Association of serum C-reactive protein and leptin levels with wasting in childhood tuberculosis

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ABSTRACT

Introduction: Wasting is a systemic manifestation of tuberculosis (TB) and is often thought to affect the severity and outcome of the disease. Leptin and several cytokines/proteins are thought to play a role in the relationship between TB, nutritional status and host immune response. The aim of this study was to determine the association of C-reactive protein (CRP), an inflammatory response protein and serum leptin levels with wasting in childhood TB.

Methods: A cross-sectional observational analytic study was conducted at two hospitals in West Java from January to March 2010. The subjects were 13 children aged 2–120 months who were infected with TB and 26 healthy children of the same age and gender as the comparison group. History-taking and anthropometric, physical, serum CRP and leptin examinations were conducted for each subject. The association of CRP and serum leptin levels with wasting in childhood TB was studied.

Results: Serum leptin levels were lower (95 percent confidence interval [CI] 314.0–1,228.9 pg/mL, p-value less than 0.001) and serum CRP levels were higher (95 percent CI 16.5–81.1 mg/L) in the subjects than in the comparison group. There were positive correlations between leptin and body mass index (p-value less than 0.001) and between CRP and wasting (p-value less than 0.001), but a negative correlation between leptin and wasting (p-value less than 0.001).

Conclusion: Elevated serum CRP levels and a decrease in serum leptin levels are associated with an increase in wasting in childhood TB.

Keywords: childhood tuberculosis, C-reactive protein, leptin, wasting

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INTRODUCTION

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide, especially in Asia and Africa. Under the surveillance and results of the survey conducted by the World Health Organization (WHO) in 2006, the number of new TB cases worldwide is estimated to be 9.2 million (139/100,000 population), accounting for 1.7 million deaths.[1] An estimated 10% of TB cases occur in children under 15 years of age.[2] Indonesia has the third highest prevalence and incidence of TB in the world (after India and China). In 2006, the prevalence of TB in Indonesia was estimated to be around 578,000 (253/100,000 population), with a mortality of 88,000 per year (38/100,000 population).[1] Wasting (loss of body mass) is a systemic clinical manifestation of TB, and it is often found to affect the severity and outcome of the disease.[3] The cause or pathogenesis of TB-associated wasting is still unclear;[4,5] however, it is thought to be due to microbial products that stimulate proinflammation and cytokine production, which then trigger the host acute phase response, causing anorexia during infection.[6]

Leptin, a 16-kDa protein encoded by the obese gene (ob), is mainly produced by adipocytes. It is known to have a strong positive correlation with body mass index (BMI), which increases when one is overweight and decreases in cases of wasting.[4,5,7] Leptin is thought to be a mediator that is involved in the complex relationship between TB, nutritional status and host immune response; hence, it likely plays an important role in the regulation of food intake, energy expenditure and control of body weight.[5] Leptin regulates appetite and energy expenditure at the level of the hypothalamus by binding to its specific receptors.[8] A study involving healthy human subjects reported that circulating leptin concentrations are proportional to fat mass and decrease during starvation.[9] A study conducted in animals has shown a rise in leptin levels due to inflammatory mediators.[10] Various studies conducted on the functions of leptin have found that leptin not only works to suppress appetite and body weight, but is also a multifunctional hormone. It regulates energy homeostasis and food intake,[11,12] neuroendocrine function,[13] angiogenesis,[14]

Table I. General characteristics of the study subjects.

Characteristic	No. (%)	
	TB patients (n = 13)	Comparison group (n = 26)
Age (yrs)		
≤ 5	4 (31)	8 (31)
> 5–10	9 (69)	18 (69)
Gender		
Male	5 (39)	10 (39)
Female	8 (61)	16 (61)
BMI (kg/m^2)		
Mean ± SD	11.7 ± 1.0	15.9 ± 1.3
Median; range; 95% CI	11.6; 10.4–13.1; 11.0–12.3	15.8; 13.7–18.4; 15.2–16.4
BMI/A		
Normal	0 (0)	26 (100)
Wasting	4 (31)	0 (0)
Severe wasting	9 (69)	0 (0)

