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The Correlation between 25-Hydroxyvitamin (OH) D Levels and Anemia in Paediatric Patient under 6 Years Old with Family History of Atopy

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KEYWORDS

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ABSTRACT:

Introduction: Children atopic disease is associated with anemia because multiple comorbid chronic inflammation can lead to anemia. In other hand, there was association between Vitamin D and anemia. Children with atopic disease also could run into vitamin D deficiency.

Objectives: The prevalence of low Vitamin D level in atopic children found to be higher than healthy children. Lack of Vitamin D and anemia in children, especially those with atopy disease, will affect their growth, development, and immunological functions. Therefore, this study investigates the relationship between 25hydroxyvitamin D (OH) D levels and anemia in pediatric patients with a family history of atopy

Methods: A Cross sectional study with purposive sampling in Diponegoro University Hospital, Semarang, Indonesia from June - October 2021, a total of 78 pediatric patients aged 6 months - 6 years meeting the inclusion criteria were enrolled in the study.

Results: Both subject with deficiency 25(OH)D levels and anemia was found in 3.8% patients. The result showed from nutritional diary analysis (3 days food recall) that most of the pediatric patients had deficient level of iron (62.8%) and calories (62.8%) intake, but most of them also had sufficient intake of zinc (85.9%), protein (98.7%), and fat (52.6%).

Conclusions: There was no correlation between 25(OH)D levels and anemia in subjects, also between nutrition $intake\ and\ type\ of\ atopic\ disease\ with\ anemia\ in\ subjects.\ This\ is\ \overline{like} ly\ because\ the\ association\ is\ multifactorial$ in nature. Further research is warranted to elucidate the underlying mechanisms and clinical implications of this results.

Childhood atopic disease is associated with chronic systemic inflammation, therefore multiple comorbid chronic inflammation can be found, such as iron deficiency and disruption of iron homeostasis that leads to anemia [1,2]. Some patients with atopic disease are

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also at higher risk for low bone mineral density that affect the erythropoiesis process. All these factors are associated with anemia. Study from US found that atopic disease such as eczema, asthma, hay fever, and food allergy is associated with increased odds of anemia, with prevalence was 9.5% for eczema, 12.8% for asthma, 17.1% for hay fever, 4.2% for food allergy, and 1.1% for anemia [2]. Meanwhile another study from Korea showed that the odds ratio of iron deficiency anemia was significantly higher across patient with atopic disease, which even higher in children with multiple atopic disease, and associated with three kinds of atopic disease (atopic dermatitis, allergic rhinitis, and asthma) [3]. Beside the condition of atopic disease, anemia also caused by insufficient nutritional intake, especially iron. Children go through periods of rapid growth, then the exogenous diet of iron should adequate to facilitate more red blood cells (RBC) needed, specially between 6 to 23 months of age. The need of RBC also increasing if the child run into chronic disease such as atopy [4].

In another hand, vitamin D serves a significant function in metabolism, nutritional well-being, and influences immune responses to allergens by interacting with Vitamin D receptors found on numerous immune cell types such as B cells, T cells, dendritic cells, and macrophages. Vitamin D also plays a crucial role in improving anemia and iron levels by stimulating the growth of precursor cells for red blood cells, increasing the expression of erythropoietin receptor mRNA, and boosting red blood cell production. Additionally, the involvement of vitamin D in iron metabolism has been linked to its ability to suppress proinflammatory cytokines, which are known contributors to the development of anemia [5,6]. Numerous elements, including nutritional intake, race, sex, sun exposure, and obesity, might impact children's vitamin D levels [7].

However, lack of vitamin D was found in all age groups including children, belonging to different factors, one of them is insufficient nutritional intake. The conditions of Vitamin D deficiency and run into anemia in children, especially under 5 years old, will affect their growth, development, and immunological functions. Patients with known food allergies, such as those with cow's milk allergies, may experience vitamin D insufficiency due to reduced intake from strict avoidance of a particularly good source of vitamin D [8]. Although micronutrient deficiencies in vitamin D were the main cause of atopic

diseases, which can also result in anemia through a different pathogenesis, it's important to remember that an excess of these micronutrients can also cause inflammation through different mechanisms and may also play a role in the development of atopic diseases [9].

