ROLE OF COMBINED ZINC, VITAMIN A, AND FISH OIL SUPPLEMENTATION IN CHILDHOOD TUBERCULOSIS

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Abstract. This objective of this study was to determine benefit of one month combined supplementation (zinc, vitamin A, fish oil) along with anti-tuberculosis drugs (ATD) on increasing serum leptin levels and decreasing tumor necrosis factor-α (TNF-α) in children with tuberculosis (TB). A quasi experimental study was conducted on 22 children (aged 5-14 years) with a positive acid-fast bacilli (AFB) smear. The children were divided into 2 groups. A history, physical examination, anthropometric measurements, serum leptin levels, TNF-α levels, retinol and zinc levels were examined in all subjects before and after treatment. Nutritional supplementation and ATD were given to group I while ATD only were given to group II. The change in leptin, TNF-α, retinol and zinc levels were analyzed with the Mann-Whitney test, while a *t*-test was used to determine changes in body mass index (BMI). Group I had a higher significant increase in serum leptin levels than group II (p=0.034). Group I had a significantly greater decrease in TNF-a levels than group II (p=0.032). No significant differences in retinol or zinc levels were seen between the two, but both groups had an increase after treatment. Both groups had a significant increase in BMI (p=<0.001) post-treatment compared to pre-treatment. Supplementation with zinc, vitamin A and fish oil is associated with a significant increase in leptin levels and a significant decrease in TNF-α levels among children treated for TB. No significant benefit was seen in BMI among children receiving supplementation compared to those without it, although ATD resulted in a significant increase in BMI in both groups.

Keywords: tuberculosis, zinc, vitamin A, fish oil, leptin, TNER, children

INTRODUCTION

The incidence of childhood tuberculosis (TB) is starting to decline but slowly (less than 1% per year) (Vashishtha, 2009). In 2008, the prevalence of TB in Indonesia was about 229/100,000 population and the mortality was 27/100.000 (Lolekha *et al*, 2008; WHO, 2009); about 10% of the cases were found in children aged <15 years (WHO, 2009). However, the prevalence of childhood TB report may be underestimated (Donald, 2004). Studies of childhood TB are rare.

Malnutrition is common in TB and may contribute to a poorer prognosis; therefore, this also needs appropriate management (Sarraf *et al*, 1997; Zachariah

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et al, 2002). The mechanism of wasting associated with TB unclear (van Crevel *et al*, 2002; Buyukoglan *et al*, 2007) but one study (Mootoo *et al,* 2009) has reported this is due to increased inflammation caused by increasing proinflamatory cytokines such as TNF-α. This results in anorexia, wasting and inadequate production of leptin (Sarraf *et al*, 1997; Dinarello, 2000; van Crevel *et al*, 2002; Mootoo *et al*, 2009). Inadequate leptin production can affect the immune system (Zarkesh *et al*, 2004; Claycombe *et al*, 2008; de Andrade *et al*, 2008). Micronutrients (Ray *et al*, 1998; Karyadi *et al*, 2002; Lettow *et al*, 2004; Range *et al*, 2005; Villamor *et al*, 2008) and fish oil (Rossi *et al*, 2005; Damsgaard *et al*, 2007) are thought to be involved in boosting the immune system though lymphocyte homeostasis. The aim of this study was to determine the effect of providing suppelementation with zinc, vitamin A and fish oil along with anti-tuberculosis drug (ATD) treatment on leptin and TNF-α levels in children with TB.

MATERIALS AND METHODS

This study was conducted from October 2010 to May 2011 at Hasan Sadikin General Hospital, Bandung, Indonesia. Twenty-three children aged 5-14 years were recruited from the outpatient department, the Pediatrics Ward and parents who were AFB positive and attending the direct observed treatment shortcourse (DOTS) ward. All the children had a positive AFB smear obtained from the sputum or gastric lavage. The subjects were randomly divided into two groups, group I was given the supplement [one capsule (350 mg Ω-3) of fish oil, one tablet of vitamin A (1,500 UI), and 10 mg of zinc] for one month and anti-tuberculosis drug (ATD) treatment following WHO guidelines, while group II was only given ATD treatment. Each group consisted of eleven subjects. The parents of each subject gave written informed consent prior to participation. This study was approved by Hasan Sadikin General Hospital.

