ORIGINAL ARTICLE

Bali Medical Journal (*Bali Med J*) 2018, Volume 7, Number 1: 244-248
P-ISSN.2089-1180, E-ISSN.2302-2914

Published by DiscoverSys

The effect of overnutrition toward the risk of dengue shock syndrome in pediatric patient: in-depth investigation of sVCAM-1 and adiponectin level

Ni Kadek Elmy Saniathi,[1,2*] Bambang Udji Djoko Rianto,[3] Mohammad Juffrie,[4] Soetjiningsih[5]

ABSTRACT

Introduction: Overnutrition has become an emergent health problem worldwide and could significantly alter the normal human physiology. It still unknown how overnutrition could affect the outcome of pediatric patient with dengue infection. This study aimed to unveil how overnutrition affects the risk of dengue shock syndrome (DSS) in dengue pediatric patient by evaluating the adiponectin which has immune-modulatory property and the inflammation marker sVCAM-1.

Methods: An analytic observational nested case-control study was conducted in Paediatric Division Sanglah General Hospital, Bali, Indonesia from January 2015 to October 2016. Initially, patient's anthropometric parameters were measured and the diagnosis of the dengue infection was established according to the medical record and blood plasma was isolated to determine the level of adiponectin and sVCAM-1.

Result: The study showed that overnutrition increased the risk of DSS as much as 2.67 times greater than normal but it was not statistically significant (95%CI: 0.72 - 9.95). The mean level of sVCAM-1 was observed to be higher in overnutrition patients in almost all categories of dengue infection, but none is statistically significant. However, its level appears to be lower in over nutritious DSS patients compared to their non-DSS counterparts (4638.64 (±934.99) vs. 5680.80 (±601.77); P = 0.013). The adiponectin level was found to be consistently lower in patients with overnutrition regardless the category of dengue infection, but significant differences only observed in over nutritious DHF and overnutritious non-DSS comparisons.

Conclusion: It can be concluded that overnutrition increase the risk of DSS in pediatric patient with dengue infection whether it was not statistically significant. The lower level of adiponectin and elevated expression of sVCAM-1 could be the explanation of this phenomenon.

Keyword: Overnutrition, Pediatric, dengue hemorrhagic fever, adiponectin, sVCAM-1

Cite This Article: Saniathi, N.K.E., Rianto, B.U.D., Juffrie, M., Soetjiningsih. 2018. The effect of overnutrition toward the risk of dengue shock syndrome in pediatric patient: in-depth investigation of sVCAM-1 and adiponectin level. *Bali Medical Journal* 7(1): 244-248. DOI:10.15562/bmj.v7i1.912

[1]Postgraduate Student of Faculty of Medicine Gadjah Mada University
[2]Faculty of Medicine and Health Warmadewa University
[3]Ear, Nose, and Throat-Head and Neck Department Faculty of Medicine Gadjah Mada University/ Sardjito General Hospital
[4]Pediatric Department Faculty of Medicine Gadjah Mada University/ Sardjito General Hospital
[5]Pediatric Department Faculty of Medicine Gadjah Mada University/ Sardjito General Hospital

*Correspondence to:
Ni Kadek Elmy Saniathi;
Postgraduate Student of Faculty of Medicine Gadjah Mada University/ Faculty of Medicine and Health Warmadewa University;
elmye84@gmail.com / pspd_warmadewa@yahoo.co.id

Received: 2017-10-19
Accepted: 2017-12-14
Published: 2017-12-21

INTRODUCTION

Adiponectin is one of the several kinds of adipokines that produced by adipocytes which has anti-inflammatory properties such as inhibit the secretion of TNF-α (*Tumor necrosis factor-α)*, IL-6 (*Interleukin-6*), and reduced the activation of *(Nuclear Factor Kappa-light-chain-enhancer of activated-B)*. It also inhibits the expression of several inflammatory adhesion molecules like ICAM-1(*Intercellular Adhesion Molecule-1)*, VCAM-1 (*Vascular Cellular Adhesion Molecule -1)*, and E-selectin as well as Scavenger class A (SR-A) receptor expression.[1-7] VCAM-1 is one of the several molecules that play an important role in leukocytes extravasation by stabilizing diapedesis process and transendothelial migration, enabling the leukocytes to pass through the vascular wall to the tissue.[8-11]

