JMCH Journal of Maternal and Child Health (2023), 08(01): 12-22
Masters Program in Public Health, Universitas Sebelas Maret
Meta-analysis

The Effect of Vitamin D Deficiency on Anemia in Children and Adolescents: Meta-Analysis

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ABSTRACT

Background: Anemia in children and adolescents has an impact on central nervous system damage that can continue into adulthood. Some evidence states that one of the causes of anemia is vitamin D deficiency. This study aims to estimate the magnitude of the effect of vitamin D deficiency on the incidence of anemia in children and adolescents, through a meta-analysis of primary studies conducted by previous authors.
Subjects and Method: This study is a systematic review and meta-analysis with the following PICO, population: children and adolescents, intervention: vitamin D deficiency, comparison: no vitamin D deficiency, outcome: anemia. The articles used in this study were obtained from three databases, namely PubMed, Google Scholar, and Science Direct, using the search key "Vitamin D" OR "25-Hydroxy Vitamin D" OR "25(OH)D" AND "Anemia" OR " Anemia". The included article is a full-text cross-sectional study design from 2010 to 2022 and reports the adjusted odds ratio (aOR) in multivariate analysis. The selection of articles is done using PRISMA flow diagrams. Articles were analyzed using the Review Manager 5.4 application.
Results: A total of 9 cross-sectional studies involving 18,608 children and adolescents from the Americas and Asia were selected for meta-analysis. Children and adolescents with vitamin D deficiency had a risk of anemia 2.87 times compared to those without vitamin D deficiency (aOR= 2.87; 95% CI= 2.36 to 3.48; p<0.001).
Conclusion: Vitamin D deficiency increases the risk of anemia in children and adolescents.

Keywords: vitamin D deficiency, anemia, children, adolescents

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Cite this as:
Dianika B, Dewi YLR, Murti B. (2022). The effect of vitamin d deficiency on anemia in children and adolescents: Meta-Analysis. J Matern Child Health. 08(01): 12-31. https://doi.org/10.26911/thejmch.-2023.08.01.02.

BACKGROUND

WHO has targeted that in 2025 there will be a 50% reduction in anemia rates from 2012, but the prevalence rate of anemia from 2015 to 2019 continues to increase. In 2019, the prevalence of anemia in women of reproductive age globally reached 29.9%, while in pregnant women it was 36.5%. Likewise for children aged 6-59 months, the prevalence rate of anemia in 2019 was 39.8% (WHO, 2021).

One group that is prone to anemia is children and adolescents. Anemia in children and adolescents is caused by increased iron requirements during periods of rapid growth, low iron stores at birth, non-exclusive breastfeeding, inappropriate complementary feeding, low quantity plant-based fee-

ding, poor iron bioavailability due to high inhibitor content, iron loss due to infectious diseases, weak iron absorption due to poor hygiene (Kapil, 2019).

There is some evidence that anemia is significantly associated with damage to the central nervous system. This damage can continue into adulthood. Anemia is also independently known to have a relationship with cognitive decline in terms of intelligence, impaired language coordination, skills, poor attention, and decreased memory and learning ability (Yehuda et al., 2009). In fact, according to Kumar (2014), anemia is the second highest cause of disability in the world, because anemia can cause various kinds of complications, including decreased endurance, cognitive impairment, stunted growth, decreased activity, and changes in behavior

The magnitude of the problems caused by anemia requires an analysis of risk factors in prevention efforts. Malnutrition is one theory that is believed to be one of the causes of anemia. Vitamin D is one of the micronutrients that is often associated with bone health, but recently, researchers have found a correlation caused by vitamin D deficiency to anemia

Red blood cells require the process of erythropoiesis to form mature cells in sufficient numbers so that anemia does not occur. Given that erythropoiesis occurs in the bone marrow, this allows for the role of vitamin D deficiency in anemia. This theory is in line with Lee's research (2014), from 2526 children and adolescents aged 10-20 years, it was found that there was a relationship between vitamin D deficiency and an increased risk of anemia (aOR= 2.15; 95% CI= 1.13 to 2.88). Likewise, Atkinson (2014), found that vitamin D deficiency had a risk of increasing the incidence of anemia in children (aOR= 1.93; 95%CI= 1.21 to 3.08)

The results of several primary studies that have been carried out need to be processed again to obtain a higher level of statistical significance. This data processing is carried out by focusing on statistical summaries aimed at drawing conclusions that are more valid and reliable through meta-analytic studies. Based on the above background, the authors are interested in conducting research on the effect of vitamin D deficiency on the incidence of anemia in children and adolescents.