Children with a history of other chronic diseases, including HIV/AIDS, malignancy, rheumatoid arthritis, liver disease, chronic cardiovascular disease, diabetes mellitus or inflammatory bowel disease were excluded from the study. Anthropometric measurements (weight, height, BMI) and leptin, TNF-α, zinc and retinol levels were obtained at onset and after one month of treatment. Subjects who were non-compliant with the medical regimen for at least one day were excluded from the study.

The leptin and TNF-α levels were measured using an ELISA (Quantikine® and Quantikine HS R&D Systems, Minneapolis, MN). The zinc level was measured using an atomic absorption spectrophotometer (GBC 933 AA) and the retinal level was measured using high performance liquid chromatography (HPLC 515 Pump). The leptin, TNF-α, zinc and retinol levels were evaluated using the Mann-Whitney test while the BMI was evaluated using the *t*-test. Statistical significance was set a *p*-value of <0.05. Analyses were performed using SPSS version 16.0 for windows (SPSS, Chicago, IL).

RESULTS

In this study of 23 subjects, 1 patient was lost to follow-up. Of the 22 remaining subjects, 12 had pulmonary TB, 3 had abdominal TB, 3 had TB lymphadenitis and pulmonary TB, 2 had TB spondylitis and pulmonary TB with femoral abscesses, 1 had scrofuloderma and pulmonary TB

Tabel 1
General characteristics.

Subject characteristics	Subject group	
	Group I[a] (n = 11)	Group II[b] (n = 11)
Age (years)		
Mean (SD)	9.9 (2.7)	9.64 (3.6)
Median	8.0	10.0
Range	5-13	5-14
Gender		
Male	7	7
Female	4	4
BMI (kg/m^2)		
Mean (SD)	12.9 (3.1)	11.8 (2.7)
Median	12.5	11.5
Range	9.5-20.5	7.5-18.5

[a]Supplemented group; [b]Non-supplemented group.

and 1 had milliary TB. There were no significant differences between the 2 study groups in general characteristics (gender, age, and BMI) (Table 1). Eighteen subjects had wasting and the other 4 did not. Of the wasted subjects, 8 were in the supplement group (Group I) and 10 were in the other group (Group II).

The mean baseline leptin concentrations in the two groups were not significantly different from each other (p=0.300). The mean leptin concentrations in both groups increased at the time of second exam (1 month) but were significantly different (p=0.034) from each other (group I : 59.9% increase at 1 month; group II : 17.9% increase) (Table 2).

The baseline TNF-α levels in both groups were not significantly different from each other (Z_{MW} =0.559; p=0.606). After treatment, the mean TNF-α levels decreased in both groups (group I: 22.6%; group II: 6.9%) but the mean TNF-α level in group I was significantly lower than in group II (Table 3).

The mean baseline retinol levels in both groups were not significantly different from each other (p=0.606). After treatment, the mean retinol levels in both groups increased significantly (group I: 79.7%; group II: 83,8%) but they were not significantly defferent from each other (p=0.949) (Table 4).

The mean baseline zinc levels in the two groups were not significantly different from each other (p=0.625). After treatment the mean zinc level in group I had a significant increase (p=0.006) but the mean zinc level in group II did not have a significant increase (p=0.501) (group I: 34.7%; group II: 31.7%) but the post-treatment mean zinc levels were not significantly different from each other (p=0.784) (Table 5).

The mean BMI levels at baseline were not significantly different from each other (p=0.395). Both groups had a significant increase in the mean BMI levels post-treatment (group I: 21.5%; group II: 6.4%) and the two groups post-treatment were significantly different from each other (p<0.001) (Table 6).

Tabel 2
Comparison of leptin levels by group pre- and post- treatment.

