

Original article:

Association of 25-hydroxyvitamin D and Anemia parameters in elderly with anemia of inflammation and non-inflammation

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Abstract

Background: Vitamin D deficiency and anemia are conditions commonly in elderly. Both result in significant morbidity in elderly. Relationship between 25-hydroxyvitamin D and anemia need to be concern, particularly in the elderly. including those characterized by inflammatory processes. The aim of this study is to analyze association and differentiations between of 25-hydroxyvitamin D levels and anemia parameters in elderly with anemia inflammation and non-inflammation. **Methods:** An observational study, at Posyandu Lansia Puskesmas Sokaraja, was conducted among 40 subjects aged ≥ 60 years consecutively, between September - November 2015. 25-Hydroxyvitamin D, hemoglobin levels, hematocrit and red blood cell counts were measured. 25-Hydroxyvitamin D deficiency defined in level < 30 ng/mL and anemia defined by the World Health Organization. **Results:** After adjustment for CRP levels and leucocyte count, 25-Hydroxyvitamin D was inversely associated with anemia inflammation ($p=0,018$; $p=0,010$; $p=0,000$) and non-inflammation ($p=0,002$; $p=0,002$; $p=0,000$). There was significantly differences 25-hydroxyvitamin D levels ($p=0,003$) in elderly with anemia of inflammation and non-inflammation. **Conclusion:** 25-Hydroxyvitamin D levels was associated with anemia inflammation and non-inflammation in elderly. Vitamin D may suppress inflammatory mechanism, and studies to determine whether chronic disease involves anemia inflammation are warranted.

Keyword: elderly; anemia; 25-hydroxyvitamin D; hemoglobin; hematocrit; red blood cell count

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Introduction

Epidemiological studies showed that elderly increasing all around the world, including Indonesia. Anemia of any degree is an independent contributor for morbidity, mortality, and frailty in elderly populations.¹ Data from the Third National Health and Nutrition Examination Survey (NHANES III) showed incidence of anemia in men and women older than age 65 was 11% and 10%, respectively. The prevalence of anemia after the age of 50 was rise rapidly, especially a rate greater than 20% in those individuals aged 85 years and over.² Riset Kesehatan Dasar (Riskesdas 2013) showed that Indonesians in 55-64 years (25,0%), 65-74 (34,2%) and ≥ 75 years (46%).^{3,4}

Anemia has often been considered in elderly, the pathophysiology of such an age-related with erythrocyte production is obscure and need to be understand. Anemia in elderly individuals have become a target to analyze in research interest.⁵ Anemic in the elderly, divided into nutritional deficiency, anemia of inflammation, and “unexplained” anemia. Vitamin D was one of nutritional deficiency commonly occurred in elderly.⁶ Deficiency vitamin D status is common among elderly people and become important public health issue worldwide.⁷ The pathophysiology role of vitamin D in calcium absorption and bone metabolism is well known. The major storage and circulating serum concentrations of 25 hydroxyvitamin D (25 (OH) D), depend on

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the supply of cholecalciferol (sunlight exposure) and ergocalciferol (dietary sources). 25 (OH) D is converted to its metabolically active form, 1,25 dihydroxyvitamin D (1,25 (OH) D), in the kidney, although hydroxylation of 25 (OH) D to 1,25 (OH) D can also occur in many different tissues throughout the body, including bone and muscle.⁸ Deficiency 25 (OH) D or 1,25 (OH) D can promote to secondary hyperparathyroidism. Parathyroid hormone induced hydroxylation of 25 (OH) D to 1,25 (OH) D in the kidney, which results in a compensatory rise in 1,25 (OH) D concentrations to normal or elevated levels. Low serum levels of 1,25 (OH) D can occur due to impaired renal conversion of 25 (OH) D, which in commonly occur in elderly.^{8,9} 1,25 (OH) D regulates erythropoiesis by stimulating erythroid progenitor cells in bone marrow with cytokines and hormone, including erythropoietin (EPO), and it has been reported that vitamin D metabolites are needed for red blood cell production.⁹ Vitamin D have important role on erythropoiesis including cellular proliferation and differentiation and induction of erythroid progenitors in bone marrow. Low 1,25 (OH) D levels may therefore lead to anemia. Serum concentration of 1,25 (OH) D mediates cellular actions of vitamin D. It modulates the level of systemic cytokine production, thus can be reduce the inflammatory milieu that leads to anemia inflammation. Studies have demonstrated that 1,25 (OH) D reduces cytokine production in inflammation mechanism.^{6,8}

