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RESEARCH ARTICLE

Lower IGF-1 and Alkaline Phosphatase Activity Blood Levels in Stunted Children with Soil-Transmitted Helminth Infections

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Abstract

BACKGROUND: Stunting due to malnutrition and soil-transmitted helminth (STH) chronic infections can cause disturbances in bone formation processes during growth. Reduced levels of insulin-like growth factor 1 (IGF-1) lead to reduced osteoblast activity which can be monitored through alkaline phosphatase (ALP) and osteocalcin (OCN). Unfortunately, studies that assessed IGF-1, OCN, and ALP together in stunted children with STH infection are still limited. Therefore, in this study, the osteoblast activity was monitored by measuring IGF-1, OCN, and ALP in stunted and normo-stature children with STH infection.

METHODS: A case-control study involving 28 stunted and 33 normal-stature children was conducted. Blood plasma IGF-1 and OCN levels were measured using the enzyme linked immunosorbent assay (ELISA) method, while ALP activity was measured using the colorimetric method. STH infection was examined by direct smear method. The parameters were analyzed and compared between the stunted and normo-stature children.

RESULTS: Although in stunted boys there was no significant difference, however in stunted girls with positive STH infection, a decrease in IGF-1 (<36.60 ng/mL *vs.* ≥36.60 ng/mL) showed an impact on the decrease of OCN levels (54.68 (42.22-144.54) ng/mL *vs*. 104.55 (86.14-392.73) ng/mL; p=0.047). ALP activity in children with positive STH infection showed that ALP activity in stunted children was lower than in normo-stature children (18 (10-671) U/L *vs.* 228.50 (16-574) U/L; p=0.005).

CONCLUSION: The blood levels of IGF-1 and ALP activity in stunted and positive STH infected children tend to be lower than in normo-stature children.

KEYWORDS: stunted, normo-stature, IGF-1, bone alkaline phosphatase, osteocalcin

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Introduction

Based on the data from The Indonesia Basic Health Research 2018, Indonesian children are still among the shortest in the world.(1,2) One of the factors that was thought to be causing the problem of low height growth in Indonesia is stunted. According to the Indonesian Nutritional Status Survey (SSGI) by the Ministry of Health of the Republic of Indonesia and the Health Profile of Buton Regency in 2021, the prevalence of stunted in Indonesia is 24.4%, Southeast Sulawesi is 30.2%, and Buton Regency is 21.3%.(3,4) Environmental factors, such as access to clean water, proper sanitation, and hygiene, are significant determinants of stunting in Indonesia, particularly in areas with economic limitations.(5-7) Poor sanitation increases the risk of soil-transmitted helminth (STH) infections, such as those caused by Ascaris lumbricoides and Trichuris trichiura.(8) These

infections can trigger the host's immune response, leading to chronic inflammation and nutrient deficiencies. The resulting chronic inflammatory state may interfere with essential physiological processes, such as bone formation which is critical for children's growth. Consequently, STH infections contribute to growth disturbances and stunting, particularly in preschool and school-age children.(9,10)

Stunted is a disruption or failure of growth and development in children due to chronic undernutrition and recurrent infections, characterized by failure of linear growth.(11) Growth of long bones is a complex process driven by a variety of endogenous (growth hormone such as insulin-like growth factor-1 (IGF-1) which plays an important role in stimulating the proliferation of chondrocyte and osteoblast cells, growth factors) and exogenous (dietary, environmental) variables.(12) Linear growth is highly influenced by the process of endochondral ossification in the bone growth plate, where chondrocyte cells undergo remodelling through the bone resorption by osteoclasts and bone formation by osteoblasts.(13) During the growth period, the chondrocyte cells in the growth plate will secrete type I collagen, osteocalcin (OCN), and bone alkaline phosphatase (ALP), and undergoes proliferation and hypertrophy, which cause widening of the bone plate resulting in bone elongation (linear growth/height growth). (14,15)

Most ALP in serum originates from the mucosal cells of the small intestine, proximal cortical tubules of the kidney, placenta, and liver, and especially from osteoblasts during the growth period. Consequently, serum ALP activity testing is essential in the investigation of bone disorders associated with increased osteoblast activity. Elevated ALP activity in osteoblasts signifies their active participation in bone matrix formation and mineralization through regulating the balance of phosphate and calcium ions.(16-18)

STH infections are known to impair growth by triggering chronic inflammation and reducing levels of growth hormones, such as IGF-1, which play a critical role in bone growth by stimulating osteoblast activity. Despite this, limited research has explored the biochemical interactions between IGF-1 and bone-specific markers, such as OCN and ALP, in stunted children affected by STH infections. This gap in understanding hinders the development of targeted strategies to address the disruption in bone formation caused by these infections.