SUBJECTS AND METHOD

1. Study Design

This study uses a systematic review and meta-analysis using secondary data, namely data from previous research results. Search for articles using 3 databases, namely: Pubmed, Google Scholar and Science Direct which were published in 2010-2022. This is done using the following keywords: "Vitamin D" OR "25-Hydroxy Vitamin D" OR "25(OH)D" AND "Anemia" OR "Anemia" AND "Children" AND "Adolescent".

2. Steps of Meta-Analysis

The meta-analysis was carried out in five steps as follows:

1. Formulate research questions in the PICO format (population, intervention, comparison, outcome).
2. Search for primary study articles from various electronic databases including Google Scholar, PubMed, and Science Direct and non-electronics.
3. Conduct screening and critical appraisal (Critical Appraisal) of primary research articles.
4. Perform data extraction and synthesize effect estimates into RevMan 5.3.
5. Interpret and conclude the results.

3. Inclusion Criteria

Inclusion criteria in this study included articles with a cross-sectional study design, full text available articles, results of analysis used adjusted odds ratio (aOR), year of

article used range 2010-2022 and study outcomes were children and adolescents who have anemia.

4. Exclusion Criteria

Exclusion criteria in this study included not clearly explaining the results, in vivo or in vitro research articles, different interventions and populations, paid or locked articles and analysis results not using an adjusted odds ratio (aOR).

5. Operational Definition of Variables

Article search was carried out by considering the eligibility criteria determined using the PICO model. The population of this study were children and adolescents, the intervention used was vitamin D deficiency, the comparison in this study was no vitamin D deficiency and the outcome was anemia.

Vitamin D deficiency is a condition in which the body lacks serum 25(OH)D (less than 30 ng/mL).

Anemia is a condition in which the body lacks hemoglobin concentration (less than 11 g/dL).

6. Instrument

The instrument in this study was an article on the effect of vitamin D deficiency on anemia in children and adolescents. This research was conducted using a checklist for critical assessment from a cross-sectional study (survey) sourced from the Center for Evidence Based Management (CEBMa) (2014).

7. Data Analysis

Research that has been collected is selected with predetermined criteria and then the results of the research will be analyzed based on variations between studies with the fixed effect model analysis model and random effect model using RevMan 5.4 software. issued by the Cochrane collaboration. RevMan software functions to calculate the overall OR value by describing the 95% Confidence Interval (CI) using the effects model as well as data heterogeneity.

RESULTS

Search for articles in this study through databases that include PubMed, Google Scholar, and Science Direct. With keywords including: "Vitamin D" OR "25-Hydroxy Vitamin D" OR "25(OH)D" AND "Anemia" OR "Anaemia" in the period 2010-2022. The process of reviewing related articles can be seen in the PRISMA flow diagram in Figure 1. The initial search process yielded 8,785 articles, after the process of deleting published articles, 8,461 articles were found, with 1,023 of them meeting the requirements for further full-text review. A total of 9 articles that met the quality assessment were included in the quantitative synthesis using meta-analysis. The selected research articles came from two continents, namely 3 articles from America and 6 articles from Asia, can be seen in Figure 2.

Table 1, researchers conducted an assessment of the quality of the study. The nine articles that have been obtained from several databases need to be assessed to determine the quality of the articles before being used as material for meta-analysis. This research was conducted using a checklist for critical assessment from a cross-sectional study (survey) sourced from the Center for evidence-based management (CEBMa) (2014). This assessment criteria consists of 12 question criteria with each given a score of 2 = if the answer is yes, 1 = if the answer does not know, and 0 = if no answer, then added up. The results of the summation of the scores that have been carried out, obtained 5 articles with a total score of 24 answers, namely Chang et al. (2012), Jang et al. (2019), Lee et al. (2014), Nikooyeh et al. (2018), Yoon et al. (2011). In addition, there are 3 articles with a total answer score of 23, namely Atkinson et al. (2014), Sharma et al. (2014) and Syed et al. (2017), as well as one article with a total score of 22, namely Altemose et al. (2018).

