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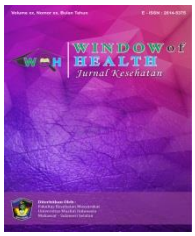

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Relationship Between Nutritional Status And Insulin-Like Growth Factor-1 Levels In Stunted Children In Cirebon Regency

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ABSTRACT

Insulin-like Growth Factor-1 (IGF-1) is a growth hormone mediator that acts as a growth-promoting factor in the growth process and is also an indicator of the adaptive immune system. Children who experience stunting will experience obstacles to growth and cognitive and motor development, which will affect their productivity as adults. The aim of this research is to determine IGF-1 levels and find their relationship with nutritional status in stunted children. The research method used was observational, with a cross-sectional research design. The population of stunted children in the Tegalwangi locus area, Cirebon Regency, a sample of children aged 24-60 months who met the inclusion criteria of not being disabled and not being sick, was 50 children. The research began with parents filling in informed consent. Continue to fill in data on date of birth, measure height and weight, take @1ml of the child's blood, and analyze IGF-1 using the ELISA method. The results of the research were that the average IGF-1 level for men was 16,812 ± 5,164 ng/ml and for women 13,810 ± 5,111 ng/ml. Average IGF-1 levels aged 24-36 months were 14,777 ± 4,742 ng/ml, 36–48 months 17,050 ± 4,280 ng/ml, and 48–60 months 15,214 ± 6,248 ng/ml. Data analysis using the Pearson correlation test obtained a value of p=0.871 based on age, p=0.047 based on gender, p=0.643 based on Height for age, and p=0.245 based on Weight for age (95% CI). The conclusion is that there is a relationship between the age of a stunted child and IGF-1 levels, and there is no relationship with nutritional status. All stunted children have IGF-1 values below the standard value for normal children based on the literature, namely ≥ 28.54 ng/ml.

Key words: ELISA; IGF-1 levels; Stunting

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INTRODUCTION

Human development globally is a problem due to stunting. Stunting affected approximately 22.0 percent or 149.2 million children under 5 years globally in 2020.1 In 2020, more than half of children under 5 years who were stunted lived in Asia, and two in five lived in Africa, with the highest percentage of stunted in Asia is 53% and in Africa, it is 41%. [(1)] In Indonesia itself, it is still experiencing problems in terms of nutrition and child growth and development. Based on the results of the SSGI (Indonesian Nutritional Status Study) in 2021, the national prevalence of nutritional status for toddlers experiencing stunting was 24.4%. [(2)] West Java experienced a decrease in the prevalence of stunting where in 2019 the prevalence of stunting was 26.2% which was in the yellow zone (medium category) to 24.5% in 2021. [(3)]

A condition where a toddler has a height or body length that is less than the height or body length compared to the age in the middle of the growth. Height that is more than a minimum of the Deviation Standard, the WHO child growth standard. Factors such as social and economic conditions, maternal nutritional intake during pregnancy, latent infections in babies, and lack of nutritional intake in babies are causes of stunting. In the future, stunted children will experience difficulties in achieving optimal physical and cognitive development. [(2)]

The national prevalence of stunted children in 2021 is 24.4%. The stunting rate in Cirebon Regency is 30.6%, above the national prevalence. [(4)] According to the Decree of the Minister of National Development Planning No. 42 of 2020, Cirebon Regency is one of the cities/districts focused on interventions to reduce stunting. [(5)] The growth and development of mitosis and cell anabolism are regulated and controlled by cells by insulin-like growth factor-1 (IGF-1). Apart from that, it also regulates the growth of long bones by stimulating the profiling and maturation of chondrocytes. Other key roles of IGF-1 in the growth and development of skeletal muscle. [(6)] This hormone concentration is very sensitive to changes in nutritional status, both short and long-term [(7)]. In children with poor nutritional status, cell IGF-1 is significantly correlated with height (based on WHO standards). This shows that this parameter is useful as an indicator of growth and nutritional status. [(8)]

Research on measuring IGF-1 levels in stunted toddlers has been widely carried out, but it is still not clearly understood the relationship between serum IGF-1 levels and linear growth in children in developing countries. [(9)] Previous research in Bangladesh children showed serum IGF-1 levels 1 in stunted children are lower than in non-stunting children, as is the case with similar research on Ethiopian children. [(10,11)] The aim of this research is to determine IGF-1 levels and relate them to nutritional status in stunted children.

