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Original Article

Serum zinc levels and clinical severity of dengue infection in children

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Abstract

Background Immunopathogenesis of dengue infection reveals the aberrant immune response. Zinc deficiency alters immune response and therefore may associated with clinical severity of dengue infection.

Objective To indentify the relationship between serum zinc levels and clinical severity of dengue infection in children.

Methods A comparative study was conducted at the Department of Child Health Hasan Sadikin Hospital Bandung, from February to March 2007. We included children aged ≤ 14 years fulfilled the clinical criteria for dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) according to WHO (1997), confirmed with serologic test. Subjects were selected consecutively until met the sample size for each group. Serum zinc level were measured with atomic absorption spectroscopy (AAS) on admission. Data were analyzed using Kruskal-Wallis and Pearson chi square test. Significance was considered if $P<0.05$.

Results The serum zinc levels were low in 47 (78.3%) children. The serum zinc level in DF, DHF, and DSS subjects were 56-81 ($X \pm SD = 68.2 \pm 8.3$) μg/dL; 50-77 ($X \pm SD = 61.6 \pm 8.7$) μg/dL; and 35-52 ($X \pm SD = 42.7 \pm 5.4$) μg/dL, respectively ($P<0.001$). The prevalence ratio of DF to DHF and DHF to DSS were 1.444 ($P=0.311$) and 3.353 ($P=0.077$), respectively.

Conclusion Low serum zinc level were significantly different in each clinical severity of dengue infection. However, low serum zinc level was not a risk factor for the development of severe dengue infection in children. **[Paediatr Indones. 2009;49:309-14]**.

Keywords: *serum zinc levels, dengue infection, clinical severity*

Dengue infection is an immediate problem and it has become a leading cause of child mortality in South and Southeast Asia and Central and South America.[1] The World Health Organization (WHO) estimates that more than 2.5 billion people are at risk of dengue infection.[2] Of an estimated 500,000 cases of DHF/DSS requiring hospitalization each year, roughly 5% die according to WHO statistics, although case fatality could be twice as high.[1,3,4] The disease mostly affected children, and 95 percent of cases occured in the age group below 15 years.[1-6]

The pathogenesis of severe dengue is not well understood. Various mechanisms have been suggested to explain the pathogenesis of severe dengue, including antibody-dependent enhancement or ADE, complement activation by virus-antibody complexes, T-cell mediated immunopathology, and differences in virulence of viral genotypes.[4-10]

Serum zinc levels have been studied more recently to be correlated with severity of dengue infection. Widagdo[11] found the mean serum zinc

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levels was lower than normal, but there was no association between serum zinc levels and severity of the disease. However, in that study, the numbers of subjects for each group were not proportional. Zinc is essential for life with its involvement in immune function, including the skin barrier, gene regulation within lymphocytes, and normal development and function of cells mediating nonspecific immunity, such as neutrophils and natural killer cells. Zinc deficiency can result in impairment of the immune system and may increase the risk of morbidity and mortality due to infection.[12-14]

The aim of this study was to indentify the relationship between serum zinc levels and clinical severity of dengue infection in children.

Methods

We conducted a comparative study at the Department of Child Health Hasan Sadikin Hospital Bandung, from February to March 2007. The subjects in the study were children aged ≤ 14 years admitted to the Department of Child Health Hasan Sadikin Hospital Bandung who fulfilled the clinical criteria for DF, DHF, or DSS according to WHO (1997), confirmed with serologic test. Subjects were selected consecutively until met the sample size for each group (20 subjects). The exclusion criteria were severe malnutrition or obesity, had been diagnosed of other illness besides dengue infection during hospitalisation, had been taking immunosuppresion therapy or zinc suplementation for two weeks before admission.

The serologic test was taken with rapid test dengue blot using Panbio dengue duo cassette kit. Primary infection if the result was negative IgM and IgG, secondary infection if the result was positive IgG with positive or negative IgM. Serum zinc level were measured with atomic absorption spectroscopy (AAS) on admission, with normal level was 70-90 μg/dL.

The patients were categorized into DF and severe dengue infection (DHF/DSS). Data was analyzed using Kruskal-Wallis and Pearson chi square test. Significance were considered if $P<0.05$. All statistic analyse was performed using SPSS version 15.0 for Windows 2007, SPSS Inc, Chicago-Illinois, USA.

