BLOOD ZINC LEVELS AND CLINICAL SEVERITY OF DENGUE HEMORRHAGIC FEVER IN CHILDREN

Widagdo

Department of Child Health, Faculty of Medicine, Trisakti University, Jakarta, Indonesia

Abstract. The aims of the study were to investigate the relationship between blood zinc levels and the severity of DHF. A cross-sectional study was conducted involving 51 children at Budhi Asih Hospital, Jakarta, from March to May, 2005. Blood zinc levels were measured with a colorimeter using 5-BR-PAPS. The diagnosis and grading of DHF was determined according to WHO criteria. On analyzing the data, *z* test, ANNOVA, chi-square, linear regression and coefficient of correlation, were applied appropriately with $p \leq 0.05$. Forty-five samples were obtained consisting of 15 boys and 30 girls, age 75±35 months, body mass index of 15.1±3.2, pre-admission fever for 4.4±1.6 days, a hematocrit of 38.4±5.1%, a thrombocyte count of 77,200±32,100/dl, and a zinc level of 6.4±6.3 μmol/l, of with 34 had low and 11 were high zinc level cases (cutoff point: 9.18 μmol/l). The clinical severity in the low and high zinc groups were not significantly different. The blood zinc level in DHF grade 4 was not significantly lower than in the other three grades ($p>0.05$). The numbers of lymphocytes in the four grades were significantly different ($p<0.01$), between the low and high zinc groups ($p<0.05$). The association between lymphocytes and zinc levels showed a linear regression of Yc: 42.04+1.30X, $p<0.05$ and a coefficient correlation of *r*: 0.47. Therefore, no association was found between zinc levels and clinical severity of DHF in children.

INTRODUCTION

DHF is a major problem in Indonesia. It was first reported when the outbreaks occurred in Jakarta and Surabaya. The disease then spread to the other provinces of the country, reaching maximal incidence of 44,573 cases with 1,527 deaths in 1988. With considerable effort to control the vectors the incidence has decreased to a level of around 9.7/100,000. In 1997 there were 30,730 cases with 681 deaths (Prasittisuk *et al*, 1998).

Dengue is an acute infection caused by any of 4 serotypes of dengue virus. The clinical presentations include: dengue fever, dengue hemorrhagic fever and dengue shock syndrome. The pathogenic effects of dengue can result in death due to shock from extravascular fluid leakage and/or massive hemorrhage (WHO, 1999).

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 (Shankar and Prasad, 1998). Zinc deficiency can result in impairment of the immune system and may increase susceptibility to infection (Black, 2003; Fischer Walker and Black, 2004; OSU, 2007). Zinc supplementation has been shown to reduce the incidence (Sazawal *et al*, 1997; Widagdo *et al*, 2001) and diminish the severity of infections (Black, 1998). The aim of this study was to investigate the relationship between blood zinc levels and the clinical severity of DHF.

Correspondence: Widagdo, Department of Child Health, Faculty of Medicine, Trisakti University, 140 Kiyai Tapa, Grogol, Jakarta 11440, Indonesia.
Tel: 62 21 565 5786; Fax: 62 21 530 1688
E-mail: widiant@centrin.net.id

MATERIALS AND METHODS

Research design

We carried out a cross-sectional study at the Department of Child Health, Budhi Asih Provincial General Hospital, Eastern Jakarta from March to May 2005.

Population and sample

The subjects in the study were children admitted to the Department of Child Health Budhi Asih Hospital with a diagnosis of DHF and gave informed consent to participate in the study.

Data collection

For the study, the data were collected through (1) patient particulars and history, including name, age, gender and symptoms such as fever; (2) physical finding, including patient weight, height, and body mass index, temperature, pulse rate, blood pressure, bleeding manifestations, tourniquet test, and liver enlargement; and (3) laboratory examinations, consisting of hemoglobin, hematocrit, platelet count, white blood cell count, serum zinc level, and serology for dengue virus. The clinical diagnosis and grading of severity were determined according to WHO criteria (1999).

Inclusion and exclusion criteria

The patients were included in the study if they fulfilled the clinical criteria for DHF and had a measurement of a serum zinc level. Failure to meet these criteria resulted in exclusion from the study.