TB: tuberculosis; SD: standard deviation; CI: confidence interval; BMI/A: body mass index/age

bone formation,[15] reproduction,[16] haematopoiesis[17] and immunity.[18]

To date, studies concerning circulating levels of leptin in TB have only been conducted in adult TB patients and have shown varying results. Cakir et al found elevated levels of plasma leptin and inflammatory response with a good correlation between these two parameters and thus interpreted that an increase in leptin levels causes a loss of body mass and contributes to the inflammatory process.[19] Several earlier studies had found a negative correlation between leptin levels and proinflammatory response (C-reactive protein [CRP] and tumour necrosis factor [TNF]-α).[4,5,7] Based on these studies, leptin levels in TB may be the result of two opposite mechanisms, namely chronic inflammation of TB that causes a loss of body fat mass, thereby reducing the production of leptin,[4,7] as well as the host's inflammatory responses leading to increased leptin levels, which theoretically would suppress the appetite, causing anorexia and loss of body mass.[4,20] Furthermore, low leptin levels may worsen the prognosis of TB, as leptin is important for cellular immunity, which is essential to fight against *Mycobacterium* (*M.*) *tuberculosis*.[4,5,20] To our knowledge, no data regarding the circulating levels of leptin in childhood TB is yet available. Hence, the aim of this study was to determine the association of CRP, an inflammatory response protein and serum leptin levels with wasting in childhood TB. This could in turn help to predict the prognosis and aid in better management of the disease.

METHODS

A cross-sectional observational analytic study was conducted in Hasan Sadikin General Hospital and Rotinsulu Hospital from January to March 2010. The subjects were 13 consecutive children aged 2–120 months who had TB and who showed positive acid-fast bacilli (AFB) (obtained from direct smear by gastric lavage and/or culture) and 26 healthy children of the same age and gender as the comparison group. History-taking, anthropometric and physical examinations, serum CRP and serum leptin levels were conducted for each subject. Informed consent was obtained from the parents of all subjects, and the study was approved by the Health Research Ethical Committee of the Faculty of Medicine, Padjadjaran University, Bandung, West Java, Indonesia.

Patients found to be suffering (through history-taking and physical examination) from other chronic diseases, such as HIV/AIDS, malignancy, rheumatoid arthritis, chronic liver disease, chronic cardiovascular disease, diabetes mellitus and inflammatory bowel disease, were excluded from the study. All subjects underwent anthropometric measurement (weight, height, BMI), tuberculin test and chest radiography. The subjects were determined to be showing signs of wasting (or wasted) if their BMI for age (BMI/A) was < –2 SD and showing signs of severe wasting (or severely wasted) if their BMI/A was < –3 SD, based on the growth charts developed by the WHO Multicentre Growth Reference Study (WHO-MGRS) 2007.[21]

Serum leptin and CRP levels were obtained from blood samples taken from the cubital vein. The blood was then transferred into a test tube, allowed to settle for 30 minutes to coagulate and subsequently centrifuged at 3,000 rpm for ten minutes to obtain a serum. Serum leptin levels were examined using ELISA (Quantikine®, Minneapolis, MN, USA), while serum CRP levels were checked using the immunoturbidimetric method (Cobas®, Roche, Basel, Switzerland). Serum CRP, leptin and BMI

Table II. Comparison of serum leptin and CRP concentrations in each group.

Variable	TB patients (n = 13)	Comparison group (n = 26)	p-value
Serum leptin (pg/mL)			
Mean ± SD	771.46 ± 756.99	3,792.92 ± 2,763.52	< 0.001*
Median; range; 95% CI	266.00; 156.00–2,169.00; 314.02–1,228.91	2,841.00; 1,538.00–12,588.00; 2,676.71–4,909.13	
Serum CRP (mg/L)			
Mean ± SD	48.81 ± 53.50	1.01 ± 0.72	< 0.001*
Median; range; 95% CI	25.90; 8.20–192.20; 16.48–81.14	0.75; 0.30–2.60; 0.72–1.31	

* p-value is calculated based on Mann-Whitney test.
CRP: C-reactive protein; TB: tuberculosis; SD: standard deviation; CI: confidence interval