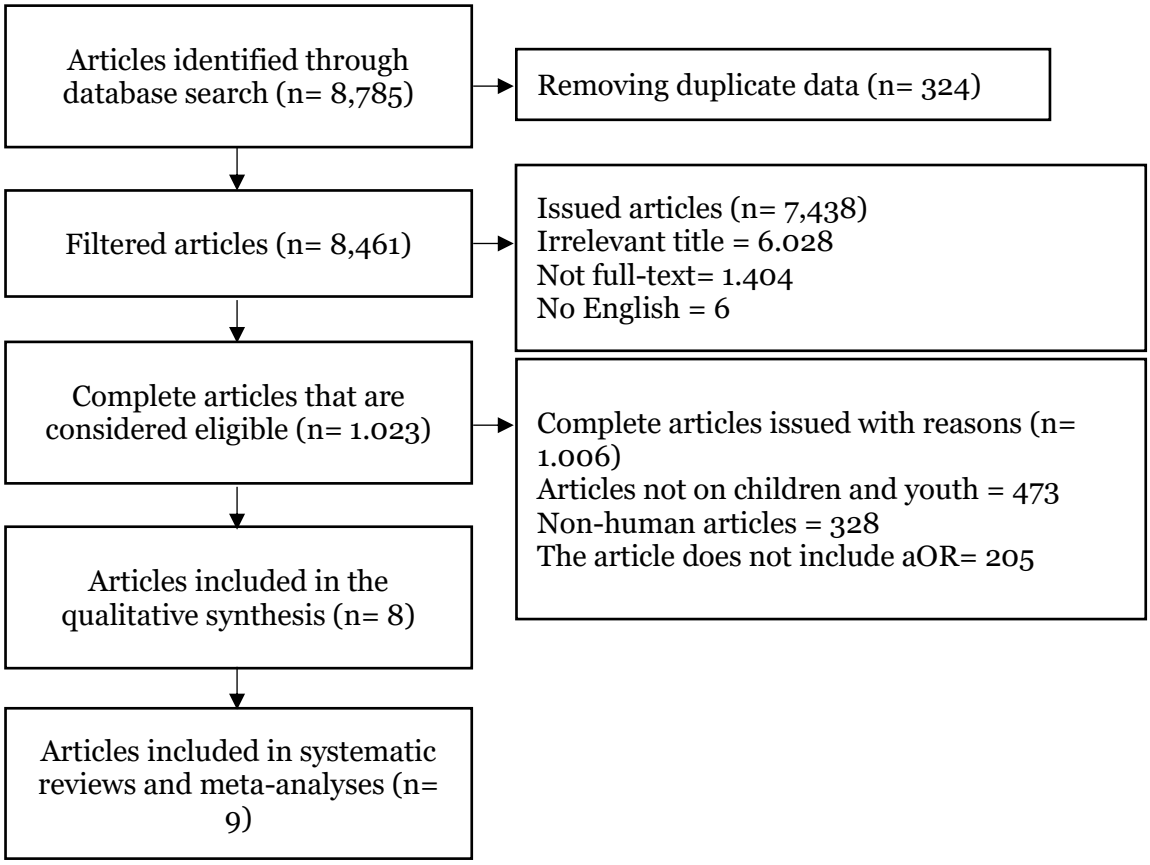

Figure 1. PRISMA flowchart diagram of the effect of vitamin D deficiency on the incidence of anemia in children and adolescents

Figure 2. Map of the research area on the effect of vitamin D deficiency on the incidence of anemia in children and adolescents

Table 1. Critical appraisal checklist for cross-sectional studies in meta-analysis

Primary Study	Criteria of the Questions												Total
	1	2	3	4	5	6	7	8	9	10	11	12	
Altemose *et al.* (2018)	2	2	2	1	2	2	2	2	2	2	2	1	22
Atkinson *et al.* (2014)	2	2	2	1	2	2	2	2	2	2	2	2	23
Chang *et al.* (2012)	2	2	2	2	2	2	2	2	2	2	2	2	24
Jang *et al.* (2019)	2	2	2	2	2	2	2	2	2	2	2	2	24
Lee *et al.* (2014)	2	2	2	2	2	2	2	2	2	2	2	2	24
Nikooyeh *et al.* (2018)	2	2	2	2	2	2	2	2	2	2	2	2	24
Sharma *et al.* (2014)	2	2	2	1	2	2	2	2	2	2	2	2	23
Syed *et al.* (2017)	2	2	2	2	2	2	2	2	2	2	2	1	23
Yoon *et al.* (2011)	2	2	2	2	2	2	2	2	2	2	2	2	24

Description of the question criteria:

1 = Does the study address the clinical problem clearly?
2 = Are the research methods appropriate for answering the research questions?
3 = Is the method for selecting research subjects clearly described?
4 = Does the method of obtaining the sample avoid selection bias?
5 = Is the sample representative of the research target population?
6 = Was the study subject sample size estimated taking into account the results of preliminary studies of statistical power?
7 = Is the minimum response rate achieved?
8 = Is the measurement (questionnaire) valid and reliable?
9 = Was a statistical significance test carried out?
10 = Did the researcher report the confidence interval?
11 = Are there any confounding factors that have not been taken into account?
12 = Are the results applicable in practice or in the community?