This study was approved by Ethics Committee of Medical Faculty of Padjadjaran University, Hasan Sadikin Hospital, Bandung. Written consent was obtained from the parents for each patient before enrolling in the study.

Results

The characteristics of the subjects and clinical manifestations of both groups are shown in **Table 1** and **Table 2**, respectively.

Table 3 shows that 92.5% children who suffered severe dengue infection (DHF/DSS) revealed secondary infection compared to 65% children who suffered mild dengue infection (DF) ($P<0.05$). The elevated hemoglobin and hematocrit were found in DHF and DSS ($P<0.05$). Leukopenia was found in 41 children (68.3%). The serum zinc levels was low in 47 children (78.3%). The serum zinc level in DF, DHF, and DSS subjects were 56-81 ($X \pm SD = 68.2 \pm 8.3$) μg/dL; 50-77 ($X \pm SD = 61.6 \pm 8.7$) μg/dL; and 35-52 ($X \pm SD = 42.7 \pm 5.4$) μg/dL, respectively ($P<0.001$).

Mann-Whitney analysis as seen in **Table 4** revealed the comparison of serum zinc level for each clinical severity was significantly different. However, the prevalence ratio of DF to DHF and DHF to DSS **(Table 5 and Table 6)** were 1.444 (P=0.311) and 3.353 (P=0.077), respectively.

Discussion

We found no differences in age distributions, sex and length of fever among the clinical severity of dengue infection ($P<0.05$). Hung et al[15] and Taweepong and Supapan[16] found no association between sex or age and the severity of the disease. However, Hammond et al[17] and Junia et al[18] found that children between five and nine year old have higher risk for severe dengue infection. In this study, fifteen (70%) children in DHF had normal nutrition. Thisyakorn and Nimmannitya[19] found that most patients with DHF were not undernourished.

Upper respiratory symptoms such as cough and injected pharynx was found in 15% subjects. Although upper respiratory symptoms were not included in

the diagnostic criteria of dengue infection, but some DHF patients may complain of sore throat, and an injected pharynx may be found in examination.[2,3] Hepatomegaly was mostly found in DSS and DHF. Narayanan et al[20] in a study of 59 DHF cases found hepatomegaly in 31 cases (52.5%) and elevated liver enzymes in more than 60% of cases. The elevated hemoglobin and hematocrit were found in DHF and DSS ($P<0.05$). Taweepong and Supapan[16] found the risk for DHF was an increase in hemoconcentration of more than 20% from baseline.

We found that children who suffered severe dengue infection (DHF/DSS) revealed secondary infection compared to children who suffered mild dengue infection (DF). Tantracheewathorn and Tantracheewathorn[21] and Hammond et al[17] found secondary infection was a risk factor for severe dengue infection. Secondary infections have been shown to lead to higher viral loads and the manifestations of severe dengue are believed to be due to virus replication which induces infected monocytes to release vasoactive mediators.[4,7,8] This observation can

Table 1. Characteristics of the subjects

	Clinical severity		
	DF (n=20)	DHF (n=20)	DSS (n=20)
Sex			
Male	13	9	11
Female	7	11	9
Age (years)			
0-4	9	7	5
5-9	6	7	10
10-14	5	6	5
Nutritional status			
Normal nutrition	7	15	8
Under nutrition	13	5	12
Length of fever (day)			
2-3	1	0	0
4-5	14	12	6
6-7	5	8	14

Note: * = Pearson's chi square test (χ^2)

Table 2. Clinical manifestations of the subjects

	Clinical severity		
	DF (n=20)	DHF (n=20)	DSS (n=20)
URTI			
Negative	18	15	18
Positive	2	5	2
Spontaneous Bleeding			
Negative	17	17	14
Positive	3	3	6
Epigastric pain			
Negative	5	4	0
Positive	15	16	20
Hepatomegaly			
Negative		11	6
Positive	6	9	14
Rumple Leede			
Negative	6	8	10
Positive	14	12	10

Notes: * = Pearson's chi square test (χ^2)
URTI = Upper respiratory tract infection