(calculated as weight/height^2, kg/m^2) were compared between the TB patients and healthy children. Mann-Whitney test was used for analysis. Spearman's rank correlation analysis was used to assess the association of serum CRP with age, BMI and leptin. The same method was also used to assess the association of serum leptin with age and BMI. Point biserial correlation analysis was used to assess the associations of serum CRP and leptin with the categorical variable (wasting, severe wasting) in TB group. Significance test results were determined based on p-value < 0.05. Data were analysed using the Statistical Package for the Social Sciences version 16.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

The characteristics of the patients and the comparison group are shown in Table I. Comparisons of serum leptin and CRP levels between the two groups of subjects are shown in Table II. None of the results were statistically significant. We found a positive association with a very strong correlation (point-biserial correlation coeffecient [rpb] = 0.867) and high statistical significance (p < 0.001) between CRP and BMI/A in the TB patients. It showed that an increase in serum CRP level was followed by an increase in wasting (decrease in BMI/A). We could not perform the correlation test in the comparison group, as BMI/A in that group was a constant value (Table III).

There was a significant positive correlation between leptin levels and age (rs = 0.737, p = 0.004) in TB patients. This implied that an increase in the patients' age was accompanied by an increase in their leptin levels, while no significant correlation was observed in the comparison group (Table IV). We also found a positive association and strong correlation (rs = 0.639, p < 0.001) between serum leptin levels and BMI in the comparison group, which showed that an increase in BMI corresponded with an increase in serum leptin levels, while TB patients showed a non-significant, weak positive correlation (rs = 0.244, p = 0.421) (Table IV). TB patients showed a highly significant negative correlation, with a strong correlation (rpb = –0.786, p < 0.001) between serum leptin and BMI/A. This implied that a decrease in serum leptin levels was in line with an increase in wasting in childhood TB (Table IV). It also showed that a very weak negative correlation existed between serum leptin and CRP levels in TB patients (rs = –0.053), although it was not statistically significant (p = 0.864). The comparison group showed a weak positive correlation between serum leptin levels and serum CRP (rs = 0.294), but it was also not statistically significant (p = 0.145).

DISCUSSION

This study showed that TB patients had a lower mean BMI compared to that of subjects in the comparison group, and that all these patients showed wasting. Serum leptin levels in TB patients were almost five times lower than those for the comparison group, and the difference was statistically significant (p < 0.001). These results are consistent with those reported by previous studies on adult TB patients,[4,5,7] which reported that adult TB patients had lower BMI and circulating leptin levels compared to healthy control adults, thus concluding that low leptin levels due to loss of body mass subsequently lead to reduced leptin production. Cakir et al,[19] however, reported that although adult TB patients had lower BMI compared to the control group in their study, serum leptin levels were higher and showed a simultaneous increase in the inflammatory response of TNF-α. Therefore, they concluded that leptin and TNF-α may play a role in the loss of body mass in adult TB.[19] Based on the above studies, the actual pattern of leptin production in adult TB is still contradictory. However, to our knowledge, no data of previous studies in childhood TB is available.

In our study, we found that serum CRP levels, which represented inflammatory response, were significantly higher in TB children (almost 50-fold) when compared with the comparison group, and the difference was statistically significant (p < 0.001). There

Table III. Association of serum CRP level with age, BMI, and BMI/A.

Association	TB patients		Comparison group	
	r	p-value	r	p-value
Serum CRP (mg/L) and age (mths)	−0.041†	0.893	−0.116†	0.573
Serum CRP (mg/L) and BMI (kg/m^2)	0.245†	0.419	0.319†	0.112
Serum CRP (mg/L) and BMI/A	0.867¶	< 0.001	*	*

Note: p-values were calculated based on Spearman's rank correlation analysis or point-biserial correlation test, as appropriate.
† Spearman's rank correlation coefficient ¶ Point-biserial correlation coefficient
* There is no correlation value, as BMI/A in this group was a constant value.
TB: tuberculosis; CRP: C-reactive protein; BMI/A: body mass index/age

Table IV. Association of serum leptin concentration with age, BMI, BMI/A and serum CRP level.