2. Objectives

A study amongst Indonesian children has found that the prevalence of Vitamin D deficiency was 33% [10], and many studies stated that its prevalence was higher in children with allergic disease. A study among atopic pediatric population found that most of the subjects had insufficient vitamin D level (68%), 17% had deficient level, and only 15% had normal level of Vitamin D [11]. Similar results also found among children with atopic dermatitis (66.7% with vitamin D deficiency and mean value of 15,51 ± 3,01 ng/ml), among children with asthma (75.9% subjects had vitamin D level <30 ng/ml). among children with cow's milk allergy (55%) and other food allergy (62%) [12-14]. Therefore, numerous evidences showed that children with vitamin D insufficiency and deficiency generally also develop atopy, which raises the risk of iron deficiency anemia [14]. However, there haven't been studies definitively assessing whether the persistent inflammatory state seen in atopic diseases affects the likelihood of developing

Atopy refers to a tendency towards an immune response to different antigens and allergens, resulting in the differentiation of CD4+ Th2 cells and an overproduction of immunoglobulin E (IgE). This leads to an increased likelihood of hypersensitivity or allergic reactions in clinical settings [15]. Atopic conditions develop due to a combination of individual genetic susceptibility and exposure to environmental factors. Literature stated that there was a genetic contribution to developing allergies in 50% cases of children with atopic disease, and the heritability estimates ranging from 36-79% [15]. Therefore, the subjects of our study were pediatric children under 6 years old with any family history of atopic disease. The aim of his study was to investigated the relationship between levels of 25-hydroxyvitamin (OH) D and anemia in children under 6 years old with a history of atopy in the family.

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3. Methods

In accordance with the ethical precepts outlined in the Declaration of Helsinki, this study was carried out at Diponegoro University Hospital in Semarang after the protocol (Protocol Number No. 97/EC/KEPK/FK-UNDIP/IV/2021) was approved by the hospital's ethics committee. Retrospective analysis of the data was done from June - October 2021. The design of this study is cross-sectional. The data was collected between June -October 2021 with purposive sampling. This study's inclusion criteria were: pediatric patient aged 6 months until 5 years, there was a history of atopic disease in the patient's family (parents and sibling) for asthma, allergic rhinitis and eczema. Exclusion criteria for this study were: patients who were diagnosed with any chronic disease other than atopic disease, patients with current infection, and patients without detailed medical data

Primary data, such as iron, zinc, calories, protein, and fat consumption were collected by 3 days food recall methode from each of pediatrics patient's parent. These recall methods involve gathering information such as dietary histories, completing food frequency questionnaires, and conducting multiple daily recalls [16]. The level of each nutriotion intake then categorized as sufficient and deficient, based on Regulation of the Minister of Health of Indonesia number 28 of 2019 about Recommended Nutritional Adequacy Figures for the Indonesian Community, calculated per person per day. For children age 6 - 11 months, the sufficient recommendations for nutritions are >800 kkal calories, >35 g protein, >35 g fat, >11 mg iron, and >1.1 mg zinc. For children age 1 - 3 years old, the sufficient recommendations for nutritions are >1350 kkal calories. >45 g protein, >45 g fat, >7 mg iron, and >3 mg zinc. For children age 4 - 6 years old, the sufficient recommendations for nutritions are >1400 kkal calories, >50 g protein, >50 g fat, >10 mg iron, and >3 mg zinc. For the history of exclusive breatfeeding, the data was asked from the parents.