Description of the answer score:

0 = No
1 = Hesitant
2 = Yes

Table 2 shows that 9 articles from observational studies as evidence of the linkage of the effect of vitamin D deficiency on the incidence of anemia in children and adolescents. Primary studies that met the criteria totaled 9 cross-sectional research articles, with 3 articles from the Americas (North America, New York, Atlanta) and 6 articles from the Asian continent (China, Iran, India, and 3 articles from Korea) so that a total of 18,608 samples with an age range of 0-29 years.

Table 3 displays the amount of aOR in each selected article. It is necessary to pay attention to the collection of articles by selecting articles that have controlled for confounding factors. According to Murti (2018), confounding factor is mixing the estimation of the relationship between exposure and the disease being studied, by other factors that are related, both to disease and exposure.

Table 2. Summary of cross-sectional primary study articles in the meta-analysis

Author (year)	Country	Sample	P	I	C	O
Altemose et al. (2018)	The USA	580	1-16 years	25(OH)D serum <30 ng/mL	Non def vit. D	Hb <11 g/dL
Atkinson et al. (2014)	New York	10410	1-21 years	25(OH)D serum <30 ng/mL	Non def vit. D	Hb <12 g/dL
Chang et al. (2012)	China	110	4-7 years	25(OH)D serum <30 ng/mL	Non def vit.D	Hb <11 g/dL
Jang et al. (2019)	South Korea	3643	Girls 12-29 years	25(OH)D serum <20 ng/mL	Non def vit.D	Hb <12g/dL
Lee et al. (2014)	South Korea	2526	10-20 years	25(OH)D serum <15ng/mL	Non def vit. D	Hb <12 g/dL
Nikooyeh et al. (2018)	Iran	937	9-12 years	25(OH)D serum <25 ng/mL	Non def. vit. D	Hb <11.5 g/dL
Sharma et al. (2014)	India	263	1-5 years	25(OH)D serum <30 ng/mL	Non def vit. D	Hb <11 g/dL
Syed et al. (2017)	Atlanta	69	5-19 years	25(OH)D serum <30 ng/mL	Non def. vit. D	Hb <11.5 g/dL
Yoon et al. (2011)	South Korea	79	Girls 0–13 years	25(OH)D serum <20 ng/mL	Non def vit. D	Hb <11 g/dL

Table 3. Adjusted Odds Ratio (aOR) effect of vitamin D deficiency on the incidence of anemia in children and adolescents

Author	aOR	95% CI	
		Lower Limit	Upper Limit
Altemose et al. (2018)	1.93	1.22	3.04
Atkinson et al. (2014)	1.93	1.21	3.08
Chang et al. (2012)	12.16	7.61	19.44
Jang et al. (2019)	1.61	1.04	2.49
Lee et al. (2014)	2.15	1.25	3.68
Nikooyeh et al. (2018)	3.45	1.21	9.81
Sharma et al. (2014)	3.60	1.77	7.35
Syed et al. (2017)	3.20	0.50	22.70
Yoon et al. (2011)	5.66	1.66	19.31