Physiologically, adiponectin would regulate the expression of VCAM-1 which helps to keep the inflammatory response in check. However, in the condition of overnutrition, the production rate of adiponectin is lowered which theoretically decrease the inhibitory effect toward VCAM-1 expression. In addition, there is also increased production of TNF-α, IL-1β, IL-6, and IL-8 which enhance VCAM-1 expression. All of those processes eventually lead to the proinflammatory condition in over nutritious patients.[5-7]

In dengue virus infection, the infected cells produce TNF-α, IL-1β, IL-8, dan IFN-γ which have a direct effect on the endothelial cells activation.[12-14] They induce the expression of such as ICAM-1, VCAM-1, E-selectin, P-selectin dan *von Willebrand Factor* (vWF) expression by endothelial cells which ultimately lead to local inflammation, endothelial damage, and vascular leakage.[15-18]

Because of increased prevalence of overnutrition among children, there is great concern about the impact of such nutritional status on the inflammatory process in pediatric patient with Dengue Haemorrhagic Fever (DHF). However, the exact effect of overnutrition and adiponectin level on the inflammatory process is still poorly understood. Therefore, this study aimed to unveil the effect of overnutrition status and adiponectin level on the

inflammatory response, represented by sVCAM-1 expression, in pediatric patient with DHF

METHODS

An analytic observational nested case-control study was conducted in Paediatric Division Sanglah General Hospital, Bali, Indonesia from January 2015 to October 2016. This study was ethically approved by Research and Development Division of Sanglah General Hospital. The pediatric patients with Dengue Haemorrhagic Fever as defined by World Health Organization (WHO) in 1997 which includes clinically diagnosed DHF and age between 6 months old to 12 years old were included in the case group. The control groups consisted of pediatric patients with just Dengue Fever (DF) with the same age range. Those which the parent disagreed to participate and having other infectious diseases were excluded from this study.

The DHF and DD were differentiated using 1997 WHO criteria. Dengue Shock Syndrome (DSS) is defined as DHF grade 3 and four while DD was defined as DHF grade 1 and 2. For nutritional status, the subjects would be classified into two categories namely normal nutritional status and overnutrition. To properly classify the subjects, the body weight (BW) and body length (BL)/Body Height (BH) were measured and plotted as the WHO curve for children under two years of age and CDC for those who were older so the Ideal Body Weight (IBW) could be determined. The nutritional status then determined using Waterlow percentage which estimates the IBW according to the standard. Overnutrition was established if IBW > 110 % of standard and normal status if the IBW ranged between 90-110%.

3 ml of blood was collected from each subject and centrifuged at 2000 RCF to isolate the serum. The serums were stored at -80°C before sent to Eijkmann Laboratory in Jakarta for further analysis. The sVCAM-1 was measured using R & B system ELISA Kit according to manufacturer instruction.

The categorical data were analyzed using *Chi-square test* or its alternative while numerical data were assessed using independent sample student t-test or its alternatives. Normality test was conducted prior further analysis for numerical data. Finally, odds ratio (OR) were calculated to estimate the extent of the risk of the case groups to control group. P-value < 0.05 was considered significant and all of the statistical analyses were conducted using SPSS version 17 for Windows.