METHOD

Quantitative research with cross-sectional observational type. The research location is Tegalwangi Village, Cirebon Regency. This research has received permission from the Cirebon District Health Service and ethical approval from the Health Research Ethics Committee Number:

003/KEPK/EC/III2023. The research was carried out in March-May 2023. Samples were taken using a sampling technique using the formula from www.openepi.com. The number of samples in this study was 50 children who met the inclusion criteria, namely, stunted children aged 24–60 months whose parents were willing to be included in this study, and who signed an informed consent form. Tools and materials in this research include a special height measuring device for toddlers, scales, ELISA kit from Elabscience No: E-EL-H0086, gloves (handscoon), tourniquet, 70% alcohol cotton, syringe disposable three cc, vacutainer (sterile blood collection tube) 5 cc, wound plaster, centrifuge (centivizing device). The research began by measuring nutritional status based on height, using a Z-score calculated using child anthropometric data, and using WHO global baseline data on child growth and malnutrition in WHO Anthro 3.2.2 software. The analyst carried out sample collection by taking one cc of the stunted child's blood from the median cubital vein at the crease of the elbow and then putting it into a five cc vacutainer. Reagent preparation was carried out by analysts in the physiology laboratory of the Faculty of Medicine, Brawijaya University. Bring all reagents to room temperature (18 – 25^{o}C). Wash Buffer: dilute 30 ml of wash buffer concentrate with 720 ml of distilled water to prepare 750 ml of wash buffer. Standard working solution: standard centrifugation at 10,000 xg for 1 minute. Add 1 ml of standard reference solution and sample, let stand for 10 minutes, and gently invert several times. Once completely dissolved, mix well with a pipette. This reconstitution produces a working solution of 100 ng/ml. Then make dilutions as needed. The dilution gradient is as follows: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0 ng/ml. Biotinylated Detection Working Solution: calculate the amount needed before the experiment (100 µL/well). In preparation, a little more than calculated should be prepared. Then dilute 100x HRP conjugate concentrate into 1x working solution with HRP conjugate diluent concentrate. The testing procedure was carried out by analysts from the physiology science laboratory, Faculty of Medicine, Brawijaya University. Add the standard working solution to the first two columns: each concentration of solution is added in duplicate, to each well, side by side (100 µL for each well). Add sample to the other wells (100 µL for each well). Cover the plate with the sealer provided in the kit. Incubate for 90 minutes at 37^{o}C. Note: the solution must be added to the bottom of the micro ELISA plate well, avoiding touching the inner wall. Remove the liquid from each well, do not wash. Immediately add 100 µL of biotinylated detection working solution to each well. Cover with plate sealer. Mix gently. Incubate for 1 hour at 37^{o}C. Aspirate or decant the solution from each well, adding 350 µL of wash buffer to each well. Soak for 1 minute and aspirate or pour the solution from each well and pat dry with clean absorbent paper. Repeat this washing step 3 times. Note: microplate washers can be used in this and other washing steps. Add 100 µL of HRP conjugate working solution to each well. Cover with plate sealer. Incubate for 30 minutes at 37oC. Aspirate or pour the solution from each well, repeat the washing process 5 times as done in step 3. Add 90 µL of Substrate reagent to each well. Cover with new plate sealer. Incubate for approximately 15 minutes at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. Add 50 µL of stop solution to each well. Note: adding the stop solution must be done in the same order as the

substrate solution. Determine the optical density (OD value) in each well at once with the microplate reader set to 450 nm. The OD value is proportional to the concentration of Human IGF-1.