Zinc measurement

For the purpose of zinc measurement a blood specimen was taken from a peripheral vein on the day of admission before receiving treatment. The specimens were directly transferred to the Laboratory Division of Budh Asih General Hospital. The serum zinc level was measured with a Cobas Mira Plus Colorimeter (1997) using 5-BR-PAPS solution.

Statistical analysis

Data were collected and recorded on the study form. Stratification was made according to the type of variable, then means and standard deviations were calculated. The patients were categorized into grades 1 to 4, and into two groups of low and high blood zinc levels, with 9.18 μmol/l as the cut-off point (Sazawal *et al*, 1997). The statistical formulas for the *z* test, analysis of variance (*F* test), chi-square, linear regression, and coefficient of correlation were applied with the significant level set at $p \leq 0.05$ (Sanders, 1990), and the computations were performed using of Windows Excel Microsoft XP Professional, copyright 1998-2001.

Ethical clearance

The study was approved by the Committee for Research Ethics at the Faculty of Medicine, Trisakti University. Written consent was obtained from the mothers for each patient before enrolling in the study.

RESULTS

Of 51 participants enrolled in the study, six children were not analyzed because they did not fulfill study criteria. There were 15 boys and 30 girls, 75 ± 35 months, with 1 infant (2%), 18 preschool age children (40%), and 26 school age children (56%). The parents had 11± 2 years of education on everage, with an average monthly income per capita of US$ 37 ± 17. The patients present to hospital with an average length of fever of 4-5 days and symptoms of headache, myalgia, abdominal pain, vomiting, diarrhea, anorexia, cough, runny nose, sore throat, and bleeding. Some patients had seizures or respiratory symptoms. The average body weight was 20 ± 7.4 kg, an average height of 115 ± 19 cm and an average body mass index of 15.1 ± 3.2 kg/m^2, slightly lower than the average for age, with an average nutritional status of being under-nutrition (mild, moderate, or severe) in 20 children (44%), normal nutrition in 21 children (47%), and over-nutrition in 4 children (9%). The other physical

findings included fever, bleeding manifestations, a positive tourniquet test or petechiae, signs of pneumonia or pleural effusion, or hepatomygely. The laboratory results on the first day of hospitalization included an average hemoglobin of 12.9 ± 1.6 g/dl, a hematocrit of 38.4 ± 5.2%, a platelet count of 77,200 ± 32,100 / dl, a leukocyte count of 5,000 ± 3,300 /dl, and a blood zinc level of 6.4 ± 6.3 μmol/l. Table 1 depicts the characteristics of the 45 cases of DHF, of which 29 were grade 1, 12 were grade 2, and 2 cases each were grades 3, and 4. The age distributions, genders and nutrition levels for each of the four grades of DHF were not significantly different (p>0.05), nor were the height, duration of pre-admission fever, respiratory symptoms, liver enlargement, or diarrhea (p>0.05). The distribution of spontaneous bleeding and pleural effusions among each of the four grades were significantly different (p<0.05). The hematology tests on the first day of admission showed hemoglobin levels of 12.5 ± 2.0, 12.9 ± 1.6, 13.1 ± 0.7, and 12.7 ± 0.7 of grades 1, 2, 3, and 4, respectively (p>0.05), and at convalescence the results were 11.9±1.3, 11.2 ± 1.2, 11.3 ± 1.8, and 9.6±0.4, respectively (p>0.05); the hemoglobin changes for each grade were 0.9±1.3, 2.1 ± 1.4, 2.5 ± 0.2, and 2.7 ± 0.2, respectively (p<0.05). The average results of hematocrit on the day of admission were 37.6 ± 5.6, 40.0 ± 4.4, 37.5 ± 4.9, and 40.5 ± 3.5 for grades 1, 2, 3, and 4, respectively (p>0.05), and during recovery were 34.3 ± 3.5, 33.4 ± 3.3, 33.0 ± 7.1, and 27.0 ± 1.4, respectively (p>0.05), with changes of 4.2 ± 4.3, 6.9 ± 5.5, 6.5 ± 2.1, and 13.5 ± 2.1 for

Table 1
The distribution of variables according to the grade of DHF.