Table 1 **The outline of the baseline characteristics of the case and control group**

Variable	Case Group (DBD; N: 40)	Control Group (DD; N: 40)	p-value
Age (years)			
Mean (±SD)	7,40 (±3.6)	7,21(±3.5)	0.993
Sex			
Male	19 (47.5%)	25 (62.5%)	0.261
Female	21 (52.5%)	15 (37.5%)	
BW (kilogram)			
Mean (±SD)	30.39 (±16.37)	26.52 (±15.22)	0.622
BL/BH (centimeter)			
Mean (±SD)	122.01(±26.66)	118.04 (±24.74)	0.869
Nutritional Status			
Overnutrition	18 (45%)	11(27.5%)	0.081
Normal	22 (55%)	29 (72.5%)	
Type of Infection			
Primary	5 (12.5%)	10 (25%)	0,152
Secondary	35 (87.5%)	30 (75%)	

Table 2 **Risk assessment of nutritional status toward DSS risk**

	Case (DSS)	Control (non-DSS)	N	OR	95%CI
Overnutrition	9	6	15	2.67	0.72 - 9.95
Normal	9	16	25		
N	18	22	40		

RESULTS

80 subjects were enrolled in this study with 40 subjects for each group. The baseline characteristics (age, sex, BW, BL/BH, nutritional status, and type of infection) were measured and compared between case and control group (Table 1). The result of the statistical analysis showed no significant differences in the baseline characteristics between case and control group.

Initially, the effect of nutritional status on the risk of DSS was assessed. The risk assessment between nutritional status and the DHF status (DSS vs. non-DSS) reveal that overnutrition elevates the risk of DSS to 2.67 times greater than subjects with normal nutritional status. However, this finding was not statistically significant (Table 2).

The associations between sVCAM-1 and serum adiponectin level with nutritional status in DHF and DF were depicted in Table 3. It revealed that the concentration of sVCAM-1 was significantly higher in overnutrition DHF group compared to normal one while no significant differences were found in DF groups. The same goes for serum adiponectin level which was significantly higher in DHF with normal nutritional status while no differences were observed in DF groups.

Then, further analysis was conducted to compare the level of sVCAM-1 and serum adiponectin

Table 3 The comparison between sVCAM-1 and serum adiponectin level in normal and subject with overnutrition both in DHF and DD groups

Variable	DHF with overnutrition (N: 18)	Normal DHF (N: 22)	p-value	DF with overnutrition (N: 11)	Normal DF (N: 29)	p-value
sVCAM-1 Level (ng/mL) Mean (±SD)	5333.86 (±1048.18)	4703.90 (±956.64)	0.054	4666.44 (±950.74)	4549.40 (±1027.11)	0.745
Serum adiponektin level (ng/mL) Mean (±SD)	7.44 (±5.03)	16.82 (±10.98)	0.015	15.93 (±10.72)	16.99 (±8.33)	0.876

Table 4 Comparison between the level of sVCAM-1 and serum adiponectin in overnutrition vs. normal subjects in both DSS and non-DSS groups

Variable	DSS with overnutrition (N: 9)	Normal DSS (N: 6)	p-value	Non-DSS with overnutrition (N: 9)	Normal Non-DSS (N: 16)	p-value
sVCAM-1 level (ng/mL) Mean (±SD)	4638.64 (±934.99)	4358.37 (±1350.35)	0.641	5680.80 (±601.77)	5029.37 (±999.75)	0.090
Serum adiponektin level (ng/mL) Mean (±SD)	5.99 (±2.86)	8.49 (±2.73)	0.115	8.89 (±6.39)	19.95 (±17.80)	0.036

Table 5 Comparison between the level of sVCAM-1 and serum adiponectin in overnutrition DSS vs. non-DSS groups

Variable	DSS with overnutrition (N: 9)	Non-DSS with overnutrition (N: 9)	p-value
sVCAM-1 level (ng/mL) Mean (±SD)	4638.64 (±934.99)	5680.80 (±601.77)	0.013
Serum adiponektin level (ng/mL) Mean (±SD)	5.99 (±2.86)	8.89 (±6.39)	0.232

within DHF group. The DHF group was divided into DSS and non-DSS group and the difference between the two variables were assessed. It was found that a significant difference was only found in serum adiponectin level between the over nutritious non-DSS group and the normal one while no significant difference was found in DSS groups. Furthermore, no significant differences were found in sVCAM-1 variables either in DSS and non-DSS groups.

