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Association of hematology profile with serum 25-hydroxy vitamin D and BsmI polimorphism in community-dwelling older adults

Associação do perfil hematológico com os níveis séricos de 25-hidroxivitamina D e polimorfismo BsmI de idosos não institucionalizados

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ABSTRACT

Objective

To investigate the association between serum level of 25-hydroxy vitamin D and the Vitamin D Receptos gene BsmI polymorphism in the blood profile of community-dwelling older adults.

Methods

This cross-sectional study included 142 older males and females. A questionnaire collected socio demographic information, medical history, and factors associated with sun exposure. Weight, height, and waist circumference were measured. Biological material was collected to analyze biochemical parameters 25-hydroxy vitamin D, parathormone, serum calcium, urea, creatinine, liver enzymes, and blood profile) and to verify the presence of the vitamin D receptos gene BsmI polymorphism.

Results

Most participants were female (80.3%). The mean levels of 25-hydroxy vitamin D, hemoglobin, and hematocrit were 32.1±7.3 ng/dL, 13.5±1.5 d/dL, and 40.0±4.4%, respectively. Fifty-eight (40.8%) participants had vitamin

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D insufficiency/deficiency (25.7±3.3 ng/mL), and 18 (12.6%) had anemia. Serum vitamin D was associated with hemoglobin (p=0.030) and hematocrit (p=0.032). However, when subjects were categorized as anemic or not anemic, said association was not maintained (p=0.270). Moreover, the BsmI polymorphism was not associated with hemoglobin and hematocrit levels, regardless of vitamin D status.

Conclusion

The serum level of vitamin D is associated with hematocrit and hemoglobin levels in older adults. However, these blood parameters were not associated with the vitamin D receptor gene BsmI polymorphism.

Keywords: Anemia. Aged. Hemoglobins. Polymorphism. Vitamin D.

RESUMO

Objetivo

Investigar a associação do perfil hematológico com os níveis séricos de 25-hidroxivitamina D e polimorfismo BsmI do gene vitamina D receptor de idosos não institucionalizados.

Métodos

Estudo transversal realizado com 142 idosos de ambos os sexos. Foram coletadas, por meio de um questionário, informações sociodemográficas, antecedentes clínicos e fatores associados à exposição solar. Além disso, foram aferidas medidas antropométricas de peso, altura e circunferência da cintura. Material biológico foi coletado para análise dos parâmetros bioquímicos (25-hidroxivitamina D, parathormone, cálcio sérico, ureia, creatinina, enzimas hepáticas e perfil hematológico) e para identificação do polimorfismo BsmI do gene vitamina D receptor.

Resultados

Os participantes do estudo eram, em sua maioria, do sexo feminino (80,3%) e apresentaram concentrações médias de 25-hidroxivitamina D, hemoglobina e hematócrito de 32,1±7,3 ng/dL, 13,5±1,5 d/dL e 40,0±4,4%, respectivamente. Da amostra total, 40,8% (n=58) apresentaram insuficiência/deficiência de vitamina D (25,7±3,3 ng/mL) e 12,6% (n=18), anemia. Encontrou-se associação entre as concentrações séricas de vitamina D com as de hemoglobina (p=0,030) e hematócrito (p=0,032). Entretanto, quando os sujeitos foram categorizados quanto à presença ou não de anemia, essa associação não se manteve (p=0,270). Além disso, não foi observada relação entre o polimorfismo BsmI e as concentrações de hemoglobina e hematócrito nos grupos de idosos com suficiência ou insuficiência/deficiência de vitamina D.

Conclusão

O presente estudo encontrou associação entre as concentrações séricas de vitamina D e as de hematócrito e hemoglobina nos idosos analisados. Contudo, não foram observadas associações entre esses parâmetros hematológicos e o polimorfismo BsmI do gene vitamina D receptor.

Palavras-chave: *Anemia. Idoso. Hemoglobina. Polimorfismo. Vitamina D.*

INTRODUCTION

Vitamin D deficiency and anemia are two conditions that have been frequently investigated and reported in the literature in many populations and age groups[1,2]. In older adults the incidences of anemia are estimated to be around 11% in men and 10% in women[3]. Similarly, there are many literature reports of high prevalences of vitamin D insufficiency/deficiency in this population[4,5].

Vitamin D has an important role in bone health. However, in the last years, an important body of evidence has associated this metabolite with many extraskeletal outcomes, such as diabetes, cancer, and metabolic syndrome[6]. More recently, observational studies reported a relationship between vitamin D level and hemoglobin in many populations, especially in older adults[7-10].