Based on the description above, this study addresses this gap by examining IGF-1, OCN, and ALP levels in stunted children compared to normo-stature children with STH infections in Buton Island, Southeast Sulawesi. It might provides insights that may contribute to improved diagnostic and therapeutic strategies to combat stunted.

Methods

Study Design

A case-control study involving 61 subjects consisting of 28 stunted children and 33 normo-stature children was conducted in Sampuabalo Village, Buton Regency, Southeast Sulawesi during August – November 2023. The sample size in this study was determined based on time constraints, ensuring that data collection remains feasible within the available research period.(19,20)

The inclusion criteria of subjects were children aged 24-60 months (both boys and girls) who have not taken worm medicine for the last 6 months. The exclusion criteria were children suffering from autoimmune disease based on medical history, suffering from HIV/AIDS based on medical history, and having a fever during the sampling process. A child was classified as stunted if their Height-for-Age Z-Score (HAZ) was less than -2 standard deviations (< -2SD) from the WHO reference population. (11) The protocol of this research was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo Hospital (No. Ket62/un2.f1/etik/ppm.00.02/2023), and written consent was obtained from the subjects' parents.

Measurement of Anthropometrics Data

To obtain subjects' nutritional status, specifically their stunted status, subjects' height was measured by using a stadiometer or height-measuring device placed on a flat surface. Subjects must stand upright with the heels, buttocks, upper back and head touching the tool and the feet together with the heels touching each other. Subjects' head must be in a parallel position, i.e., the line between the ear hole and the lower edge of the eye must be parallel to the floor. Measurements were taken twice to ensure consistency, and if the results differ by more than 0.1 cm, the height was measured. This method followed Indonesian Ministry of Health guidelines and WHO growth standards, so the results are reliable for assessing children's nutritional status based on height.(11,21)

Blood Collection

Blood specimen collection was carried out according to routine hematology methods of 3-5 mL for each child by certified health analysts. After the blood was collected, it was

separated using a centrifuge to collect blood plasma which will be used to examine IGF-1, OCN, and ALP activity. Samples measurements were carried out at the laboratory of the Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta.

IGF-1 and OCN Measurement

IGF-1 and OCN examination were carried out by the enzyme linked immunosorbent assay (ELISA) method using the Human IGF-1 ELISA kit (Cat. No. E-EL-H0086; Elabscience, Houston, TX, USA) and the Human Osteocalcin ELISA kit (Cat. No. E-ELHI343; Elabscience). The IGF-1 examination used antibodies specific to Human IGF-1 antibodies and biotinylated detection antibodies specific to Human IGF-1, while the OCN examination used antibodies specific to Human OCN/BGP and biotinylated detection antibodies specific to Human OCN/BGP. The principle of Sandwich ELISA was used for the ELISA examination.

ALP Activity Measurement

Examination of serum ALP enzyme activity was carried out by colorimetric method using a commercial Alkaline Phosphatase Reagent (Cat. No. GD-ALP100; Glory Diagnostic, Seoul, South Korea). The measurement of ALP activity involved a biochemical reaction that produces a measurable color change proportional to the ALP activity in the sample. ALP catalyzed the hydrolysis of a phosphate group from a specific chromogenic substrate, such as p-nitrophenyl phosphate (p-NPP), under alkaline conditions. The reaction results in the production of p-nitrophenol, which is yellow in color and can be measured spectrophotometrically at a wavelength of 405 nm.

Feces Collection and STH Infection Analysis

Stools successfully collected from subjects were processed using the original method (direct slide) to detect intestinal worm or STH infections. Intestinal parasites, including protozoan cysts, trophozoites, and helminth eggs or larvae, were identified based on morphological characteristics.

