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Leptin Level in Obese Children with Vitamin D Deficiency

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Abstract

INTRODUCTION: Vitamin D contributes to leptin synthesis through its effects on adipogenesis, the regulation of pro-inflammatory cytokines, and its effect on mRNA leptin.

AIM: The purpose of this study is to determine the correlation of leptin and Vitamin D deficiency and in children with obesity.

METHODS: This cross-sectional study was conducted from December 2019 to February 2020 and was chosen using multistage cluster random sampling with the target of junior high and high school students in Makassar city who met the obesity criteria. The subjects were divided into obese children with Vitamin D deficiency group and obese children without Vitamin D deficiency group.

RESULTS: The occurrence of hyperleptinemia in children with obesity and Vitamin D deficiency was 42 (82.4 %), while it was 1 (2.2%) in children with obesity but no Vitamin D deficiency, with a statistical analysis showing a value of p = 0.000 and an OR value of 205.333. The leptin levels' mean value in children with obesity and Vitamin D deficiency was 31.67 ng/mL, while it was 5.13 ng/mL in the group without Vitamin D deficiency. A Spearman correlation analysis of Vitamin D and leptin levels revealed a negative correlation with p = 0.000 and r = −0.817.

CONCLUSION: The level of leptin was higher in children with obesity and Vitamin D deficiency than in children with obesity but no Vitamin D deficiency.

Introduction

Obesity is a medical condition characterized by an abnormal buildup of body fat. This causes a slew of problems, including an increase in morbidity and mortality. There has been a significant increase in the number of obese people in recent years. Obesity is becoming more common in Indonesia. The percentage of overweight children has risen from 1.4% to 7.3%. Obesity affects 10.8% of children aged 13–15 in Indonesia, with 8.3% overweight and 2.5% obese [1].

Childhood obesity has been linked to a variety of medical conditions, including asthma, liver steatosis, cardiovascular disease, hypertension, glucose intolerance, and insulin resistance, menstrual disorders, and orthopedic issues [2]. Obesity is frequently associated with a lack of Vitamin D. Obesity-related parameters such as body mass index (BMI), waist circumference, and fat mass were found to be inversely related to serum 25 (OH) D3 concentrations (or body fat percentage). Obese people have lower Vitamin D levels, according to clinical and epidemiological studies [3].

Global estimates of Vitamin D deficiency in children and adolescents in 2008 ranged from 29% to 100%, and the survey discovered that it was related to the degree of adiposity, with Vitamin D deficiency occurring at 21% normal body weight, 29% overweight, 34% obese, and 49% very obese. As a result, obese children are more likely to be Vitamin D deficient, exacerbating the effects of obesity on overall health problems [4]. Obesity has been shown in several studies to cause oxidative stress and disruption in adipokine production. Leptin is an adipokine that plays a role in maintaining energy homeostasis in the body [5], [6].

Leptin is a pro-inflammatory cytokine that plays an important role in obesity, metabolic syndrome, and its components such as atherosclerosis. Leptin causes an inflammatory response by activating the long arm of the leptin (ob-R) receptor, which is expressed by various types of immune cells. Concomitantly, inflammation and certain infections, such as interleukin-1, lipopolysaccharide, and tumor necrosis factor, can raise leptin levels. As a result, the interaction between leptin and inflammation is a two-way direction, the pro-inflammatory cytokines increasing leptin synthesis and secretion of leptin will be act as a pro-inflammatory cytokine in obesity [7].

Increased leptin resistance causes reduced lipid oxidation in insulin-sensitive organs, which is linked to high levels of free fatty acids and inflammatory cytokines, resulting in lipid accumulation (lipotoxicity) and insulin resistance. Furthermore, leptin promotes macrophage cholesterol absorption, angiogenesis, platelet aggregation, and endothelial cell oxidative

stress, inhibits vasorelaxation, and raises the risk of atherosclerosis [8].