Previous studies showed that association between vitamin D status and anemia with inflammation was significant.^{10,11} The mechanism underlying this relationship involved hepcidin, a hormone produced from hepatocytes that involved in the regulation of Fe recycling in the body. Hepcidin was induced by pro-inflammatory cytokines including IL-6.^{12,13} Chronic inflammatory conditions made Fe become sequestered in reticuloendothelial system and distinguished for erythropoiesis, which may lead to anemia. Recently studies showed vitamin D has been reported to lower inflammatory cytokines implicated in the pathophysiology of anemia with inflammation involving hepcidin reduced. Thus, vitamin D has anti-inflammatory effect that can may reduce the risk of anemia.¹⁴

Increasing risk of vitamin D deficiency in elderly due to several factors including lack of sun exposure and declining in synthesis and metabolism of vitamin D. Thus, anemia and low active metabolic vitamin D levels are conditions that both increase in elderly and may result in morbidity.^{8,15} While there was some

studies for an association between deficiency serum 25 (OH) D levels and anemia.¹² The previous studies were involved patients with chronic kidney disease (CKD), end-stage renal disease,¹⁶ congestive heart failure^{17,18} or diabetes¹⁹.

The primary aim of our study was to investigate a significant correlation between 25 Hydroxyvitamin D and anemia parameters in elderly community of 60 years and older with anemia inflammation and non inflammation.

Material and Methods

An observational study, was conducted in Posyandu Lansia Puskesmas Sokaraja, among 40 subjects aged ≥ 60 years consecutively, between September - November 2015. Exclusion was based on history of malignancy during the previous 5 years; autoimmune disease, hypertension, chronic kidney disease, cardiovascular, diabetes mellitus, rheumatoid arthritis, haematological disorders, infectious and vitamin D supplementation.

The 25-hydroxyvitamin D(25 (OH) D) levels was measured by ELISA methods. 25 (OH) D insufficiency was defined as a level <30 ng/mL and deficiency was defined as a level <10 ng/mL.^{8,20} Levels of hemoglobin, hematocrit and red blood cell count were measured by flowcytometry, Mindray BC5200. Data was correlated with spearman-test and compared with independent t-test, significantly $p < 0,05$. Anemia criteria used in this study were those defined by the World Health Organization: hemoglobin level <12 g/dL for women and <13 g/dL for men.. Inflammation defined as CRP (+)/positif and leukocyte $>11.000/mm^3$ or $<4000/mm^3$. All participants engaged voluntarily and signed informed consent forms. The institutional ethical review approved from Medical Faculty Diponegoro University/RSUP Dr Kariadi Semarang (no 584/EC/FK_RSDK/2015)

Results

Hematological data from 40 elderly subjects showed that anemia inflammation had lower vitamin D levels, haemoglobin levels, hematocrit and red blood cell counts, compare with anemia non inflammation. This study demonstrates a higher prevalence of anemia in individuals with 25 (OH) D deficiency compared with those with normal 25 (OH) D levels. Anemia were higher in the 25 (OH) D deficiency group compared with those with normal 25 (OH) D levels. Subject with 25 (OH)D deficiency had a lower Hb level compared with those with normal 25 (OH) D levels.

Table 1. Main characteristics of the population

Characteristic	Anemia inflammation (n=20)	Anemia non inflammation (n=20)
Age(years)	66,55±4,33	63,7±3,33
Leukosit counts(x10 ³ /μL)	8,37±2,86	7,01±1,19
25-hydroxyvitamin D(ng/ml)	17,06±9,55	28,34±14,99
Haemoglobin (gr/dl)	9,90±1,29	10,22±1,05
Hematocrit (%)	39,79±3,99	40,71±3,02
Red blood cell counts (x10 ⁶ /uL)	4,46±0,48	4,549±0,40

The subject age average was 65,13 years: 17,5% of male and 82,5% of female. The majority serum 25(OH) D level was in deficiency status: 17.06 ng/mL in anemia inflammation and 28,34 ng/mL in anemia non inflammation. Approximately 20% of subjects with vitamin D deficiency (<10 ng/mL): 15% of males and 75% of females.