Both level of vitamin D and Haemoglobin was collected via blood sampling using aseptic technique. Three ml of venous blood sample from each subject were collected on EDTA coated plastic tubes and runs through hematology analyzer. In order to measure vitamin D levels, 25-OH-D3 Elisa kits were used [17]. In this

research, the vitamin D status was categorized as deficiency, insufficiency, and normal. Vitamin D deficiency was defined as levels less than 20 ng/ml, insufficiency as less than 30 ng/ml, and normal levels as between 30 and 50 ng/ml.

The characteristics of the data were analyzed univariately. The levels of 25-hydroxyvitamin D, hemoglobin levels, history of atopy, adequacy of breastfeeding, adequacy of iron intake, adequacy of zinc intake, adequacy of protein intake, and adequacy of fat intake were presented categorically with n and percentage. For numerical data with two categories, a normality test was conducted, followed by an independent t-test and Mann-Whitney test if the data were not normally distributed. For data with more than two categories, if normally distributed, a one-way ANOVA was conducted, and if not normally distributed, a Kruskal-Wallis test was conducted. The relationship between variables was assessed using the chi-square test. In particular, Vitamin D level and Haemoglobin level were presented numerically. For those numeric data a normality test was coducted using Kolmorgov-Smimov, and if not normally distributed, the correlatiion was assessed using Spearman's test. Statistically significant values were defined as p-values less than 0.05, using the device IBM SPSS statistical software version 25.

4. Results

During the study period, a total of 78 pediatric patients, who had a history of atopy in the family members, were included in this study. Demographic and clinical categorical data are summarized in Table 1, representing all gender, pediatrie age, patient's atopic type, father's history of atopy, mother's history of atopy, sibling history of atopy, 25(OH)D levels, hemoglobin level, iron sufficiency, zinc sufficiency, calories sufficiency, protein sufficiency, fat sufficiency, and exclusive breastfeeding status. Among the 78 patients, 47 (60.3%) were males and 31 (39,7%) were females. The age range of the patients varied from 6 months to 6 years.

For the atopic disease, 28.2% (n = 22) of the patients did not manifest any atopic type until this research took place. Among 22 patients, 81% (n = 18) of them had a history of atopic disease in one of their families, and the rest (19%; n = 4) had a atopic disease found in 2 members of their families. None of pediatric patients without atopy come without any history of atopic disease

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in their family, nor found atopic disease manifested to all of their family members. The same results were shown in the family's history of atopic disease, most of the pediatric family members did not manifest any atopic disease. Total of 52.6% (n = 41), 39.7% (n = 31), and 96.2% (n = 75) of the pediatric patients did not have father, mother, nor siblings with atopic disease, respectively. However, 71% (n = 56) of total participants still manifested atopic disease, moreover 32% (n = 18) of them had more than 1 atopy. This research also found that most of the pediatric mothers had a history of atopy (60.3%; n = 41), then only 70.7% of them passed down the atopy to their children.

The results also showed that most of the pediatric patients had normal 25(OHD) levels (76.9%), and mayority of them (96.2%) were not categorized as anemia. Except for calories and iron intake, most of the patients had sufficient intake of zinc (85.9%), protein (98.7%), and fat (52.6%) intake. The result showed that most of the pediatric patients had deficient level of iron (62.8%) and calories (62.8%) intake. For the exlusive breastfeeding status, 59 (75.6%) subjects were done exclusive breastfeeding.

This study also investigated the distribution between vitamin D level and haemoglobin level with atopy manifestation in children showed in table 2. It was found that most of children with atopy manifestation has a normal level of vitamin D and haemoglobin. Besides that, we also found that all of children with vitamin D deficiency also had atopic allergies. Meanwhile from tabel 3, we can conclude that there was no significant correlation between any of the nutrition intake with the haemoglobin level. In table 4-5, an analysis of corellation between numeric data of Vitamin D levels and Hemoglobin levels was presented using Spearman's test correlation. There were no independently variable that significant correlation was found (CI 95%; p>0.05) either for all subjects or only subjects with manifestation included.