Obesity, which is defined by an increase in body fat mass cold, be rising of leptin levels. Leptin directly stimulates the synthesis of FGF23 which reduces renal tubular reabsorption. Leptin has also been shown to affect 1.25(OH)2D3 biosynthesis by downregulating 25-hydroxy Vitamin D 1a-hydroxylase. In addition, leptin can directly suppress the binding of circulating 25-hydroxy Vitamin D with 1a-hydroxylase (CYP27B1) and 1,25-hydroxy Vitamin D with 24-hydroxylase (CYP24) in the kidney and adipose tissue. Low Vitamin D levels can also increase adipogenesis by influencing preadipocyte cell transcription factors, which raise leptin levels [9]. Kaneko *et al*. previously reported that 1.25 (OH) 2 D3 directly suppressed leptin mRNA in 3T3-L1 adipose tissue [10]. Besides that, 1.25 (OH) 2 D3 strongly inhibits the activation of the nuclear factor kappa beta pathway and the (mitogen-activated protein kinase [MAPK]), MAPK signal, both of which are gene transcription factors for several pro-inflammatory factors [11]. Other studies, however, have shown that leptin can also inhibit Vitamin D synthesis. So that, it is critical to investigate the correlation between leptin and Vitamin D deficiency in children with obesity who are at risk.

The previous studies found that Vitamin D status and fat mass are inversely related. Recent intervention trials have shown that correcting low Vitamin D status caused by obesity can prevent a variety of metabolic diseases, lowering the risk of mortality and morbidity due to fatty tissue hemostasis failure. Existing research still yields erratic results in explaining the relationship between Vitamin D deficiency and pro-inflammatory cytokines, particularly leptin levels, implying that additional study is necessary.

Material and Methods

Study design

This cross-sectional study was conducted at Zion High School in Makassar, South Sulawesi, from December 2019 to February 2020. Blood samples were examined at the Hasanuddin University Medical Research Center Laboratory. Ethics approval has been obtained from the ethics committee for research in Humans of Faculty of Medicine, Hasanuddin University with approval number UH20070319.

Data were collected using *Multistage Cluster Random Sampling (total sampling).* Researchers obtained data from the Makassar City Education Office regarding junior and senior high schools which have students with middle and above socioeconomic levels. After *a* school *cluster* with a middle and upper socioeconomic level is obtained, then it is carried out randomly to select schools that will be used as research sites. The study population was obese children aged 11–17 years who have been screened through several criteria such as not having endocrine or renal disease (based on history taking and physical examination) and not consume anti tuberculosis, corticosteroid or anti-epileptic drugs. Informed consent was obtained in person from the parents/guardians of the participating students on the day of inclusion.

Sampling and laboratory methods

Bodyweight was measured with a standardized CEBA digital body scale, and body height was measured with a microtome with 0.1 cm accuracy. Obesity was evaluated using the BMI and classified using the National Center for Health Statistic (CDC-NCHS) growth chart for children >2 years based on age and sex; children were classified as obese if their BMI was higher than the 95th percentile. The puberty classification based on physical examination and classified by I–V of Tanner scale.

The child who includes all the study criteria must having fasting 8–12 h before blood sample was taking. Serum Vitamin D (25[OH]D) level measured by using the Human Vitamin D (VD) enzyme-linked immunosorbent assay (ELISA) assay method and Leptin level measured by Leptin Human ELISA Kit (Sigma, No. catalogue RAB0333). Child who had (25[OH]D) level < 20 ng/ml categorized with Vitamin D deficiency. The study subjects were grouped into two main groups, obese children with Vitamin D deficiency and obese children without Vitamin D deficiency. All of them will be grouping again, based on leptin level (hyperleptinemia > 20 ng/ml and not hyperleptinemia < 20 ng/ml).

Statistical analysis

The data analysis was using SPSS statistic for Windows, version 23. 90 (IBM Co., Armonk, NY, USA). Data characteristics such as frequency, distribution, mean, median (range), and standard deviation, while Chi-square or Fisher Exact test was used to determine the significance of incidence of Vitamin D deficiency and hyperleptinemia. The risk factor was determined using Crude odds ratio (OR) analysis with 95% confidence interval (CI). Statistical significance was indicated with $p \leq 0.05$.

Results

A total of 96 obese children were classified into

Vitamin D deficiency group (51 children) and the without Vitamin D deficiency group (45 children). According to sex, there were 32 boys (62.7%) and 19 girls (37.3%) in the Vitamin D deficiency group. Meanwhile, there were 25 boys (55.6%) and 20 girls (44.4%) in the group with no Vitamin D deficiency (Table 1). Statistical analysis revealed that there was no significant difference in sex distribution between the two groups with p = 0.474 (p > 0.05). In the Vitamin D deficiency group, the age median was 14.00 years, while in the no Vitamin D deficiency group was 13.75 years. There was no significant difference age between the two groups with p = 0.579.