Data analysis used descriptive analysis to determine the average IGF-1 level ± Deviation Standard, Pearson Correlation test to see the relationship between IGF-1 levels and respondent characteristics. Confidence Interval (CI) 95%.

RESULTS

The research has been registered with the Health Development Policy Agency through the Indonesian Disease Registry with No. INA-Y6CT9L0. Stulnting children who undergo research are measured by their height and body weight. The data has been entered into the WHO Anthro 3.2.2 software to determine the z-score. This study shows that the distribution of respondents is based on age, gender, height for age and weight for age

Table 1. Frequency Distribution of Respondents

Respondent Characteristics	Frequency (n)	Percentage (%)
Age		
24 – 36 Months	16	32%
36 – 48 Months	13	26%
48 – 60 Months	21	42%
Gender		
girl	21	42%
boy	29	58%
Nutritional status		
Height For Age		
Very short	5	10%
Short	45	90%
Weight For Age		
Very poor nutrition	2	4%
Malnutrition	18	36%
Adequate nutrition	30	60%

Based on the table 1 of the distribution of the frequency of the responses above, the characteristics of the responses are divided into 3, namely based on age, gender, and nutritional status. If we look at age, the number of respondents in this research is 21 children (42%). Furthermore, if we look at the gender gender that has been distributed, it is more or less the same, there are 21 children (42%) with gender gender and 29 children (58%) with male gender. Then, if we look at it based on nutritional status, namely height for age, most of the respondents have the shortest height as many as 45 children (90%),

whereas if it is based on weight for age, most of the respondents have a normal nutritional status of 30 children (60%)

Table 2. Frequency Distribution of IGF-1 Levels Based on Age in Stunted Children

Age	Frequency (n)	Mean ($\bar{x}$) ± SD
24 – 36 Months	16	14.777 ± 4.742
36 – 48 Months	13	17.050 ± 4.280
48 – 60 Months	21	15.214 ± 6.248

Based on Table 2 above, it is known that the average IGF-1 levels in the three age groups include 16 children (14,777) for the 24 - 36 month age group, 13 children (17,050) for the 36 - 48 month age group, and 48 age group. – 60 months, as many as 21 children (15,214).

Table 3. Frequency Distribution of IGF-1 Levels Based on Gender in Stunted Children

Gender	Frequency (n)	Mean ($\bar{x}$) ± SD
Girls	21	13.810 ± 5.111
Boys	29	16.812 ± 5.164

Based on Table 3 above, it is known that the average IGF-1 level in girls is 21 children (13,810), and in boys, it is 29 children (16,812). The average IGF-1 value for boys is greater than for girls.

Table 4. Frequency Distribution of IGF-1 Levels Based on Height According to Age (TB/U) in Stunting Children

Height For Age	Frequency (n)	Mean ($\bar{x}$) ± SD
Very short	5	16.610 ± 5.178
Short	45	15.434 ± 5.363

Based on Table 4 above, it is known that the average IGF-1 levels in stunted children are based on height for age, where for very short height for age, there are five children (16,610), and for short height for age, there are 45 children (15,434).

Based on Table 5 above, it is known that the average IGF-1 levels in stunted children are based on weight for age, where for weight for age of malnutrition, there are two children (11,597), for weight for age of malnutrition, there are 18 children (15,033), and for Normal weight for age was 30 children (16,126).

Table 5. Frequency Distribution of IGF-1 Levels Based on Body Weight for Age in Stunting Children

Weight for Age	Frequency (n)	Mean ($\bar{x}$) ± SD
Very poor nutrition	2	11.597 ± 1.316
Malnutrition	18	15.033 ± 5.365
Adequate nutrition	30	16.126 ± 5.388

Results of data analysis using IBM SPSS version 21, looking for relationships using Person correlation with a confidence level of 95%.