Finally, the concentration between the level of sVCAM-1 and serum adiponectin between over-nutritious DSS and over-nutritious non-DSS were compared (Table 5). It revealed that significant difference was only found in the sVCAM-1 level. Although the level of serum adiponectin was different between both groups, the difference was not statistically significant.

DISCUSSION

Overnutrition has become a significant health problem around the world, especially in urban areas. It often associated with many metabolic and endocrine alterations that could affect patient response toward the infectious agent. Most notably, overnutrition and obesity are associated with decreased level of plasma adiponectin level, thus, lowering its metabolic and immuno-modulatory properties.

Consistent with the accepted theory, this study found a lower level of serum adiponectin in the over-nutritious group. In over nutritional state, pro-inflammatory adipokines (e.g. leptin, TNF-α, IL-6, IL-8, PAI-1, dan IL-1β) secretion is elevated accompanied with decreasing production of adiponectin. The primary cause of decreasing production of adiponectin is TNF-α which inhibit the expression of adiponectin gene in adipose tissue. The immunological consequences of this process is diminishing of physiological immuno-modulatory capability provided by adiponectin.[4,6,7,20,21] The effect is more prominent in over-nutritious children who contract infectious diseases such as dengue viral infection. In case of heterologous dengue infection, there is increased production of TNFα, IL-1 β, IL-8, and IFN-γ followed by an elevated level of adhesion molecules expression (ICAM-1, VCAM-1, E-selectin, P-selectin and factor vWF). Because there is only limited modulation effect by low adiponectin level, the inflammatory consequences of dengue infection such as plasma leakage occur in significantly larger scale compared with non-over nutritious dengue patients.

Published by DiscoverSys | *Bali Med J* 2018; 7(1): 244-248 | doi: 10.15562/bmj.v7i1.912

In our study, adiponectin level was found to be lower in over nutritious group regardless of their dengue status (DF, DHF, DSS, and non-DSS). However, it is only significant in normal DHF and normal non-DSS group compared with their over nutritious counterparts. However, it was possible that a low number of the sample affects the analysis of the other group so studies with larger number of sample are required to confirm it. In addition, this is the first study that evaluates the serum level of adiponectin in pediatric dengue patients.

Increased expression of adhesion molecules in dengue infection is caused by increased cytokines production and stimulation by target cells. The cytokines above activate the endothelial cells and have a direct effect in altering the expressional pattern of endothelial cells.[12-14] The increased expression of adhesion molecules such as ICAM-1, VCAM-1, E-selectin, P-selectin, and vWF induce local inflammation by facilitating the margination and migration of leukocytes which result in endothelial damage and plasma leakage.[15,16,18] Of note, the exposure of dengue virus to endothelial cells is not sufficient to induce the expression of the adhesion molecules. However, the presence of TNFα and IL-1 β would result in marked increased in the expression profile of adhesion molecules.[17]

Consistent with our objective, the mean serum concentration of sVCAM-1 was also found to be higher in over nutritious group compared with the normal one in every category of dengue infection (DF, DHF, DSS, and non-DSS). Among all of the dengue categories, the highest mean level of sVCAM-1 was found in the group with DSS and overnutrition while the lowest was found in over nutritious group with DF. These findings should denote that overnutrition is related with the higher level of inflammation. However, these findings were not statistically significant.

VCAM-1 is one of principal vascular adhesion molecule that plays a crucial part in inflammatory response by facilitating leukocyte extravasation and transendothelial migration to the inflamed tissue.[8-11] Massive outward migration of leukocytes might result in leukopenia which often observed in dengue infection. According to the literature and explanation above, it could be assumed that the dissolved form of VCAM-1 could also migrate outward which results in decreased level of sVCAM-1 detected in the serum of the patient with dengue shock syndrome.