Statistical Analysis

Data were reported as mean with standard deviation (mean±SD) if homogenous and normally distributed, and with median and minimum-maximum range (median (min-max)) if not homogenous and normally distributed. Statistical analysis was performed using the Mann-Whitney U test for independent samples. A two-sided p-value<0.05 was considered statistically significant. Correlation of IGF-1, OCN, and ALP activity concentrations was tested using the Spearman rank correlation test. Statistical analysis was performed with the IBM Statistical Package for Social Sciences (SPSS) Statistics, version 21.0.1 (IBM Corporation, Armonk, NY, USA).

Results

Characteristics of Study Subjects

The number of subjects taking part in the study was 61 children, aged 2 to 5 years (24-60 months), which consist of 35 boys and 26 girls. The number of stunted children in this study was 45.9%, while the normo-stature children were 54.1%. Based on gender, more girls (46.2%) suffered from stunting compared to boys (45.7%). The average height of girls in the stunted group was 87.71±6.25 cm, and in normo-stature group was 90.17±6.84 cm. Meanwhile, the average height of boys in the stunted group was 85.84±3.99 cm and in normo-stature group was 93.12±5.18 cm (Table 1). Based on the feces examination, there was a statistically significant between the incidence of STH infection and the incidence of stunting (p=0.047). Nine of 28 children without STH infection suffered from stunting, and 19 of 33 children with positive STH infection suffered from stunting (Table 2).

Lower IGF-1 Level in Stunted Children

The median IGF-1 level in the group of stunted children tends to be lower than those in the group of normo-stature children (13.40 (4.45-296.45) ng/mL *vs.* 24.55 (4.25-241.65) ng/mL), even though, it was not statistically significant (p=0.393) (Table 3). Likewise, in children with positive STH infection, the IGF-1 level was lower in stunted children compared to the normo-stature children (39.25 (4.85-296.45) ng/mL *vs.* 41.95 (5.20-241.65) ng/mL) though not statistically significant (p=0.423) (Table 4). While, in children with normo-stature growth and not STH-infected, there was significant difference in IGF-1 concentrations between boys and girls (10.22 (6.65-222.55) ng/mL *vs.* 36.60 (4.25-145.40) ng/mL; p=0.032).

In the group of stunted girls infected with STH, the decrease in IGF-1 (IGF-1<36.60 ng/mL) showed an impact on the decrease in OCN (54.68 (42.22-144.54) *vs*. 104.55(86.14-392.73) ng/mL; p=0.047) (Figure 1). Similarly, in stunted and STH-infected boys, although not statistically significant, a decrease in IGF-1 (IGF-1<10.22 ng/mL) showed an impact on decreasing OCN (78.97 (40.66-153.11) *vs*. 88.50 (38.57-191.96) ng/mL; p=0.806).

Table 1. Demographic data and nutritional status.

Characteristics	Stunted (n=28)	Normo-stature (n=33)	*p*- value
Total sample, n (%)	28 (45.9)	33 (54.1)	0.000*
Gender, n (%)			
Boys (n=35)	16 (45.7)	19 (54.3)	0.000*
Girls (n=26)	12 (46.2)	14 (53.8)	
Age (months), Mean±SD			
Boys (n=35)	39.94±7.86	41.58±9.55	0.200
Girls (n=26)	42.25±8.01	36.14±10.98	
Height (cm), Mean±SD			
Boys (n=35)	85.84±3.99	93.12±5.18	0.200
Girls (n=26)	87.71±6.25	90.17±6.84	

*Significant if $p<0.05$, Kolmogorov-Smirnov test.

OCN Level in Stunted Children

Without looking at the infection factor, the median OCN concentration in the stunted group was 87.32 (38.57-392.73) ng/mL, while the median OCN concentration in the normo-stature group was 86.70 (39.42-235.49) ng/mL. There was no significant difference in OCN concentration in stunting and normo-stature subjects (p=0.761) (Table 3). While children with positive STH infections showed that the median OCN concentration in the stunted group was 86.14 (38.57-392.73) ng/mL, while the median OCN concentration in the normo-stature group was 85.35 (39.42-185.72) ng/mL; these results showed a tendency for high OCN in stunted children infected with STH, although there was no statistically significant difference between OCN concentration and the incidence of stunted (p=0.942) (Table 4).

In normo-stature children with positive STH infection, ALP activity showed significantly different values between OCN≥86.140 ng/mL and OCN<86.140 ng/mL. When the children have OCN<86.140 ng/mL, the ALP activity values were lower than the children with OCN≥86.140 ng/mL (33 (16-512) U/L vs. 513 (63-574) U/L; p=0.013) (Table 5).