Variables	DHF 1 (*N*=29)	DHF 2 (*N*=12)	DHF 3 (*N*=2)	DHF 4 (*N*=2)	Statistical analysis
Gender (M / F)	9 / 20	6 / 6	0 / 2	0 / 2	χ^2: 3.6; p>0.05
Age (mo)	73±33	79±41	80±57	71±50	*F*: 0.09, p>0.05
Pre-admission fever (d)	4.5±1.1	4.2±1.3	3.5±0.7	3.0±0.1	*F*: 1.66, p>0.05
Temperature on admission (ºC)	37.7±1.0	37.5±1.5	37.4±0.6	37.7±0.8	*F*: 0.15, p>0.05
Nutrition: Over-/Normal-/Under-	2 /13 /14	01/05/2006	0 / 2 / 0	01/01/2000	χ^2: 7.3; p> 0.50
BMI (kg/m^2)	15.2±3.0	14.5±3.0	14.4±4.0	18.8±7.2	*F*: 0.19, p>0.05
Spontaneous bleeding	0	12	2	2	χ^2: 44.9; p< 0.01
Upper respiratory infection	18	6	0	0	χ^2: 5.5; p> 0.10
Pneumonia	1	1	0	0	χ^2: 0.7; p> 0.90
Pleural effusion	0	0	1	1	χ^2: 21.2; p< 0.01
Hepatomegaly	15	6	0	0	χ^2: 3.8; p> 0.50
Diarrhea	3	1	0	1	χ^2: 3.5; p> 0.25
Hemoglobin (g/dl)	12.7±1.7	13.3±1.4	13.7 ± 1.6	12.2±0.1	*F*: 2.33, p>0.05
Hb changes (%)	0.9±1.3	2.15±1.4	2.5±2.2	2.7±0.2	*F*: 3.97, p<0.05
Hematocrit (%)	38.3±4.8	40.3±4.4	39.5±4.9	40.5±3.5	*F*: 0.59, p>0.05
Ht changes (%)	4.2±4.3	6.9±5.5	6.5±2.1	13.5±2.1	*F*: 4.44, p<0.05
Plateletes (000/dl)	79.7±31.6	75.0±36.5	81.0±25.5	49.5±16.3	*F*: 0.57, p>0.05
WBC (000/dl)	5.4±3.8	4.9±2.4	2.8±1.0	2.9±0.2	*F*: 0.67, p>0.05
Lymphocytes (%)	49.4±15.5	49.3±16.3	55.0±24.0	37.5±17.7	*F*: 10.40, p<0.01
Neutrophils (%)	41.9±19.4	48.0±18.4	38.5±20.5	53.5±19.1	*F*: 3.04, p>0.05
Zinc level (μmol/l)	6.1±6.6	6.9±6.4	12.3±4.8	1.2±0.2	*F*: 1.05, p>0.05
Hospital stay (d)	3.9±1.0	4.2±1.4	4.5±0.7	5.0±5.7	*F*: 0.55; p>0.05

The value is presented in mean ± SD or in number

Table 2
The distribution of DHF variables according to zinc level group.