Cumulative evidence showed that adiponectin exerts its effect mainly in innate immunity which mostly regulated the macrophages.[22] In physiological condition, adiponectin-mediated the differentiation of monocytes into M2 macrophage while preventing their differentiation to pro-inflammatory M1 macrophage.[22] Thus, allowing increased concentration of anti-inflammatory cytokines such as IL-10 in the circulation. Furthermore, recent studies found that it also affects other kinds of immune cells which result in inhibition of NK-cell, neutrophil, dendritic cell activation as well as prevent eosinophil recruitment.[23,24,25] These evidences support our findings and underlie the reason why children with overnutrition tend to have higher risk of DSS compared to normal one since lower level of adiponectin presented in the circulation is not sufficient to prevent massive inflammatory responses which resulted in massive increase in vascular permeability and plasma leakage.

CONCLUSION

From this study, it could be concluded that overnutrition has potential to increase the risk of DSS in a pediatric patient with DHF whether it is not statistically significant. It also observed that over nutritional status affect the level of sVCAM-1 and serum adiponectin level which could be one of the explanations of increased risk of DSS among over nutritious DHF pediatric patients. However, due to the limited number of subjects in this study, it is important to replicate and improve this study model in the future with larger number of subjects and more comprehensive study design as well as evaluation of other inflammatory parameter so the exact mechanism of how nutritional level affects the risk of DSS could be elucidated.

ACKNOWLEDGEMENT

The authors acknowledge and appreciate the contribution of the staffs of Pediatric Division Faculty of Medicine Udayana University/Sanglah General Hospital (dr. BNP Arhana Sp.A(K), dr Dwilingga Sp.A(K), and dr Gustawan Sp.A(K), MSc) and ERIA Subdivision (DR. dr. Dyah Kanyawati, Sp.A(K), dr IB. Suparyatha Sp.A(K), and dr. Budi Hartawan Sp.A(K)), dr. Ketut Suarta Sp.A(K), the nurses from Jempiring Ward and Pediatric Intensive Care Unit of Sanglah General Hospital, medical record division, R. Tedjo Sasmono Ph.D.; Eijkman Institute for Molecular Biology, Jakarta; Prodia Laboratory, Denpasar; Laboratorium Biomolecular Laboratory of Faculty of Medicine and Health of Warmadewa University; Pediatrician Association of Bali; Mirah; Andhiko Suryo; and Made Sutiasih.

REFERENCES

1. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS, Caprio S. Obesity and the Metabolic Syndrome in Children and Adolescents, *N Engl J Med* 2004;350:2362-74.