Table 1: The characteristic sample based on Vitamin D status

	Vitamin D		p-value
	Deficiency	Without Deficiency	
Sex			0.474*
Male	32 (62.7%)	25 (55.6%)	
Female	19 (37.3%)	20 (44.4%)	
Age			
Mean	13.82	14.34	0.579***
Median	14.00	13.75	
Standard Deviation	1.16	1.75	
Minimum–Maximum	12.08–17.08	11.83–17.92	

*Chi-square, **Fisher's exact test, ***Mann–Whitney test.

The incidence of hyperleptinemia in male with obesity was 25 (58.1%) while in female with obesity were 18 (41.9%). There was no significant difference in the occurrence of hyperleptinemia in children with obesity based on sex (p= 0.824). The occurrence of hyperleptinemia in children with obesity and Vitamin D deficiency was 42 (97.7%), while in children with no Vitamin D deficiency was 1 (2.3%) (Table 2). A very significant difference was found in the occurrence of hyperleptinemia between the Vitamin D deficiency and not deficiency group (p = 0.000). The OR = 205.333 (CI 95% from 24.924 to 1691.615) means that the hyperleptinemia risk in children with obesity and Vitamin D deficiency was 205.333 times higher than in children with obesity but no Vitamin D deficiency.

Table 2: The characteristic sample based on leptin status

	Leptin status		p-value
	Hyperleptinemia	No hyperleptinemia	
Sex			0.824*
Boys	25 (58.1%)	32 (60.4%)	
Girls	18 (41.9%)	21 (39.6%)	
Puberty Status			
Pre-Puberty	0 (0.0%)	2 (3.8%)	0.500**
Puberty	43 (100%)	51 (96.2%)	
Age (years)			
Mean	13.66	14.39	0.083***
Median	13.67	14.00	
Standard Deviation	1.14	1.64	
Minimum–Maximum	12.08–17.08	11.83–17.92	
Vitamin D status			0.000*
Deficiency	42 (97.7%)	9 (17.0%)	OR = 205,333 (95%
Not Deficiency	1 (2.3%)	44 (83.0%)	CI 24,924–1691,615)

*Chi-square, **Fisher's exact test, ***Mann–Whitney test.

The leptin levels' mean value in children with obesity and Vitamin D deficiency was 31.67 ng/mL, while in children with obesity but no vitamin deficiency was 5.13 ng/Ml (Table 3). The statistical analysis using the Mann–Whitney test showed there was a significant difference between these two groups with P = 0.000. Based on the Spearman correlation analysis,

Table 3: Leptin levels between obese children with Vitamin D deficiency and no Vitamin D deficiency

Leptin levels	Vitamin D Status		p-value
	Deficiency (n = 51)	Not Deficiency (n = 45)	
Mean	31.67	5.13	0.000***
Median	31.11	2.57	
Standard Deviation	11.00	5.60	
Minimum–Maximum	13.92–55.27	1.17–28.29	

***Mann–Whitney.

Vitamin D and leptin levels had a negative correlation with P = 0.000, indicating a very strong correlation −0.817) (Table 4).

Table 4: Correlation of Vitamin D levels with leptin levels in obese children

	Leptin levels
Vitamin D levels	r = −0.817 p = 0.000 n = 96

****Spearman.

Discussion

According to this study, hyperleptinemia was found in 82.4% of obese children with Vitamin D deficiency and 2.2% of obese children without Vitamin D deficiency. Leptin level was higher in children with obesity and Vitamin D deficiency than in children with obesity but no Vitamin D deficiency.

We found 1 child (2.2%) with obesity and Vitamin D deficiency who had not developed hyperleptinemia in this study. It is possible that this is due to the length of time the study subjects were obese and deficient in Vitamin D, which was not investigated. Therefore, the degree and duration of obesity, as well as the duration of Vitamin D deficiency, did not significantly contribute to hyperleptinemia. However, because the process will continue, it is critical to monitor Vitamin D and leptin levels and treat obesity at a young age. On the contrary, we discovered 9 (19.6%) obese children who did not have Vitamin D deficiency but had hyperleptinemia. Aside from Vitamin D intervention, other mechanisms can contribute to the occurrence of hyperleptinemia in obese children. As previously stated, plasma leptin levels are determined by the presence of a large number of adipocytes in comparison to the general population. This is also demonstrated by the study's findings, which show that the median leptin value of the study subjects was 17.70 ng/ml, which is significantly higher than the normal value of leptin in children of normal weight, which is 3 ng/ml [12].

In this study, we found a significant difference in leptin levels between the Vitamin D deficiency and not deficiency. Mateani *et al*. discovered that individuals with 25 (OH) D levels >100nM have a lower leptin level [13], which is consistent with our findings of a significant relationship between increased body mass

index, low 25-OH-VitD levels, and increased plasma leptin concentration.