Variables	Low zinc group (*N*=34)	High zinc group (*N*=11)	Statistical analysis
Zinc level (μmol/l)	2.68 ± 2.38	14.55 ± 4.68	*z*: -9.92; p<0.05
DHF grade 1/2/3/4	23/8/1/2	6/4/1/0	χ^2: 0.74; p>0.25
Sex (M / F)	9 / 6	25 / 5	χ^2: 2.94; p>0.05
Age (mo)	77.6 ± 32.7	65.0 ± 42.5	*z*: 0.90; p>0.05
Pre-admission fever (d)	4.6 ± 1.7	3.9 ± 0.8	*z*: 1.75; p>0.05
Body temperature (ºC)	37.4 ± 1.1	37.8 ± 1.3	*z*: -0.63; p>0.05
BMI	15.0 ± 3.2	15.6 ± 3.3	*z*: -0.48; p>0.05
Nutrition:Over-/Normal-/Under-	2/6/6	2/5/4	χ^2: 1.62; p>0.5
Spontaneous bleeding	11	5	χ^2: 0.62; p>0.25
Upper respiratory infection	18	6	χ^2: 0.1; p>0.5
Pneumonia	0	2	χ^2: 0.5; p>0.25
Pleural effusion	2	0	χ^2: 0.7; p>0.25
Hepatomegaly	16	5	χ^2: 0.1; p>0.5
Diarrhea	3	2	χ^2: 0.7; p>0.25
Hemoglobin (g/dl)	12.9 ± 1.7	11.7 ± 1.9	*z*: 2.2; p>0.05
Hematocrit (%)	39.2 ± 4.9	35.6 ± 5.5	*z*: 1.95; p>0.05
Platelets (/dl)	77,100 ± 34,800	72,700 ± 30,700	*z*: 0.54; p>0.05
Leukocytes (000/dl)	4.8 ± 3.4	5.7 ± 3.2	*z*: -0.77; p>0.05
Lymphocytes (%)	45.1 ± 14.2	66.3 ± 15.3	*z*: -4.08; p<0.05
Neutrophils (%)	46.9 ± 18.4	31.3 ± 16.3	*z*: 1.47: p>0.05
Length of admission	3.8 ± 1.4	4.6 ± 1.4	*z*: -1.72; p>0.05

The value is presented in mean ± SD or in number

grades 1, 2, 3, and 4, respectively (p<0.05). The number of leukocytes and neutrophiles for each of the 4 grades were not significantly different, but the lymphocyte count was significantly lower in grade 4 (p<0.01). The average platelet count was lower in grade 4 (p>0.05). The average blood zinc level was lower in grade 4, but the difference was not significant (p>0.05).

Table 2 shows that 34 cases had zinc levels less 9.18 mol/l, the cutoff point, and 11 cases had zinc levels of 9.18 mol/l or higher. The distribution among the four grades of DHF for the low and high levels of zinc was not significant different (p>0.25). The average number of lymphocytes in the high zinc level group was significantly higher than in the low zinc group (p<0.05), and the relationship between the blood zinc level and the number of lymphocytes is expressed by the regression slope of Yc = 42.0 + 1.3X with p <0.05 with a coefficient of correlation of *r* 0.47.

DISCUSSION

We found no differences in age, gender, or nutrition among the four grades of DHF (p>0.05). Hung *et al* (2005), and Taweepong and Supapan (2007) found no association between sex, age or nutritional status and the severity of the disease, Pichainarong *et al* (2006) and Kalayanarooj and Nimmannitya (2005) found that obesity and malnourishment in children were related to severity of DHF, and Thisyakorn and Nimmannitya (1993) found that most patients with DHF were not undernourished.

The mean duration of pre-admission fever in this series was 4.3 ± 1.1 days; grades 3 and 4 had shorter durations than grades 1 and 2 ($p>0.05$). Narayanan *et al* (2002) recorded 4.9 ± 2.3 days of preadmission fever and found no significant differences among the grades of dengue ($p=0.26$). Respiratory symptoms and diarrhea for each of the 4 DHF grades were not significantly different ($p>0.05$).

Hemorrhagic tendencies demonstrated by a positive Rumple-Leed test were found in all patients in group 1. Spontaneous bleeding was found in all patients in grades 2, 3, and 4 and none in grade 1 ($p<0.05$). Taweepong and Supapan (2007) found bleeding as a risk factor with the severity of DHF.

Pleural effusion was found in 2 cases, one cases each in grades 3 and 4 ($p<0.05$). The effusions were detected on physical and chest X-ray. Ultrasonography can diagnose pleural effusion more sensitively (Venkata Sai *et al*, 2005; Srikiatkachorn *et al* 2007; Rojanakarin *et al*, 2008).

Hepatomegaly was found in 21 patients (47%) with grades 1 and 2, and none with grades 3 and 4. Narayanan *et al* (2002), in a study of 59 DHF cases found hepatomegaly in 31 cases (52.5%) and elevated liver enzymes in more than 60% of cases. With the use of ultrasound Venkata Sai *et al* (2005) found hepatomegaly in 21% of DHF cases.