Leptin levels (pg/ml)	Subject group		Z_{MW}	*p*-value
	Group I (*n*=11)	Group II (*n*=11)		
Pre-treatment				
Mean (SD)	2,828.5 (3,570.1)	882.3 (306.3)		
Median	950.2	826.4	1.122	0.300
Range	780.0-12,640.5	243.0-1,521.6		
Post-treatment				
Mean (SD)	4,093.9 (4,403.2)	1,040.9 (460.4)		
Median	2,185.0	980.0	2.497	0.010
Range	795.0-15,431.4	252.6-1,996.2		
Pre- *vs* Post-treatment				
Z_W	2.934	1.718		
p-value	0.003	0.086		
Percent increase	59.9%	17.9%	2.102	0.034

SD, standard deviation; Z_W, Wilcoxon test; Z_{MW}, Mann-Whitney test.

Tabel 3
Comparison of TNF-α levels by group pre- and post-treatment.

TNF-α level (pg/ml)	Subject group		Z_{MW}	*p*-value
	Group I (*n*=11)	Group II (*n*=11)		
Pre-treatment				
Mean (SD)	3.7 (1.6)	4.1 (1.5)	0.559	0.606
Median	3.3	4.2		
Range	2.0-6.5	2.4-7.7		
Post-treatment				
Mean (SD)	2.9 (1.2)	3.8 (1.7)	1.512	0.133
Median	2.2	2.9		
Range	1.6-4.6	2.2-7.8		
Pre- *vs* Post-treatment				
Z_W	2.949	1.159		
p-value	0.003	0.247		
Percent decrease	22.6%	6.9%	1.972[a]	0.032

SD, standard deviation; Z_W, Wilcoxon test; Z_{MW}, Mann-Whitney test; [a] = *t*-test.

DISCUSSION

In this study, the mean baseline leptin levels in group II were lower than I; this may be because group II was comprised of 10 wasted subjects (3 with severe malnutrition) and 1 non-wasted subject, while group I was comprised of only 8 wasted subjects (4 with severe malnutrition) and 3 subjects who were not wasted. Low leptin levels in children with TB are due to wasting (loss of body mass) which reduces

Tabel 4
Comparison of retinol level by group pre- and post-treatment.

Retinol level (μmol/l)	Subject group			
	Group I (*n*=11)	Group II (*n*=11)	Z_{MW}	*p*-value
Pre-treatment				
Mean (SD)	0.8 (0.5)	1.3 (2.2)	0.755	0.606
Median	0.8	0.5		
Range	0.3-1.8	0.2-8.0		
Post-tratment				
Mean (SD)	1.2 (0.6)	1.5 (2.4)	1.08	0.300
Median	1.1	0.7		
Range	0.4-2.0	0.4-8.6		
Pre- and post-treament				
Z_W	2.934	0.098		
p-value	0.03	0.949		
Percent increase	79.7%	83.8%	0.098	0.949

SD, standard deviation; Z_W, Wilcoxon test, Z_{MW}, Mann-Whitney test.

Tabel 5
Comparison of zinc levels by group pre- and post-treatment.

Zinc level(μmol/l)	Subject group			
	Group I (*n*=11)	Group II (*n*=11)	*t*	*p*-value
Pre-treatment				
Mean (SD)	10.9 (3.0)	10.1 (5.1)	0.49	0.625
Median	10.1	9.6		
Range	7.2-18.2	0.4-17.7		
Post-treatment				
Mean (SD)	13.9 (2.9)	11.3 (4.9)	1.5	0.153
Median	14.1	12.1		
Range	8.3-18.4	0.9-20.7		
Pre- *vs* post-treatment				
t -test	3.473	0.698		
p-value	0.006	0.501		
Percent increase	34.7%	31.7%	0.328[a]	0.784

SD, standard deviation; [a] Mann-Whitney test.

leptin production (Herlina *et al*, 2011). After treatment, the leptin levels in both groups increased but group I (supplementation group) had a greater increase; therefore, we can conclude supplementation can result in an increase in leptin levels. Our findings support a previous study that found a negative correlation between leptin levels and acute inflammation in adult with TB (Deveci *et al*, 2005; de Andrade *et al*, 2008). Fish oil, rich in Ω-3 fatty acid can raise leptin secretion

Table 6
Comparison of BMI levels by group pre- and post-treatment.