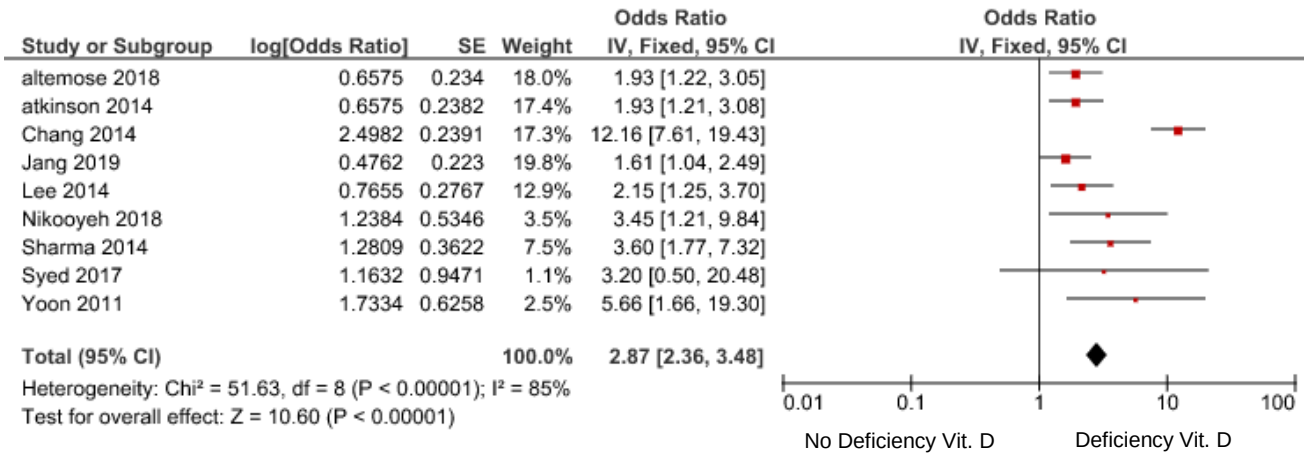

Figure 3. Forest plot of the effect of vitamin D deficiency on anemia in children and adolescents

The forest plot in Figure 3 shows that there is an effect of vitamin D deficiency on anemia in children and adolescents. Children and adolescents with vitamin D deficiency had 2.87 times the risk of developing anemia compared with those without vitamin D deficiency, and this effect was statistically significant (aOR= 2.87; 95% CI= 2.36 to 3.48; p<0.001). Forest plots show significant variation between study effect estimates (I^2= 85%; p<0.001). Thus, the calculation of the average effect estimate is carried out using the random effect model approach.

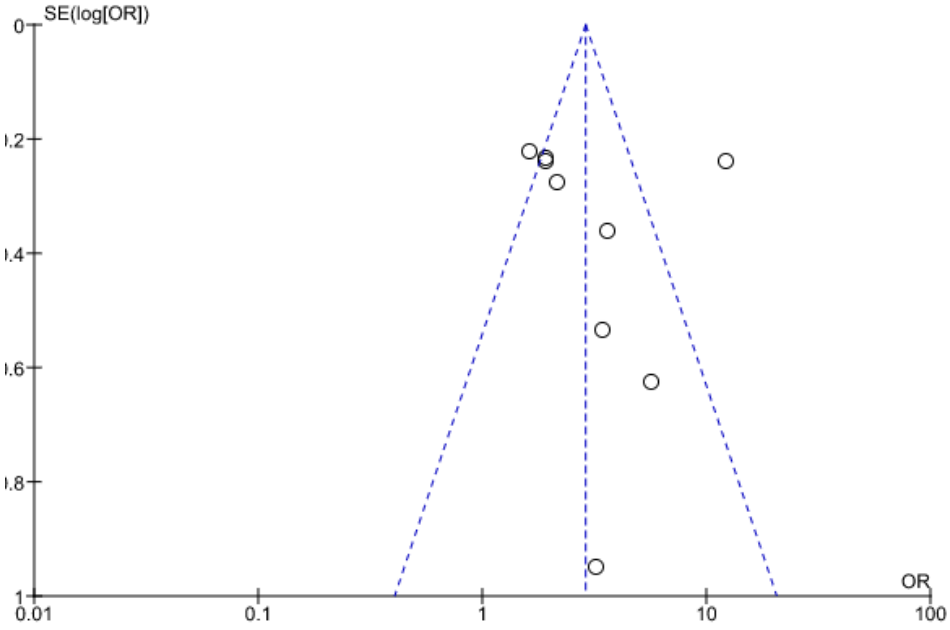

Figure 4. Funnel plot of the effect of vitamin D deficiency on anemia in children and adolescents

The funnel plot in Figure 4 shows an asymmetric distribution of effect estimates to the right and left of the average vertical line of estimates. The effect estimates are more spread out to the right of the vertical line than to the left, indicating publication bias. Because the distribution of these effect estimates is more to the right of the estimated

average vertical line, as well as the average estimated effect on the forest plot image is also to the right of the vertical line of the null hypothesis, the publication bias is over-estimating the true effect (overestimation).

DISCUSSION

A systematic review and meta-analysis in this study was conducted with the aim of increasing the generalizability of the findings and obtaining convincing conclusions from the results of similar studies regarding vitamin D deficiency on the incidence of anemia in children and adolescents. The selection of primary articles for meta-analysis processing uses a predetermined PICO reference. The selection of articles also uses research that has controlled for confounding factors, which can be seen from the study inclusion requirements, namely the adjusted odds ratio (aOR). Primary studies that met the criteria totaled 9 observational research articles as sources originating from 3 from the Americas and 6 from the Asian continent involving a total sample of 18,608.

