment to the tissue. In the same time, the permeability of the microvasculature would increase.^{22,23}

In the initial phase of inflammation, TNF α , IL-1 β , IL-8, and IFN γ increase the expression of adhesion molecules such as ICAM-1, VCAM-1, E-selektin, P-selektin and vWF.⁴⁻⁷ The higher the level of TNF α , IL-1 β , IL-8, and IFN γ , the higher the expression of adhesion molecules which lead to higher number of leukocytes bound to the endothelial surface. In this condition, the possible that the level of adhesion molecules detected would be higher compared to if the shock already occurred. Meanwhile, if the severity of plasma leakage increased, the number of leukocytes moving to the extravascular compartment is also higher together with sE-selectin, sICAM-1 and sVCAM-1. Thus, this process might explain why the plasma level of sE-selectin, sICAM-1 and sVCAM-1 in DSS group were found to be lower than in non-DSS group.

Although had incorporated the minimum number of sample, but the sample size is still considerably small. This could lead to the population and analysis bias. Thus, a more comprehensive study with larger number of subjects are needed to confirm this finding.

CONCLUSION

In conclusion, sICAM-1 and sVCAM-1, but except sE-selektin, might played a significant role in DHF, DF, DSS and non-DSS and their level were found to be consistently higher in overweight compared to normal weight despite not statistically significant.

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CONFLICT OF INTEREST

The authors declare that they don't have any competing interest regarding manuscript

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