BMI (kg/m^2)	Subject group		t	p-value
	Group I (n=11)	Group II (n=11)		
Pre-treatment				
Mean (SD)	12.9 (3.1)	11.8 (2.8)	0.87	0.395
Median	12.5	11.5		
Range	9.5-20.5	7.5-18.5		
Post-treatment				
Mean (SD)	15.5 (2.9)	12.5 (2.9)	2.39	0.026
Median	15.0	12.0		
Range	12.0-22.5	8.0-19.5		
Pre- *vs* post-treatment				
t-test	19.574	7.216		
p-value	<0.001	<0.001		
Percent increase	21.5%	6.4%	3.842[a]	<0.001

SD, standard deviation; [a]Mann-Whitney test.

and leptin receptor sensitivity through an unclear mechanism (Rossi *et al*, 2005; Damsgaard *et al*, 2007).

Subjects in this study were not categorized by severity, which could give variations in TNF-α levels. Baseline TNF-α levels in both groups were high, but declined significantly with treatment in group I. A decrease in TNF-α levels parallels good outcomes (de Andrade *et al*, 2008).

Malnutrition is a common finding in TB, but the micronutrient status of children with TB is not well documented. Zinc is a micronutrient that plays an important role in lymphocyte homeostasis. (Karyadi *et al*, 2002). Seventeen of 22 subjects in our study had zinc deficiency. After supplementation the mean zinc level increased significantly in the supplemented group, but the percent increase was not significant. We only provided one month of intervention. Reza *et al* (2005) found zinc levels were increased after four months of ATD.

Vitamin A is another micronutrient important for immunity, cell reproduction, and has been proven to impede mycobacterial multiplication in macrophages and proliferating lymphocytes in order to maintain the epithelium layer (Mathur, 2007; Yamada *et al*, 2007). In this study we observed an increasing retinol level in both groups with treatment but this was not significant. This finding is thought to be due to the interaction between zinc and vitamin A, where zinc deficiency can lead to secondary vitamin A deficiency (Mathur, 2007). This postulate had been proved by some studies who found vitamin A levels improved more in subjects given both vitamin A and zinc rather than vitamin A alone (Karyadi *et al*, 2002; Villamor *et al*, 2008).

BMI increased significantly in both groups in this study, also group I had a greater increase than group II which is in agreement with a previous study (Herlina *et al*, 2011). An improvement in the clinical

picture is thought to be responsible for the increase in BMI, as is seen by the decreasing TNF-α level. The increasing BMI also correlates with the increasing zinc levels, similar to another study (Range *et al*, 2005). High TNF-α level are associated with wasting in TB which is also associated with decreasing leptin (Herlina *et al*, 2011). Low leptin levels can indicate a poor prognosis, since leptin is known as an essential factor in cellular immunity against *Mycobacterium tuberculosis*. Giving leptin would be worth considering but leptin recombinant is too costly and has unclear proven benefit. The role of zinc, vitamin A and fish oil in lymphocyte homeostasis and elevation of leptin levels has been studied (Ray *et al*, 1998; Lettow *et al* 2004; Range *et al*, 2005; Rossi *et al*, 2005; Damsgaard *et al*, 2007). Supplementation with zinc and vitamin A have been shown to have beneficial affects in adults with TB, (Karyadi *et al*, 2002) but combined supplementation with zinc, vitamin A and fish oil have never been studied in children with TB.

Macronutrients provided at home can affect the BMI; the short supplementation period in our study (1 month) might not have allowed adequate time to determine the maximum response. Providing three supplements at once limits our ability to determine the individual affect of each of the components. The small sample size in our study, limited by finances, also could have an impact on our results. Further studies of a longer duration and with separate supplement would be beneficial.

In conclusion, this study found nutritional supplementation with zinc, vitamin A and fish oil was associated with elevated leptin levels, an increase in BMI and a decrease in TNF-α levels among children undergoing treatment for TB. The decline in TNF-α levels with clinical improvement suggests this test might also be used to follow TB treatment in children.

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