Several randomized controlled trials have also investigated the correlation between Vitamin D on leptin levels. A previous study which looked at the effect of giving oral Vitamin D to obese rat samples, found that it significantly reduced leptin levels (p = 0.001) [14]. Similarly, Tabesh *et al*. in Iran found that giving oral Vitamin D to diabetes mellitus patients reduced plasma leptin levels with p = 0.01 value [15]. Vitamin D interferes with pro-inflammatory cytokine of nuclear transcription factors by discovering Vitamin D receptors in the promoter region of cytokine genes.

Aside from inhibiting leptin synthesis through its effect on adipogenesis, Vitamin D also inhibits leptin synthesis through its anti-inflammatory effect. Increased pro-inflammatory cytokines stimulate leptin secretion in obese patients [7]. Vitamin D inhibits NF-B and the MAPK, both of which are gene transcription factors for several pro-inflammatory factors. Nobre *et al*. in a study that assessed the state of hyperleptinemia and administration of Vitamin D to 3T3L1 preadipocyte culture cells discovered that incubating these two hormones was able to neutralize Cyp27b1/1 a - hydroxylase but reduced Vitamin D sensitivity in adipose tissue [16].

According to the American Academy of Pediatrics (AAP) and the Institute of Medicine, children aged 0–12 months should receive 400 IU of Vitamin D/day, and children aged > 12 months should receive 600 IU/day. The AAP recommends giving high doses of Vitamin D for 2–3 months, as much as 1000 IU/day for neonates, 1000–5000 IU/day for children aged 1–12 months, and 5000 IU/day for children aged >12 months, in the therapies of Vitamin D deficiency. After 2–3 months of therapy, control 25 (OH) levels were checked and supplemented at a maintenance dose of 400 IU/day. Monitoring levels of 25 (OH) can be checked again after 3 months of initial therapy, and if the levels are normal, monitoring levels of 25 (OH) can be checked again 6 months or 12 months later [17].

Several guidelines have also been made in light of the high incidence of Vitamin D deficiency in obese children. AAP recommendation to those Vitamin D deficiency patients with 5,000 IU/day Vitamin D 2000 IU/day or 50,000 IU/week for at least 6 weeks or until a serum concentration of 25 (OH) D above 20 ng/mL is achieved, according to the Endocrine Society Guidelines. According to recent studies, giving 25,000 IU of Vitamin D per week for 9 weeks to obese people who are Vitamin D deficient is well tolerated [17].

This study's limitation was that it was using a cross-sectional design, there were not any analyze genetic factors, nor the obesity's degree and the Vitamin D deficiency's duration, and the dietary factors and physical activity that could affect Vitamin D levels in the blood were not analyzed. This study also did not analyze the amount of sun exposure that affects the formation of Vitamin D from keratinocyte cells.

The study's limitations can be mitigated by using samples from the same ethnic population. Furthermore, to reduce bias in dietary factors and physical activity, epidemiological assumptions can be made by collecting samples from middle and high socioeconomic schools. A causal relationship between obesity, Vitamin D, and hyperleptinemia could not be established because the data collection was done at the same time of sampling. Thus, even though a child is not yet suffering from Vitamin D deficiency or hyperleptinemia, it is important to keep track of their Vitamin D and leptin levels. This aims to treat early the incidence of Vitamin D deficiency and hyperleptinemia conditions that can occur in obese children and prevent the effects of hyperleptinemia. The strength of this study is that the participants were chosen at random from Makassar schools with middle and high economic status, allowing it to define the health conditions of children in the Makassar region.

Conclusion

This study concludes that leptin levels in children with obesity and Vitamin D deficiency were higher than in children with obesity but no Vitamin D deficiency. In obesity, there is an accumulation of adipose tissue, changes in adipokine secretion, and the formation of hypertrophic and hypoxic adipocytes that will cause the release of pro-inflammatory cytokines. Leptin is formed by the accumulation of adipose tissue and is also exacerbated by the presence of pro-inflammatory cytokines. Besides, obese children are at high risk of Vitamin D deficiency due to sequestration in fat tissue and decreased bioavailability of Vitamin D, which, in turn, will affect leptin synthesis.

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Author Contributions

There is no financial grant or funding that can be reported in this study. AJ: Conceptualization, Writing-Reviewing and Editing the manuscript. SHP: Data curation, Writing-Original draft preparation,

Visualization, Investigation. IJG: Methodology, Software, Validation. All of the authors have approved the manuscript for publication.

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