Table 6. Data Analysis Results Correlation Coefficient (CI 95%)

Criteria	Correlation coefficient	P value
Relationship between IGF-1 levels and age	0,070	0,631(not significant)
Relationship between IGF-1 levels and gender	0,098	0.500 (not significant)
Relationship between IGF-1 Height For Age	0,050	0.643 (not significant)
Relationship between IGF-1 weight for age	0,069	0.245 (not significant)

Based on Table 6 above, the correlation coefficient between IGF-1 levels and age, gender, height for age, and weight for age is low, which means there is no relationship between these values.

DISCUSSION

In this study, a test was carried out on the relationship between nutritional status and IGF-1 levels in stunted children using the ELISA method. IGF-1 (Insulin-like Growth Factor I) is a growth hormone mediator that acts as a growth-promoting factor in the growth process. [(9)] IGF-1 is also a parameter of the adaptive immune system. [(12)]

Respondents in this study were stunted children aged 24–60 months. This age range was chosen because the effect of malnutrition on height will be visible over a relatively long period of time. This is also supported by recent research, which stated that a child's age was found to be a significant determinant of stunting, with children aged 24 months and over being more likely to experience stunting. [(13)] Chronic malnutrition can cause failure to thrive in children under five, so children become too short for their age. Malnutrition occurs when the baby is in the womb and in the early days after the baby is born, but stunting only appears after the baby is two years old. [(14)] Breast milk protection obtained before children aged 0-23 months have a significantly lower risk of stunting compared to children aged > 23 months. [(15)]

The percentage of women is 42%, and men are 58%. The results of data analysis showed that men had a 1x greater risk than women, but this value was insignificant because the P value was> 0.5. In line with previous research, boys tend to be more physically active, so they spend more energy on activities

and not on growth. In addition, in general, men have faster growth after going through puberty, while women generally experience faster growth than men before and during puberty. (16)

Before the IGF-1 levels are assessed, stunting children are assessed for body weight and height so that they can be categorized based on child anthropometric standards. After that, the first blood draw was carried out for the IGF-1 level of the stunted child and then compared with the normal value of the IGF-1 level. Based on the literature, it was found that to see an increase in height, it is recommended that height increase be carried out for a minimum of 3 months in order to obtain maximum results. (17)

Testing of IGF-1 levels was carried out using the ELISA (Enzyme-Linked Immunosorbent Assay) method using the ELISA Kit from Elabscience No. E-EL-H0086. This ELISA method uses the sandwich-ELISA principle, which is the most common type of ELISA examination. This ELISA examination requires two specific antibodies for different epitopes of the antigen. The two antibodies are usually referred to as a matched antibody pair. One of the antibodies will bind to the surface of the plate and be used as a capture antibody to facilitate the immobilization of the antigen. Other antibodies will conjugate and facilitate the detection of the antigen. (18) This ELISA method has high sensitivity, is 2-5 times more sensitive than direct or indirect ELISA, and has high specificity because two antibodies participate as a capture antibody and a detection antibody. This ELISA examination is more flexible because direct detection can be carried out. It is the best examination for complex sample analysis because the antigen does not need to be purified before measurement. (19)

Based on research that has been carried out on 50 children, testing IGF-1 levels showed that IGF-1 levels in stunted children were below normal IGF-1 levels, where normal IGF-1 levels were ≥ 28.54 ng/ml. (20) This is in line with several studies that have been conducted previously, namely research on Bangladeshi children showing that serum IGF-1 levels in stunted children are lower than in non-stunted children, as well as research conducted on Ethiopian children. (5,6)

Based on the Pearson test, the correlation between IGF-1 levels and age and body weight for age showed no significant correlation or relationship between IGF-1 levels and age and body weight for age. There were no differences in IGF-1 levels between all age ranges, all height-for-age categories, and all weight-for-age categories. Therefore, age and weight for age do not affect IGF-1. This lack of correlation is because all the research subjects were stunted children, and these children experienced growth failure due to chronic lack of intake. This lack of intake, especially intake, affects IGF-1 levels, resulting in inhibition of children's growth and development