Table 3. Laboratory findings of the subjects

	Clinical Severity			*P* value
	DF (n=20)	DHF (n=20)	DSS (n=20)	
Type of Infection (IgG/IgM)				0.012*
Secondary / IgG(+)	13	17	20	
Primary / IgG(-)	7 (35%)	3 (15%)	0	
Hemoglobin				
Mean (SD)	12 (1.5)	13.9 (2.2)	14.4 (1.4)	<0.001***
Median	11.7	14.1	14.5	
Interval	9.5 – 14.8	9.8 – 17.7	11.7 – 17.1	
Hematocrit				
Mean (SD)	35.4 (4.2)	40.9 (5.1)	43 (4.8)	<0.001**
Median	34	42	43	
Interval	30 – 43	30 – 49	34 – 52	
Leukocyte count				
Decrease	17	14	10	0.088**
Normal	3	5	10	
Increase	0	1	0	
Serum zinc level (µg/dL)				
Mean (SD)	68.2 (8.3)	61.6 (8.7)	42.7 (5.4)	<0.001**
Median	68	59	41	
Interval	56-81	50-77	35-52	

Notes:
*= Pearson's chi square test (χ^2)
**= Kruskal-Wallis' chi square test (χ^2_{KW})
*** = analysis of variance/ANOVA (*F test*)
SD=Standard deviation

Table 4. Comparison of serum zinc level for each clinical spectrum

Clinical Severity		Differentiation of Serum Zinc level	Significancy
Comparing	Compared		
DF	DHF	6.95	0.009
DF	DSS	23.65	0.000
DHF	DSS	16.7	0.000

Table 5. The prevalence ratio of serum zinc level for DHF

Serum zinc level	Clinical Severity		Prevalence ratio (95% CI)	*P* value*
	DF	DHF		
Decrease	12	15	1.444	0.311
Normal	8	5	(0.672-3.107)	

Note: *= Pearson chi square test (χ^2)

Table 6. The prevalence ratio of serum zinc level for DSS

Serum zinc level	Clinical Severity		Prevalence ratio (95% CI)	*P* value*
	DHF	DSS		
Decrease	15	19	3.353	0.077
Normal	5	1	(0.547-20.569)	

Note: *= Pearson chi square test (χ^2)

be explained by the theory of immune enhancement. Cross-reactive, nonneutralizing antibodies from a previous heterologous dengue virus infection bind to the new infecting serotype and facilitate virus entry via Fc-receptor-bearing cells. This mechanism can serve to increase the number of antigen-presenting cells infected during secondary dengue, which can lead to the activation of preexisting cross-reactive dengue virus-specific T lymphocytes from the primary flavivirus infection. This self-amplifying cascade can then lead to the release of cytokines and chemical mediators that may cause plasma leakage.[22]

Zinc is crucial for normal development and function of cells mediating nonspecific immunity such as neutrophils and natural killer cells. Zinc deficiency also affects the development of acquired immunity by preventing both the outgrowth and certain functions of T lymphocytes such as activation, Th1 cytokine production, and B lymphocyte help. The macrophage, a pivotal cell in many immunologic functions, is adversely affected by zinc deficiency, which can dysregulate intracellular killing, cytokine production, and phagocytosis. The effects of zinc on

these key immunologic mediators is rooted in the myriad roles for zinc in basic cellular functions such as DNA replication, RNA transcription, cell division, and cell activation. Apoptosis is potentiated by zinc deficiency. Decreased CD4+/CD8+ cell ratios are also seen. Recent studies in an experimental human model showed that the percentage of CD8+CD73+ T lymphocytes (precursors to cytotoxic T lymphocytes) that required for antigen recognition, proliferation, and cytolysis, was decreased in zinc deficiency. Zinc deficiency may be accompanied by an imbalance of Th1 cell and Th2 cell function, resulting in dysregulated resistance to infection.[14,23-26]

This study revealed that serum zinc levels were significantly different in each clinical severity. However, serum zinc level was not a risk factor for the development of severe dengue infection.

It was concluded that low serum zinc level were significantly different in each clinical severity of dengue infection. However, low serum zinc level was not a risk factor for the development of severe dengue infection in children. Furthermore, the results of our analysis emphasize the need for additional data collection pertaining to zinc in larger studies, especially those in which study subjects are stratified according to baseline nutritional status.

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