The heterogeneity that appears in the articles that have been collected is formed because of the diversity of the population which is not taken from just one region, and the instruments used in each article vary. The population in the study were children and adolescents, but when searching for articles, several articles were found with children and adolescents as research subjects who were only female. Heterogeneity in the instrument section of the articles collected, the tools used by the researchers to measure serum 25(OH)D were Diasorin LIAISON, automatic chemiluminescent, ELIS kit, and there was one article which did not mention the instrument used, only mentioning complete blood smear in lab While for measuring Hb concentration, the instruments used include Sysmex XE-2100. Olympus AU-400 analyzer, Mythic 18, Advia 2120, and there is one article that doesn't mention the instrument, only mentions a complete blood count in the lab.

The results of this meta-analysis study showed that children and adolescents with vitamin D deficiency were affected by anemia 2.87 times compared to children and adolescents who were not deficient in vitamin D, these results were statistically significant (aOR= 2.87; 95%CI= 2.36 to 3.48; p<0.001). This result is in line with Liu et al. (2015) in a systematic review study which stated that vitamin D deficiency has a potential effect on the occurrence of anemia in the population aged 6 months to 95 years (aOR= 2.25; 95% CI= 1.47 to 3.44) with evidence of significant heterogeneity I^2= 84.0%, p<0.001. Likewise with Azizi (2016), who stated that there was a relationship between anemia and vitamin D in samples aged 3 months to 31 years.

According to Alon (2002), the mechanism of the effect of vitamin D deficiency on anemia appears to indicate that inadequate serum 25(OH)D causes a decrease in local calcitriol production in the bone marrow which can limit erythropoiesis. Calcitriol has a direct proliferative effect on the erythroid-forming unit that is synergistic with endogenously produced erythropoietin, and also regulates expression of the erythropoietin receptor on erythroid progenitor cells.

In anemia with inflammation, it shows that low serum 25-(OH)D affects the increase in the amount of hepcidin levels in the body. The amount of hepcidin levels has been shown to be inversely proportional to the amount of Hb concentration, so that it can cause anemia (Carvalho, 2011). Hepcidin, which prevents further iron absorption and release of iron from cells during periods of iron sufficiency by binding to and inducing the degradation of the cellular iron exporter, ferroportin, is also regulated by the proinflammatory cytokines inter-leukin-6

(IL-6) and interleukins -1β (IL-1β). This mechanism is the body's protective response during acute infection to reduce available iron. The absorption of iron through enterocytes is reduced and the release of iron from macrophages in the iron recycling process is reduced (Ruchala, 2014).

In chronic diseases that can carry a prolonged inflammatory stimulus, iron is pathologically sequestered in the cells of the reticuloendothelial system. Although iron stores are adequate, anemia can occur due to disturbances in iron recycling, so that iron is not sufficiently available for erythropoiesis and hemoglobin synthesis (Smith, 2015). It can be concluded that calcitriol can inhibit the expression of proinflammatory cytokinees, thereby providing negative feedback to prevent excessive inflammation (Bikle, 2009). This theory is in line with Patel (2010), who says that vitamin D deficiency is independently associated with decreased Hb levels and anemia in chronic kidney patients

Referring to the results of these studies, children and adolescents need to meet the adequacy of vitamin D in the body. Wagner (2008) in the American Academy of Pediatrics recommends that all infants, children and adolescents have a minimum daily intake of 400 IU of vitamin D starting immediately after birth, and this replaces the previous recommendation of a minimum daily intake of 200 IU. Vitamin D supplementation begins in the first 2 months after birth and continues into adolescence. Besides that, it is recommended to bask in the sun for about 5-30 minutes of sun exposure, especially between 10.00 to 16.00 at least twice a week to face, arms, hands and feet without sunscreen

The advantage of this study is that the results are more representative because they are taken from various articles. However, the researcher realizes the limitations of this study. The limitations of this study are the lack of homogeneity in the instruments used for each article, search bias because it only searches through three databases, and publication bias shown in the funnel plot results.

AUTHOR CONTRIBUTION

BD is the principal researcher who selects the topic, seeks and collects research data. YLRD and BM analyzed the data and reviewed the research documents.

FUNDING AND SPONSORSHIP

This study is self-funded.

CONFLICT OF INTERESTS

There is no conflict of interest in this study.

ACKNOWLEDGMENT

The researcher thanks the research database providers by PubMed, Google Scholar, and Science Direct.

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