Table 1. Characteristics of Subjects

Variables	Frequency (%)	p
24	(N=78)	(CI 95%)
Gender		
Male	47 (60.3)	
Female	31 (39.7)	

Age		
6 month – 1 years	14 (17.9)	0.509
1 – 3 years	38 (48.7)	
3 – 6 years	26 (33.3)	
Atopic type	20 (33.3)	
None	22 (28.2)	
Atopic dermatitis	18 (23.1)	
Allergic rhinitis	11 (14.1)	
Food alergy	4 (5.1)	
Urticaria	4 (5.1)	
Asthma	1 (1.3)	
More than 1 atopic	18 (23.1)	
Father's history of	()	
atopy		
None	41 (52.6)	0.291
Atopic dermatitis	4 (5.1)	
Allergic rhinitis	15 (19.2)	
Food alergy	5 (6.4)	
Urticaria	3 (3.8)	
Asthma	1 (1.3)	
More than 1 atopic	9 (11.5)	
Mother's history of		0.849
atopy		
None	31 (39.7)	
Allergic rhinitis	19 (24.4)	
Food allergy	8 (10.3)	
Urticaria	6 (7.7)	
Asthma	1 (1.3)	
More than 1 atopic	13 (16.7)	
Siblings history of		
atopy		
None	75 (96.2)	1.000
Atopic dermatitis	1 (1.3)	
Allergic rhinitis	1 (1.3)	
Food alergy	1 (1.3)	
25-OH D levels		
Deficiency	3 (3.8)	
Insuficiency	15 (19.2)	
Normal	60 (76.9)	
Hemoglobin levels		
Anemia	3 (3.8)	
Normal	75 (96.2)	
Iron sufficiency		

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Sufficient	29 (37.2)	0.409 [£]
Deficient	49 (62.8)	
Zinc sufficiency		
Sufficient	67 (85.9)	0.764 [£]
Deficient	11 (14.1)	
Calories sufficiency		
Sufficient	29 (37.2)	0.409 [£]
Deficient	49 (62.8)	
Protein sufficiency		
Sufficient	77 (98.7)	0.964 [£]
Deficient	1 (1.3)	
Fat sufficiency		
Sufficient	41 (52.6)	0.736 [£]
Deficient	37 (47.4)	
Exclusive breatfeeding		
Yes	59 (75.6)	0.573 [£]
No	19 (24.4)	

Notes: [£] Fisher's exact; [†] Mann-Whitney; [§] Independentt; [§] Fisher's exact (joincells); [†] Continuity Correction (joincells)

Table 2. Distribution of Vitamin D and Haemoglobin Level with Atopy Manifestation in Children

Category	Atopy Manifestation		
_	Yes	No	
Anemia	2	1	
Normal Hemoglobin Levels	54	21	
Vit. D deficiency	3	0	
Vit. D insufficiency	9	6	
Vit. D Normal Level	44	16	

Table 3. Characteristic of intake status and correlations with hemoglobin levels

Variables	Median (min – max)	p (CI 95%)
Iron intake	6,15 (1,6 – 30,2)	0.178‡
Zinc intake	5,5 (2,4 – 17,6)	0.566‡
Calories intake	1183.15 (556 – 1894,9)	0.304§
Protein intake	39,25 (20 – 90)	0.488§
Fat intake	43,85 (17,9 – 86,5)	0.171‡

Notes: \$ Spearman; \$ Independent-t

Table 4. Correlation of Vitamin D and Haemoglobin Level with Spearman's in total subjects

Variable	Mean ±	p	r
	SD		
25 (OH)D level	43,49 ±	0,328	0,112
	18,57		
Haemoglobin	11,94 ±		
level	1.02		

Table 5. Correlation of Vitamin D and Haemoglobin Level with Spearman's in subjects with allergy manifestation only

Variable	Mean ±	p	r
	SD		
25 (OH)D level	43,38 ±	0,063	0,250
	19,05		
Haemoglobin	11,94 ±		
level	1,06		

5. Discussion

The study's findings revealed no significant correlation between the nutritional intake, adequacy, vitamin D level and hemoglobin levels in pediatric patients with a history of atopy.