Table 2. Distribution of 25-Hydroxyvitamin D

25-hydroxyvitamin D	Anemia inflammation (n=20)	Anemia non inflammation (n=20)	N	%
Deficiency (<10ng/ml)	8(40%)	0(0%)	8	20
Insufficiency (10-30ng/ml)	11(55%)	12(60%)	23	57,5
Normal (30-100ng/ml)	1(5%)	8(40%)	9	22,5

Vitamin D insufficiency data showed higher in anemia inflammation (55%) rather than anemia non inflammation(60%). There was no vitamin D deficiency in anemia non inflammation. Subject with normal 25 (OH) D levels only 5% in anemia inflammation and 40% in anemia non inflammation.

Table 3. Association between 25-Hydroxyvitamin D and parameters anemia.

Variable	Anemia inflammation		Anemia non inflammation		P
	p	R	P	r	
25-hydroxyvitamin D (ng/ml)					0,003
Haemoglobin(gr/dl)	0,018	-0,521	0,002	-0,643	0,397
Hematocrit (%)	0,010	-0,562	0,002	-0,638	0,325
Red Blood Cell Counts (x10 ⁶ /uL)	0,000	-0,904	0,000	-0,818	0,511

Univariate analysis defined there was association between 25-Hydroxyvitamin D and anemia parameters (haemoglobin, hematocrit and red blood cell counts)

in elderly with anemia inflammation and non inflammation (Table 3). Significantly differentiation 25-hydroxyvitamin D between anemia inflammation and non inflammation ($p<0,005$).

Discussion

In this study we examine crosssectional associations between concentration serum vitamin D level and anemia parameters levels in elderly with anemia inflammation and non inflammation. Previous studies suggest that 25 (OH) D levels influence haemoglobin. Two crosssectional studies have showed associations between low 25 (OH) D level and anemia in older people.^{12,21} We found that after adjusting for leukosit counts and CRP levels, 25 (OH) D were significantly associated with haemoglobin levels. There was also significantly differentiated 25 (OH) D levels between anemia inflammation and non inflammation. Our findings showed the possibility of the potential role of 25 (OH) D in the pathogenesis of anemia inflammation elderly. The 25 (OH) D has potensial role in modulator of chronic inflammatory responses, 25 (OH) D is involved in downregulating inflammatory markers, anti-proliferative effect and induced cytokine interaction in chronic inflammatory diseases.^{22,23}

The vitamin D has underlying mechanism role in the suppression of the inflammatory that involved to the development of anemia inflammation. Direct effect of Vitamin D in hematopoietic tissues is proliferation of erythroid precursor cells and increased erythropoietin receptor, because vitamin D receptor is also expressed in bone marrow.^{8,24,25}

The conflicting findings in our study were accounting for potential confounders such as sunlight exposure and vitamin D intake that could be associated with vitamin D and haemoglobin.^{26,27}

The lower vitamin D status in females could be explained by type of clothing (hijab) that females wear, sun protection and deficiency vitamin D intake seen in Indonesian women. High prevalence of deficiency vitamin D in postmenopausal females may have risks of both nutritional deficiency or anemia of inflammation.^{8,28,29}

Our study strength was investigate a correlation between the biologically active form of vitamin D, 25 (OH) D, and the prevalence of anemia in

elderly population ≥ 60 years with differentiation in subtype anemia inflammation and non inflammation. The limitations of our study were cross sectional designs and we did not have any data on iron level and sun exposure to enable us to determine specific subtypes of anemia.

Conclusion

Serum 25(OH)D level were associated with haemoglobin levels, hematocrit and red blood cell

counts in elderly with anemia inflammation and non inflammation. This study can showed that vitamin D metabolites may influence anemia, through inflammation pathway.

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