Lower ALP Activity Level in Stunted Children

The median value of ALP activity in the group of stunted children was lower than in the group of normo-stature (16 (10-671) *vs.* 120 (10-613) U/L); p<0.001) (Table 3). The median value of ALP activity in stunted children with positive STH infection was lower than in the group of normo-stature (18 (10-671) *vs.* 228.5 (16-574) U/L; p=0.005) (Table 4).

Discussion

In stunting children with, ALP activity and IGF-1 levels are lower than in children with normo-stature. Though not statistically different, the results of this study showed that serum IGF-1 levels tend to be lower in stunted group than the normo-stature group. This is supported by previous study which is show that children with higher levels of IGF-1 were less likely to become stunted.(22) Low IGF-1 levels can have several health effects, including reduced muscle mass and strength, with decreased muscle protein synthesis and increased muscle breakdown; reduction in bone density;

Table 2. Analysis of nutritional status (stunting and normo-stature) for worm examination in feces.

Feces Analysis	Stunted (n=28)	Normo-stature (n=33)	*p*- value
Negative STH infection	9 (32.1)	19 (67.9)	
Positive STH infection	19 (57.6)	14 (42.4)	0.047*
Ascaris lumbricoides	13 (56.5)	10 (43.5)	
Trichuris trichiura	6 (60.0)	4 (40.0)	

*Significant if p<0.05, Chi-Square test.

Table 3. Bone protein markers in the stunted and the normo-stature group.

Variable	Stunted (n=28)	Normo-stature (n=33)	*p*-value
IGF-1 (ng/mL)	13.40 (4.45-296.45)	24.55 (4.25-241.65)	0.393
Osteocalcin (ng/mL)	87.32 (38.57-392.73)	86.70 (39.42-235.49)	0.761
ALP activity (U/L)	16 (10-671)	120 (16-613)	0.000*

*Significant if $p<0.05$, Mann-Whitney test.

and delayed growth and development. This can manifest as short stature or delayed puberty.

The results of statistical analysis that did not show any difference in IGF-1 values between stunted and normo-stature children can be caused by several factors. IGF-1 concentrations in children have a wide range depending on age, gender, genetic factors, and infection.(23-25) In addition, sleep, deep sleep or slow wave sleep (SWS), exercise, diet, age, obesity, and fasting are factors that affect the production of growth hormone in the body. Previous research on circadian rhythm (CRY) revealed that IGF-1 expression decreased at the mRNA and protein levels in the liver and other organs of CRY-deficient animals, which correlated with decreased IGF-1 levels in the bloodstream of these mice.(26-28) In this study, in children with normo-stature and not infected with STH, IGF-1 levels in boys are lower than girls. IGF-1 affects bone growth in children presumably through its role as a mediator of chondrocyte and osteoblast proliferation and maturation processes in the growth plate.(29) In this study, the decrease in IGF-1 had an impact on the decrease in OCN, which is a bone matrix protein from osteoblast cells, in stunted girls with STH infection (Figure 1). The infection that occurs causes an increase in proinflammatory factors such as interferon (INF)-γ, which has a significant impact on IGF-1 production through receptor regulation, influence on signaling pathways, and modification of immune responses.(10,30,31) Besides affecting growth in children through IGF-1 hormone, cytokines also have a role to the growth, in the context of osteoimmunology. Cytokines cause a reduction in the process of proliferation and differentiation of osteoblast cells, which is seen through a decrease in the ALP Activity. This is supported by the results of observations in this study, ALP activity has a significant correlation with incidence of stunted. ALP activity in the stunted with positive parasite STH infection was lower than the normo-stature group.

Although OCN did not show significant differences between stunted and normo-stature children, the decrease in OCN levels also reflects the decrease in ALP activity in normo-stature children. OCN, or gamma-carboxyglutamic acid (Gla) bone protein, is a γ-carboxylic protein produced by osteoblasts during bone formation but also released during bone resorption. Although OCN levels are higher in stunted children, this does not necessarily indicate increased healthy bone growth, but rather impaired bone homeostasis due to increased bone resorption and chronic inflammation. Stunted children may experience increased bone turnover either as an effort to repair impaired growth or due to metabolic disorders or chronic inflammation, so OCN levels in the blood may increase because more bone is undergoing remodeling.(32-34)

The decrease in IGF-1 and ALP in stunted children infected with STH shows the significant role of parenting, environment and nutrition in children. Parasitic infections such as STH are generally asymptomatic, so STH infection is very likely to cause long-term chronic infection. Paying attention to adequate nutrition and a clean and healthy lifestyle from an early age is a good stimulus for children's growth.(35,36)

In the context of stunted growth, inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukins (IL-1, IL-6) can disrupt normal bone

Table 4. Growth hormone and bone protein markers of osteoblast in the stunted and the normo-stature group children with positive STH infection.