2. Feng Chu N, Han Shen M, Min Wu D, Ju Lai C. Relationship between Plasma Adiponectin Levels and Metabolic Risk Profiles in Taiwanese Children, *Obesity Research* 2005;13(11)2014-20.
3. Mitsnefes M, Kartal J, Khoury P, Daniels S. Adiponectin in Children with Chronic Kidney Disease: Role of Adiposity and Kidney Dysfunction, *Clin J Am Soc Nephrol* 2007;2:46-50.
4. Renaldy O. Peran Adiponektin terhadap Kejadian Resistensi Insulin pada Sindrom Metabolik, *Medicinus* 2009;22(1):65-70.
5. Schoppen S, Riestra P, Garcia-Anguita A, Lopez-Simon L, Cano B, de Oya I, de Oya M, Garces C. Leptin and Adiponectin Levels in Pubertal Children: Relationship with Anthropometric Variables and Body Composition, *Clin Chem Lab Med* 2010;48(5):707-11.
6. Sood A. Obesity, Adipokines, and Lung Disease, *J. App. Physiol* 2010; 108(3): 744-53.
7. Shore SA. Obesity, Airway Hyperresponsiveness, and Inflamation. *J.Appl Physiol* 2010;108(3):735-43.
8. Von Andrian UH dan Mackay CR. T-Cell Function and Migration dalam Mackay IR, Rosen FS, *Advances in Immunology*, *N Engl J Med* 2000;343(14):1020-43.
9. Ulbrich H, Erikson EE, Lindbom L. Leukocyte and Endothelial cell adhesion molecules as targets for therapeutic interventions in inflammatorry disease, *TRENDS in Pharmacological Sciences* 2003;24(12:640-7.
10. Kluger MS. Vascular Endothelial Cell Adhesion and Signaling during Leukocyte Recruitment, *Adv. Derm* 2004;20:163-176.
11. Baratawidjaja KG dan Rengganis I, 2009. *Imunologi Dasar, Edisi VIII*, Balai Penerbit, FKUI, Jakarta, h. 257-283.
12. Appanna R, Huat TL, See LLC, Tan PL, Vadivelu J, Devi S. Cross-Reactive T-Cell Responses to the Nonstructural Regions of Dengue Viruses among Dengue Fever and Dengue Hemorrhagic Fever Patients in Malaysia. *Clin and Vac Immun* 2007;14(8):969-77.
13. Dong T, Moran E, Chau NV, Simmons C, Luhn K, Peng Y, Wills B, Dung NP, Thao LTT, Hien TT, McMichael A, Farrar J, Jones SR. High Pro-Inflamatory Cytokine Secretion and Loss of High Avidiy Cross-reactive Cytotoxic T-Cells during the Course of Secondary Dengue Virus Infection. *Plos One* 2007;12: e1192.
14. Trivino NH, Salgado DM, Rodriguez JA, Bosch I, Castellanos JE. Levels of Soluble ST2 in Serum associated with Severity of Dengue due to Tumour Necrosis Factor Alpha Stimulation. *J. Gen. Virol* 2010;91:697-706.
15. Anderson R, Wang S, Osiowy C, Issekutz A. Activation of Endothelial Cells via Antibody Enhanced Dengue Virus Infection of Peripheral Blood Monocytes, *J.Virol* 1997;71: 4226-32.
16. Whalen MJ, Doughty LA, Carlos TM, Wisnewski SR, Kochanek PM, Carcillo JA. Intercellular Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1 are Increased in Plasma of Children with Sepsis-induced Multiple Organ Failure, *Crit Care Med* 2000; 28:2600-7.
17. Wu, Lio, Lin. Evaluation of Protective Efficacy and Immune Mechanisms of Using a Non-structural Protein NS1 in DNA Vaccine Against Dengue Virus in Mice, *Vaccine* 2003; 21: 3919-29.
18. Srikiatkhachorn A. Plasma Leakage in Dengue Haemorrhagic Fever, *Thromb Haemost* 2009;102: 1042-9.
19. Pichainarong N, Mongkalangoon N, Katayanarooj S. Chaveepojnkamjorn W, Relationship between body size and severity of dengue hemorrhagic fever among children aged 0-14 years, *Southlast Asian J Trop Med Public Health* 2006;3(2):283-8.
20. Lagathu C, Yvan-Charvet L, Bastard JP, Maachi M, Quignard-Boulange A, Capeau J, Caron M. Long-term Treatment with Interleukin-1β Induces Insulin Resistance in Murine and Human Adipocytes, *Diabetologia* 2006;49:2162-73.
21. Saltevo J, Vanhala M, Kautiainen H, Laakso M. Levels of Adiponectin, C-reactive Protein and Inteleukin-1 Receptor Antagonist are Associated with the Relative Change in Body Mass Index between Childhood and Adulthood, *Diabetes Vasc Dis Res* 2007;4:328-31.
22. Luo Y, Liu M. Adiponectin: a versatile player of innate immunity. Journal of Molecular Cell Biology. 2016;8(2):120-128.
23. Procaccini C, De Rosa V, Galgani M, et al. Role of Adipokines Signaling in the Modulation of T Cells Function. Frontiers in Immunology. 2013;4:332.
24. Medoff BD, Okamoto Y, Leyton P, et al. Adiponectin Deficiency Increases Allergic Airway Inflammation and Pulmonary Vascular Remodeling. American Journal of Respiratory Cell and Molecular Biology. 2009;41(4):397-406.
25. Chedid P., Hurtado-Nedelec M., Marion-Gaber B. et al. Adiponectin and its globular fragment differentially modulate the oxidative burst of primary human phagocytes. Am. J. Pathol 2012;180:682–692