Children with atopic conditions such as atopic dermatitis, food allergies, rhinitis, and asthma often suffer from deficiencies in both macronutrients and micronutrients. Iron deficiency, in particular, can affect immune function, along with deficiencies in zinc and vitamin D [18]. Previous studies have indicated a higher prevalence of anemia in children with atopic diseases compared to those without atopic [1,3]. According to epidemiological studies in the United States, children with atopic diseases are 8 times more likely to experience anemia than children who do not have allergies [1,2,19]. Contrary to those study's results, most children with atopy and a family history of atopy in this study showed normal hemoglobin levels for their age. Additionally, research conducted by Sayyed, et al showed that the administration of vitamin D did not lead to improvements in hemoglobin levels [20].

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However, maintaining adequate levels of vitamin D is still crucial for children's overall health, even though this study did not find a direct impact on hemoglobin levels. To mainaining adequate level of Vitamin D, it is known that most abundant source of Vitamin D is from cutaneous syntesis on UV-B exposure from sunlight. However, some dietary source also known to contributing for Vitamin D level. Fatty fish, beef liver, egg yolk, and dairy products are some dietary sources that were found to influence the attianment of adequate Vitamin D [21,22].

As the most common hematologic abnormality identified in almost half of children under 5 years old, anemia has many causes, both inherited and acquired [23,24]. Iron deficiency is considered the most common cause of anemia, but other nutritional deficiencies such folate acid, vitamin B12, and vitamin A, also affect hemoglobin synthesis, red blood cell production, or red blood cell survival, leading to anemia conditions [4,25]. Research by Kiyon Rhew suggests a relationship between serum 25(OH)D levels with iron status and anemia in children with malnutrition and non-malnutrition in the United States [3]. In the malnutrition group, hemoglobin concentrations were significantly lower in children with lower 25(OH)D levels. In the non-malnutrition group, children with low 25(OH)D concentrations were less likely to experience anemia compared to children with higher 25(OH)D concentrations. Unlike the findings of this study where children with insufficient iron and calorie intake had normal hemoglobin levels, children with adequate zinc and protein intake also had normal hemoglobin levels. Thus, adequate nutrition in terms of iron, zinc, calories, protein, and fat did not significantly affect hemoglobin levels in this study.

The exact mechanism regarding the relationship between atopic diseases and anemia remains unknown. This is likely because the association between atopic diseases and anemia is multifactorial in nature [7]. Regardless of the underlying cause, it's vital to acknowledge the association between atopic diseases and anemia. Therefore, it is important to maintain adequate intake and nutritional adequacy of both macronutrients and micronutrients in children.

This study found that only 3 patients (3.84%) among all of the subjects that undergo anemia and had deficent level of Vitamin D (table 3). However, 2 of 3 patients

with anemia diagnosed with atopy. This finding was similar with many studies that stated about higher prevalence of anemia was found in children with allergic disease [1–3,19,26,27]. The same circumstance also happened to the patients with Vitamin D deficiency level, that all of them was found to have atopic manifestation. This finding was also similar with most of previous studies that found higher frequency of lack in Vitamin D level among children with allergic, rather than the control group [13,28]. This condition could happened due to low intake of vitamin D source of diet such as fatty fish, beef liver, egg yolk, and dairy products which are known to be the some of common food that trigger some allergic manifestations, such as atopic dermatitis [29].

This study also found that there was no correlation between Vitamin D level and anemia status that showed by hemoglobin levels among total subjects (table 4). The same results also found among pediatric patients diagnosed with atopic disease (table 5). This result was contradicted with a cross-sectional study that was conducted in South Korea [3]. Previous study suggested that atopic disease (atopic dermatitis, alergic rhinitis, and asthma) was associated with iron deficiency anemia because all atopic disease demonstrated a significant, positive association with iron deficiency anemia before and after applying covariates. The study also revealed a higher prevalence of iron deficiency anemia among pediatric patients with atopic diseases compared to those without such conditions, contrary to the result of this study. The same pattern of results also found in a large US-population-based pediatric surveys [2].