Variable	Stunted (n=28)	Normo-stature (n=33)	*p*-value
IGF-1 (ng/mL)	39.25 (4.85-296.45)	41.95 (5.20-241.65)	0.423
Osteocalcin (ng/mL)	86.14 (38.57-392.73)	85.35 (39.42-185.72)	0.942
ALP activity (U/L)	18.0 (10-671)	228.50 (16-574)	0.005*

*Significant if $p<0.05$, Mann-Whitney test.

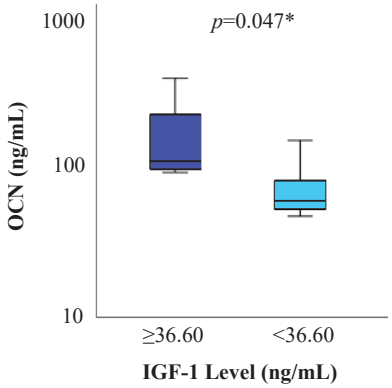

Figure 1. The decrease in IGF-1 (IGF-1 < 36.60 ng/mL *vs.* IGF-1 ≥36.60 ng/mL) in stunted girls with positive STH infection has an impact on the decrease in OCN (54.68 (42.22-144.54) ng/mL *vs.* 104.55 (86.14-392.73) ng/mL. *Significant if $p<0.05$, Mann-Whitney test.

metabolism, by preventing osteoblast differentiation, leading to decreased ALP expression and activity. These cytokines affect both osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells), leading to an imbalance that can exacerbate stunting.(37) Reducing proinflammatory chemicals and inflammation is one treatment strategy that can aid in boosting osteoblast function. Immune cells, especially T-helper type 2 or Th2 cells, naturally produce the anti-inflammatory cytokine IL-13, which has been shown to suppress proinflammatory reactions, including the generation of TNF-α. Numerous investigations have demonstrated the positive impact of IL-13 in controlling the ratio of osteoblasts (bone-forming cells) to osteoclasts (bone-resorbing cells).(38) So overcoming infections in children is expected to be another strategy in improving the health status and growth of children.

The best biochemical for predicting and monitoring treatment in stunted children was therefore ALP. Total ALP gave almost identical results to bone ALP. It could be a cheaper and simpler alternative in short of normal children, where no changes in liver ALP would be expected. The combination with IGF-1, a growth hormone, gave a better prediction of growth than height measurements alone. The study's limitations include the narrow geographic focus, small sample size and cross-sectional design, which restricts its ability to confirm causality. Additionally, uncontrolled variables such as micronutrient status, diet, and medical history may have influenced the results. Further studies with larger samples and control for confounding variables are needed to strengthen these findings.

Conclusion

The blood levels of IGF-1 and ALP activity in stunted and positive STH infection children tend to be lower than in children with normo-stature. ALP activity can be used as a marker in monitoring the handling of stunted, ALP examination is inexpensive and easy to perform in facilities with limited laboratory resources such as Primary Health Center.

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Table 5. ALP activity of osteoblast in the stunted and the normo-stature group children with positive STH infection.

Group	ALP Activity (U/L)		*p*- value
	OCN≥86.140 ng/mL	OCN<86.140 ng/mL	
Stunted (n=19)	n=9	n=10	0.935
	16 (10-569)	18 (12-671)	
Normo-Stature (n=14)	n=7	n=7	0.013*
	513 (63-574)	33 (16-512)	

*Significant if $p<0.05$, Mann-Whitney test.

Authors Contribution

TMPL lead the data collection in Sampuabalo District, Buton, Southeast Sulawesi, carry out the sample examination in the laboratory, data analysis and interpretation and prepared the draft of the manuscript. SWAJ conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript. MS advised on the data analysis and interpretation and reviewed the manuscript. HW conducted the study, data analysis and interpretation and reviewed the manuscript.

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