Association	TB patients		Comparison group	
	r	p-value	r	p-value
Serum leptin (pg/mL) and age (months)	0.737†	0.004	0.159†	0.437
Serum leptin (pg/mL) and BMI (kg/m^2)	0.244†	0.421	0.639†	< 0.001
Serum leptin (pg/mL) and BMI/A	−0.786¶	< 0.001	*	*
Serum leptin (pg/mL) and serum CRP (mg/L)	−0.053†	0.864	0.294†	0.145

Note: p-values were calculated based on Spearman's rank correlation analysis or point-biserial correlation test, as appropriate.
† Spearman's rank correlation coefficient; ¶ Point-biserial correlation coefficient
* There is no correlation value, as BMI/A in this group was a constant value.
TB: tuberculosis; CRP: C-reactive protein; BMI/A: body mass index/age

was also a strong positive correlation and high statistical significance between serum CRP levels and BMI/A, thereby showing a compatibility between elevated serum CRP levels and increase in wasting. This also indicates that the association between inflammation and wasting in TB may be caused by anorexia during infection. Our study also found a highly significant negative correlation between serum leptin and BMI/A in TB patients, which showed that a decrease in serum leptin levels was in line with increase in wasting. Therefore, this study did not support the hypothesis that leptin plays a role in the loss of body mass in TB.[19] Instead, we propose that chronic inflammatory processes lead to anorexia and reduced body mass, which corroborates with the results of previous studies on adult TB.[4,5,7]

Moreover, we found no significant correlation ($p > 0.005$) between CRP and serum leptin levels in TB patients; hence, we believe that low serum leptin levels in childhood TB is associated with the reduction of body mass due to chronic inflammation and does not depend on increased serum CRP, as reported by several studies in adults with normal weight/obesity,[22] in adult sepsis patients[23] and in children with minor acute infections.[24] However, we were not aware of the pattern of serum leptin concentrations for weeks or months before the TB diagnosis was established, although it may be predicted that a prolonged inflammatory response was likely to cause exhaustion of leptin production.[4] Since all TB patients in this study had already been in a state of wasting when they were diagnosed, their serum leptin levels were found to be low. If the nutritional status of TB patients were normal at the time of diagnosis, their leptin levels would have been different. We did not find any TB patients with positive AFB who had not been in a state of wasting. A study with a longer duration may give a clearer picture of whether TB patients and subjects in the comparison group had different nutritional statuses. This would, in turn, enable us to determine if there is an association between leptin and inflammation with wasting.

The attainment of higher serum leptin levels in the comparison group (healthy children) without accompanying anorexia and wasting indicates that anorexia and wasting in childhood TB tend to be caused by an increase in inflammatory response and not by leptin. Unfortunately, we measured serum leptin and CRP levels only once and did not measure the levels after anti-TB treatment. The notion that anorexia and wasting in childhood TB are caused by an increase in inflammatory response may be strengthened if decreased CRP levels and elevated leptin levels are found after anti-TB treatment, as reported by van Crevel et al in a study on adult TB patients who had received anti-TB treatment for two months.[4] Due to the limited funds for this study, we did not examine the role of the various pro-inflammatory cytokines such as IL-1, IL-6, TNF-α and IFNγ, which could have contributed to the increased levels of CRP. In

addition, the mediators of the cachectic process have not been studied.[6,10]

Low serum leptin levels may likely contribute to the severity of diseases, as leptin essential for cellular immunity against *M. tuberculosis*.[4,5,20] This study found low serum leptin levels in childhood TB, which indicates that it might be beneficial to prescribe leptin to patients with childhood TB in addition to anti-TB medications. However, this may not be feasible in a country like Indonesia due to cost limitations. Supplementation of micronutrients such as zinc and vitamin E[25,26] as well as unsaturated fatty acids such as fish oil,[27] which are known to increase leptin production, is essential. Umeta et al reported that zinc may also stimulate the appetite.[28] Thus, micronutrients and/or unsaturated fatty acids may be an effective alternative and cost-effective option. In addition, knowledge of the role of low leptin levels in wasting may help clinicians to make an accurate diagnosis and adopt appropriate treatment for childhood TB. This may, in turn, aid in the prevention of wasting, a condition that may worsen the disease outcome.

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