The possible action of vitamin D in the proliferation and differentiation of a variety of

cells, among them hematopoietic stem cells, and its action on erythropoiesis have been investigated[11]. In this sense, an adequate level of 25-Hydroxy Vitamin D (25(OH)D) seems to be crucial for the normal production of red blood cells, although the pathophysiological mechanisms still need to be fully elucidated[12]. Additionally, the finding that many body tissues, such as the bone marrow[13,14], have Vitamin D Receptors (VDR), a protein responsible for gene transcription and for carrying out cell function in many tissues, reinforces the hypothesis that there is a positive relationship between vitamin D level and hemoglobin[7,15,16], and that this phenomenon may be influenced by VDR gene polymorphisms[17-19]. Single-nucleotide polymorphisms are common in the human genome, and frequently, they occur in specific genes involved in the genesis of and predisposition to many human diseases. The most common VDR gene polymorphisms occur between exons 8 and 9, such as ApaI, BsmI, and TaqI[20].

Despite the great interest on the role of vitamin D on the pathogenesis of many diseases, the relationship between vitamin D insufficiency/deficiency and blood profile, and the physiological mechanisms of its activity are still controversial and based on a limited number of studies. Until now only two studies included a population of older adults without chronic diseases[7,16], and none of them investigated their relationship with genetic characteristics. Thus, the present study aimed to investigate the association of serum level of 25(OH)D with the VDR gene BsmI polymorphism and the blood profile of community-dwelling older adults from a metropolis in the Brazilian Northeast region.

METHODS

This epidemiological and cross-sectional study included a random and representative sample of community-dwelling older adults aged at least 60 years. The participants were recruited at the *Programa de Atenção à Pessoa Idosa* (PAPI, Care Program for the Elderley) in the city of *João Pessoa* (PB), a metropolis located in the Brazilian Northeast region. Data were collected between October 2012 and September 2013. In the study region, the four seasons are not well defined since the region has summer characteristics year round, with some months having more rain and cloudy days, which rarely last all day, and a temperature range of 23º to 34ºC.

This study stems from a research project designed to investigate the prevalence of hypovitaminosis D in a population of older adults, factors that affect this prevalence, and outcomes of this condition on cardiometabolic health. The sample size was calculated based on an estimated prevalence of hypovitaminosis D of 33%, an error margin of 11%, reliability of 95%, design effect of 2.0, and an additional 5% to compensate for losses[4]. The minimum sample size should be 141 individuals. Of the 148 selected individuals, six were excluded, totaling 142 older adults who agreed to participate in the study.

The inclusion criteria were the absence of factors capable of affecting the level of vitamin D (use of supplements containing vitamin D and anticonvulsive or anti-HIV/AIDS [Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome] pharmaceuticals) and absence of any of the following conditions: nephrotic syndrome, acute or chronic renal failure, hepatic disease, hypo- or hyperthyroidism, wasting, or chronic alcohol or cigarette use.

The study was approved by the Research Ethics Committee of the Center of Health Sciences of *Universidade Federal da Paraíba* (Federal University of *Paraíba)* under protocol nº 0374/12. The study procedures complied with Resolution nº 466/12 of the *Conselho Nacional de Saúde/Ministério da Saúde* (National Health Council/Ministry of Health).

First the participants were clinically assessed and answered a questionnaire about their history of chronic diseases and surgeries, chronic use of pharmaceuticals, mean time of sun exposure, and use of long clothes and sunscreen. The

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questionnaire was administered by trained researchers. To avoid memory bias, the older adults answered the questionnaire accompanied by individuals who lived in the same household.

Nutritional assessment of Body Mass Index (BMI) was given by dividing weight by the square of the height². Waist Circumference was measured by an inelastic tape measure with the individual standing up. The measurement was taken at the midpoint between the lowest rib and iliac crest. Body Mass Index and waist circumference were classified as recommended by the World Health Organization[21]. All measurements were taken three times and averaged.

Biochemical assessment of participants were instructed to fast for 12 hours. Blood count, calcium, urea, creatinine, alanine transaminase, and aspartate transaminase were determined by commercial kits (Labtest, *Minas Gerais*, Brazil), according to the manufacturer's instructions and automatic analyzer LabMax premium (*Lagoa Santa*-MG, Brazil). All analyses were performed twice. Parathormone (PTH) and 25(OH)D were determined by chemiluminescence. The intra- and inter-assay coefficients of variation were 13 and 15%, respectively.

The reference values used were those suggested by Labtest. Normal urea was defined as 15 to 45 mg/dL for men and women, and serum creatinine, as 0.8 to 1.2 mg/dL for men and 0.6 mg to 1.0 mg/dL for women. Kidney function was also estimated by creatinine clearance, using serum creatinine in the formula suggested by Cockcroft & Gault[22]. Inadequate kidney function was defined as reference values <60 mL/min/1.73 m². Normal liver function was defined as alanine transaminase values of 11 to 45 (U/L) for men and 10-37 (U/L) for women; and aspartate transaminase values of 11 to 39 (U/L) for men and 10 to 37 (U/L) for women. Normal serum calcium was defined as 4.0 - 5.0 mg/dL.