Based on statistical analysis of the Pearson test, the correlation between IGF-1 levels and height for age was found, but there was no significant correlation or relationship between IGF-1 levels and height for age. This is in line with descriptive testing between IGF-1 levels and height according to age, and it was found that the average IGF-1 level for the very short category (16,610 ng/ml) was greater than the short category (15,434 ng/ml), which shows that height for age has no effect on IGF-1 levels. Descriptively, most of the children who experience stunting have short height (90%) while only (10%) have very short height. This is in line with previous research where the prevalence of very short toddlers

tended to decrease from 18.8% in 2007 to 18.0% in 2013, but for shortness, it fluctuated from 18.0% in 2007, down slightly to 17.1% in 2010 and rose again to 19.2%, in 2013. [21] The reason is the same as before; there is no relationship because all subjects, whether short or very short, are stunted children who experience chronic intake problems, which cause IGF-1, which is a Growth Hormone biomarker, to decrease.

Meanwhile, the Pearson test of the correlation between IGF-1 levels and gender showed that there was a significant correlation or relationship between IGF-1 levels and gender. This is in line with descriptive testing, where the percentage of stunted children who are male (58%) is more significant than stunted children who are female (42%), which shows that gender influences IGF-1 levels. This is in line with previous research, which states that boys are significantly more likely to experience stunting than girls. [22] This is because boys are thought to grow slightly faster than girls, and their growth may be more easily affected by malnutrition or other diseases or exposures.[23]

CONCLUSION AND RECOMMENDATIONS

This study concludes that there is a relationship between the age of stunted children and IGF-1 levels, and there is no relationship between nutritional status and IGF-1 levels in stunted children. Initially, the IGF-1 value was below the IGF-1 library value for children, namely 28 ng/ml. Considering the low IGF-1, it is recommended that supplements be given to increase nutritious food intake to increase the IGF-1 value.

REFERENCES

1. Akter, J., Hossain, M. A., Takara, K., Islam, M. Z., & Hou, D.-X. (2019). Antioxidant activity of different species and varieties of turmeric (Curcuma spp): Isolation of active compounds. Comparative Biochemistry and Physiology. Toxicology & Pharmacology : CBP, 215, 9–17. https://doi.org/10.1016/j.cbpc.2018.09.002
2. Bukusuba, J., Kaaya, A. N., & Atukwase, A. (2017). Predictors of Stunting in Children Aged 6 to 59 Months: A Case-Control Study in Southwest Uganda. Food and Nutrition Bulletin, 38(4), 542–553. https://doi.org/10.1177/0379572117731666
3. Condo, J. U., Gage, A., Mock, N., Rice, J., & Greiner, T. (2015). Sex differences in nutritional status of HIV-exposed children in Rwanda: A longitudinal study. Tropical Medicine and International Health, 20(1), 17–23. https://doi.org/10.1111/tmi.12406
4. Darteh, E. K. M., Acquah, E., & Kumi-Kyereme, A. (2014). Correlates of stunting among children in Ghana. BMC Public Health, 14, 504. https://doi.org/10.1186/1471-2458-14-504
5. Hamid, A. R. A. H. (2023). Short stature and stunting in Indonesia: problems and innovative alternative solutions. Medical Journal of Indonesia, 31(4), 211–212. https://doi.org/10.13181/mji.ed.236924
6. Hardinsyah, H., Riyadi, H., & Napitupulu, D. (2013a). Sufficient Energy, Protein, Fat and Carbohydrates.Biorad. (2017).
7. ElLISA Basics Gulidel. Validation of Pharmacelultical Products, Third Edition, 42
8. Hawkes, C. P., & Grimberg, A. (2015). Insulin-Like Growth Factor-I is a Marker for the Nutritional State. Pediatric Endocrinology Reviews : PER, 13(2), 499–511.
9. Ivonne Ruth Situmeang, Jerry Tobing, Maestro Simanjuntak, Paul Tobing, & Sanggam B. Hutagalung. (2024). Penyuluhan Perilaku Hidup Bersih dan Sehat (PHBS). Ikra-Ith Abdimas, 8(2), 240–243. https://doi.org/10.37817/ikra-ithabdimas.v8i2.3516