The exact mechanism regarding the relationship between atopic diseases, anemia, and the level of Vitamin D remains unknown. However, previous study suggested that anemia in pediatric patient with atopic disease happened because the inflammatory immune activation, which involve role of vitamin D, that happened for long period of time [1,3]. Some difference results found in this study may happened because of the range of inflammatory process by the atopic disease in our subjects was shorter, only between 6 months until 5 years, compared with previous study, who concluded pediatric patients up to 18 years old. This condition could be the reason about low prevalence and no association was found between anemia and type of atopic disease in the patients.

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Most of our subjects has normal level of 25(OH)D (76.9%) and within normal range of hemoglobin (96.2%). Another study explained that children who were deficient in iron exhibited a higher prevalence of Vitamin D deficiency compared to those who had adequate iron levels. Therefore, no corellation was found between anemia and 25(OH)D deficiency in this study due to the low prevalence of low hemoglobin level among the subjects. A study held in Africa found a similar results with this study [30]. They stated that there was no statistically significant association between iron deficiency and anemia in children with low vitamin D status. The same results also found in this study, although 62.8% of the subjects were categorized in iron deficiency status.

It is important to consider that the active form of vitamin D can impact erythropoiesis by promoting the proliferation and maturation of erythroid progenitor cells. Consequently, a deficiency in calcitriol could potentially impair erythropoiesis, which in turn could affect iron status [20]. Condition of low iron status or anemia will affect the growth and development of children, especially in pediatric patient with inflammatory illness such as atopic disease. Sufficient level of 25(OH)D still need to be achieved in pediatric patients to improve their immune system while dealing with the atopic disease. The limitations of this study were that the subjects' recent onset of atopic history may not have sufficiently impacted hemoglobin levels to induce anemia. Nutritional assessment based solely on questionnaires necessitates supplementation with laboratory examinations to provide quantitative clarity.

For the conclusion, there are no corellation was found between 25(OH)D levels, nutrition intake, insufficient nutrition, exclusive breast feeding, and type of atopic disease with hemoglobin levels in subjects (CI 95%; p>0.05). The exact mechanism regarding the relationship between 25(OH)D level and anemia in toddlers with atopic disease or toddler's history of family with atopic disease is remains unknown. Further research is warranted to elucidate the underlying mechanisms and clinical implications of this result. However, sufficient level of 25(OH)D, hemoglobin, and adequate intake of both macronutrients and micronutrients still need to be achieved in pediatric patients to improve their immune system to optimize their growth and development, while dealing with the atopic disease.

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Conflict of Interests

None declared

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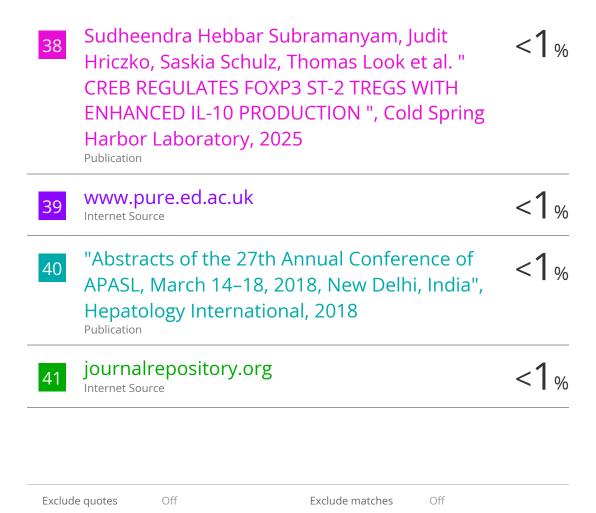
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