Anemia was defined as hemoglobin <12 g/dL for women and <13 g/dL for men, as recommended by the WHO and considering the reference values for older adults.

Serum 25(OH)D was classified as recommended by the Endocrine Society[2] as follows: Sufficient (SUF) when serum vitamin D ≥30 ng/dL, insufficient when 29≥ serum vitamin D ≥21; and Deficient (DEF) when serum vitamin D <20 ng/dL. The study older adults classified as insufficient and DEF were grouped together in a group called INSUF/DEF. The reference values for PTH were 15.0-65 pg/dL

Determination of the VDR gene BsmI genotype

Cell samples from the oral epithelium were obtained by mouthwash with a 3% sucrose solution. The genomic DNA was extracted as described elsewhere[23]. The VDR gene BsmI genotype was determined by Restriction Length Polymorphism Analysis of Polymerase Chain Reaction-Amplified Fragments (PCR/RFLP), using the primers 5'-CAACCAAGACTACAAGTACC GCGTCAGTGA-3' and 5' AACCAGCGGGAAG TCAAGGG-3'.

The annealing temperature was 58 °C. A 12-hour digestion stage at 37 °C was conducted for an initial fragment of 870 base pairs (bp) using the enzyme BsmI. The result was verified by 1.5% agarose gel electrophoresis. This enzymatic digestion process did not cleave the B allele, which remained with 870bp. The restriction site generated two fragments of 640 and 230 bp (b allele). Figure 1 represents this procedure. The BsmI polymorphism was analyzed in 126 individuals, but 16 samples were lost because of DNA extraction or amplification problems.

The data were expressed as percentage, mean, and standard deviation of the mean. All variables were tested for normality and homogeneity by the Kolmogorov-Smirnov test. Intergroup differences were tested by the independent *t*-test or its non-parametric correspondent, the Mann-Whitney test. Bivariate analysis tested the statistical associations between the blood count variables and vitamin D of the

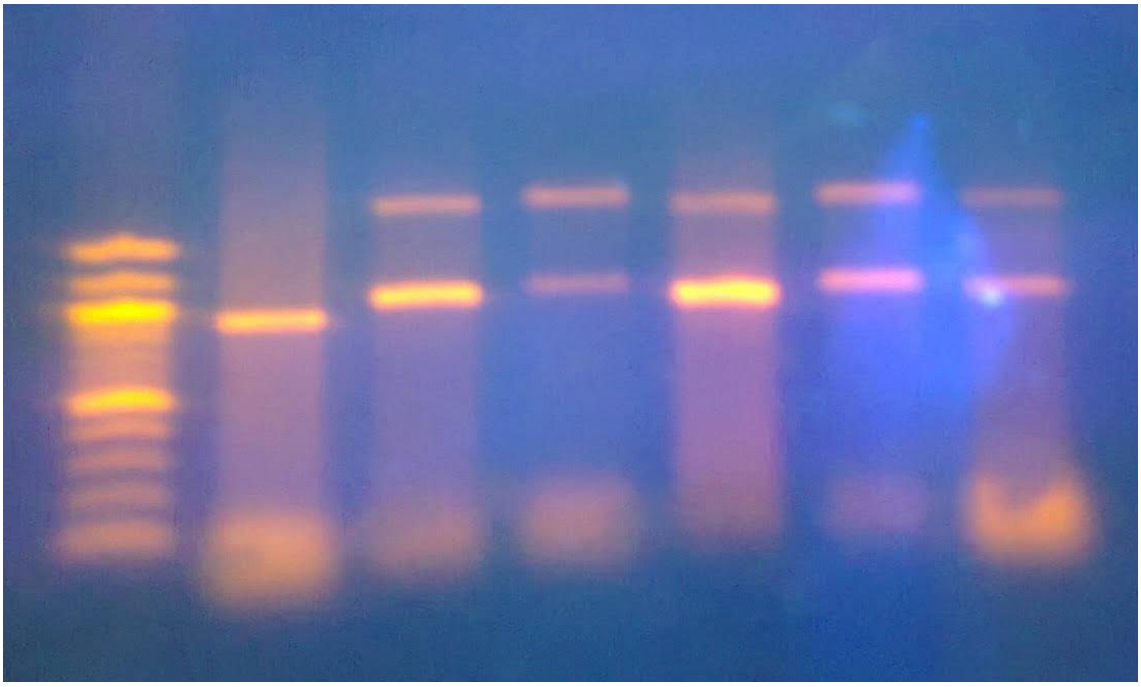

Figure 1. Agarose gel image. The procedure was conducted with a 870bp fragment. The restriction enzyme generated two fragments of 640 bp and 230 bp, respectively.

groups SUF and INSUF/DEF, using the Spearman's chi-square test and Pearson's correlation coefficient. One-way Analysis of Variance (ANOVA) compared the means of the groups categorized by polymorphism. All statistical analyses were performed by the software Statistical Package for the Social Sciences (SPSS Inc., Chicago, Ilinois, United States) version 21, using a significance level of 5%.