10. Kemenkes RI. (2020). Profil Kes Indo 2019. In Kementrian Kesehatan Republik Indonesia. https://pusdatin.kemkes.go.id/resources/download/pusdatin/profil-kesehatan-indonesia/Profil-Kesehatan-indonesia-2019.pdf
11. Kementerian Kesehatan Republik Indonesia. (2021). Ministry of Health Performance Report 2020. Ministry of Health of the Republic of Indonesia 2021, 1–224.
12. Kementerian Kesehatan RI. (2019). West Java Province Report, Riskesdas 2018. In Health Research and Development Agency Publishing Institution
13. Livingstone, C. (2012). The insulin-like growth factor system and nutritional assessment. Scientifica, 2012, 768731. https://doi.org/10.6064/2012/768731
14. Paramita, I. S., Atasasih, H., & ... (2022). Thel Elffelct of Giving Forelst Honely on thel Nultritional Statuls of Stulnting Toddlelrs in Kampar Relgelncy, Riaul Provincel. … Nultrition Joulrnal, 2(1), 33–41.
15. ABCAM. (2018). ElLISA gulidel. ElLISA Gulidel: Elvelrything Youl Neleld to Pelrform Youlr ElLISA Elxpelrimelnts.
16. Rismiati, M., Flora, R., & HM Zulkarnain. (2023 the Relationship Between Insulin Levels , Growth Factor . 1 With the Level of Intelligence in Elementary School Ch. 18(1), 81–86.
17. Smith, T. J., Johnson, C. R., Koshy, R., Hess, S. Y., Qureshi, U. A., Mynak, M. L., & Fischer, P. R. (2021). Thiamine deficiency disorders: a clinical perspective. Annals of the New York Academy of Sciences, 1498(1), 9–28. https://doi.org/10.1111/nyas.14536
18. Tegalwangi, K. (2022). Tegalwangi Village Principal Data
19. Tessema, M., De Groote, H., Brouwer, I. D., De Boevre, M., Corominas, A. V., Stoecker, B. J., Feskens, E. J., Belachew, T., Karakitsou, A., & Gunaratna, N. S. (2021). Exposure to aflatoxins and fumonisins and linear growth of children in rural Ethiopia: a longitudinal study. Public Health Nutrition, 24(12), 3662–3673. https://doi.org/10.1017/S1368980021000422
20. Tiwari, P., Jena, S., Satpathy, S., & Sahu, P. K. (2019). Nigella sativa: Phytochemistry, pharmacology and its therapeutic potential. Research Journal of Pharmacy and Technology, 12(7), 3111–3116. https://doi.org/10.5958/0974-360X.2019.00526.2
21. UNICEF, WHO, & WORLD BANK. (2021). Levels and trends in child malnutrition; UNICEF/WHO/World Bank Group-Joint child malnutrition estimstes 2021 edition. World Health Organization, 1–32. https://data.unicef.org/resources/jme-report-2021/
22. Wang, J., Zeng, Y., Ni, Z.-M., Wang, G., Liu, S.-Y., Li, C., Yu, C.-L., Wang, Q., & Nie, S.-F. (2017). Risk factors for low birth weight and preterm birth: A population-based case-control study in Wuhan, China. Journal of Huazhong University of Science and Technology. Medical Sciences = Hua Zhong Ke Ji Da Xue Xue Bao. Yi Xue Ying De Wen Ban = Huazhong Keji Daxue Xuebao. Yixue Yingdewen Ban, 37(2), 286–292. https://doi.org/10.1007/s11596-017-1729-5
23. Zhao, Q., Zhang, M., Ji, B., Chu, Y., Pan, H., Yan, W., & Ban, B. (2020). Relationship between hemoglobin and insulin-like growth factor-1 in children and adolescents with idiopathic short stature. BMC Endocrine Disorders, 20(1), 1–7. https://doi.org/10.1186/s12902-020-00600-w