On admission the hemoglobin level in grade 4 was slightly lower than grade 3, but the difference was not significant ($p>0.05$), while during convalescence the hemoglobin levels in all grades decreased and in grade 4 was the lowest ($p>0.05$), but the difference was not significant. The drop in hemoglobin was higher for grade 4 than for grades 1, 2, and 3 ($p<0.05$). The hematocrit levels on admission were higher in grade 4 than in other grades ($p>0.05$), but not significantly. Assessment during recovery revealed a non-significant drop in hematocrit for each grade ($p>0.05$), however, the average change from the initial hematocrit for each of the four grades were significantly different ($p<0.05$). Taweepong and Supapan (2007) found the risk for DHF was an increase in hemoconcentration of more than 20% from base line. Serial hemoglobin levels for all grades were low, and grade 4 was the lowest ($p<0.05$). Narayanan *et al* (2002) reported that most of 59 cases with dengue were anemic, and only two children had a hematocrit level more than 40%. A fall in hematocrit of more than 20% on treatment was found in 14 cases. The average number of leukocytes and neutrophils in grades 3 and 4 were not significantly lower than in grades 1 and 2 ($p>0.05$), but the lymphocytes were significantly lower in grade 4 ($p<0.01$). Green *et al* (1999) found the mean white blood cell count in children with DHF was lower than in those with DF, and both were lower than the controls ($p<0.001$), while the mean number of absolute lymphocytes was lower in DHF than in DF ($p<0.05$), and both were lower than the controls ($p<0.001$). Myint *et al* (2006) found that peripheral blood mononuclear cell (PBMC) apoptosis was related to the severity of DHF.

The number of thrombocytes was not significantly lower in grade 4 than in grades 1 to 3 ($p>0.05$). The platelet count was less than 100,000/dl in 35 cases (78%), and more than 100,000/dl in 10 cases (22%). Narayanan *et al* (2002) found a platelet count of more than 100,000/dl in 16 out of 59 cases (27%).

The mean blood zinc level for all cases was 6.4 mol/l, lower than the cutoff level. The mean zinc level in grade 4 was not significantly lower than in grades 1, 2, and 3 ($p>0.05$). Thirty-four cases (76%) had a low zinc level and 11 (24%) had a high zinc level. This may be due to the low nutritional status found in 20 cases (44%) low intake of zinc due to poor education or low socio economic status of the parents, or possibly due to infection since many suffer from diarrhea (Heese *et al*, 1985; OSU, 2007). The number of lymphocytes was

significantly higher in the high zinc group than the low zinc group ($p<0.05$). The relationship between the number of lymphocytes and the blood zinc level was a regression line defined by Yc = 42.0+1.3 X with $p<0.05$ and $r=0.47$. This finding is consistent with the role of zinc in the immune process, especially in the development of lymphocytes that can occur in cases with high zinc levels (Raqib *et al*, 2004; OSU, 2007; Prasad, 2007).

Besides risk factors related to the severity of DHF, such as bleeding, increased hematocrit or pleural effusion, other studies have recorded that a secondary infection, particularly with dengue virus type 2, is a risk factor for severe DHF (Sangkawibha *et al*, 1984; Thein *et al*, 1997; Vaughn *et al*, 2000; Pichainarong *et al*, 2006). A high viral titer is also a risk factor for severe DHF (Vaughn *et al*, 1997, 2000). Gubler *et al* (1997) found no relationship between dengue serotype and severity of disease, however, three fatal cases were associated with dengue serotype 3 infections. The severity of DHF is related to increased levels of TNF-alpha (Braga *et al*, 2001), elevated IL6 and IL10, serum macrophage migration inhibitory factor (Chen *et al*, 2006), increased T cells, cytokines and cross reactive antibodies (Fink *et al*, 2006), peripheral blood mononuclear cell (PBMC) apoptosis, and soluble CD95 (Myint *et al*, 2006).

Significantly lower numbers of lymphocytes were seen in grade 4 ($p<0.05$) but there was no significant link with zinc levels and grade 4. However, there was a significant correlation between blood zinc levels and the number of lymphocytes ($r=0.4$, $p<0.05$). No significant associations were seen between zinc levels and clinical severity in children with DHF.

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