RESULTS

The sample consisted of 142 older adults with a mean age of 69.9 years. Most participants were female (Table 1), and most had excess weight based on the WHO criteria. All groups had similar means.

The entire sample's mean serum level of 25(OH)D was in the normal range (mean of 32.1 ng/dL). However, a significant part (40.84%) of the sample had serum 25(OH)D <30 ng/dL, so they were included in the INSUF/DEF group. Most individuals in this group were female. The PTH and calcium levels of both groups were similar. However, serum calcium levels were close to the lower limit of normality in the INSUF/DEF group. The renal and hepatic functions of the groups did not differ (data not shown). Moreover, the sample had a 12.6% prevalence of anemia.

Table 2 shows the blood count. The INSUF/DEF group had significantly lower levels of hemoglobin and hematocrit than the SUF group, and these values were positively correlated with the level of 25(OH)D (0.177 and 0.180, respectively). Nevertheless, the mean hemoglobin of both groups was within the normal range. The groups did not differ with respect to red cell, white cell, and platelet counts.

Regarding the positive relationship of vitamin D with hemoglobin and hematocrit, screening of the SUF and INSUF/DEF groups for anemia did not confirm this correlation (Table 3).

Analysis of variance showed that the polymorphism did not affect serum vitamin D

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Table 1. Characterization of the sample and relationship of serum level of vitamin D with the sociodemographic and biochemical data of community-dwelling older adults. *João Pessoa* (PB), Brazil, 2013.

Variables	General (n=142)		25(OH)D* SUF (n=84)		25(OH)D** INSUF/DEP s(n=58)		
	Prevalence (%)	n	Prevalence (%)	n	Prevalence (%)	n	
Gender							
Male	19.7	28	28.6	24	6.9	4	
Female	80.3	114	71.4	60	93.1	54	
Skin color							
White	35.9	51	32.1	27	41.4	24	
Brown	45.1	64	45.3	38	44.8	26	
Black	19.0	27	22.6	19	13.8	8	
	M	SD	M	SD	M	SD	†*p*
Age (years)	69.9	7.0	70.1	7.2	69.5	6.9	0.601
BMI (kg/m²)	28.3	4.4	28.4	4.4	28.1	4.7	0.735
Sun exp (min)	52.3	44.0	58.0	49.0	44.0	34.4	0.141
25(OH)D (ng/ml)	32.1	7.3	36.5	5.8	25.7	3.3	0.000
PTH	42.2	23.7	42.6	27.0	41.6	18.1	0.818
Calcium (mg/dL)	8.8	1.1	9.0	1.1	8.6	1.2	0.806

Note: *25(OH)D e"75 nmol/L (30 ng/mL); **25(OH)D <75 nmol/L (30 ng/mL); †*p*-values of the comparison between the groups of older adults with sufficient and insufficient/deficient 25(OH)D according to the independent Student's *t*-test.
The data are expressed as Mean (M) and Standard Deviation (SD) of the mean.
25(OH)D: 25-Hydroxy Vitamin D; Sun exp: Sun Exposure in Minutes; BMI: Body Mass Index; PTH: Parathormone.

Table 2. Relationship between vitamin D level and the blood count of older adults from nine PAPI community groups in *João Pessoa* (PB), Brazil, 2013.

Variables	General (n=142)		25(OH)D* SUF (n=84)		25(OH)D** INSUF/DEP (n=58)		*p*†	Correlation test *p*†† (r)
	Mean	SD	Mean	SD	Mean	SD		
Hemoglobin (g%)	13.50	1.4	13.7	1.4	13.2	1.4	0.030	0.035 (0.177)
Erythrocytes (mm³)	4358.03	494.6	4457.9	529.7	4228.2	401.6	0.370	
Hematocrits (%)	40.00	4.4	40.7	4.5	39.0	4.0	0.031	0.032 (0.180)
MCV (u³)	92.00	5.8	91.7	5.9	92.3	5.6	0.979	
HCM (Pg)	31.10	2.0	30.9	2.1	31.2	1.8	0.913	
CHCM (%)	33.80	1.1	33.7	1.1	33.9	1.0	0.894	
Leucocytes (mm³)	6426.00	1717.1	6504.7	1740.0	6389.6	1744.2	0.858	
Neutrophils (%)	55.80	8.1	56.8	8.4	54.4	7.7	0.204	
Eosinophils (%)	2.50	1.1	2.6	1.1	2.4	1.1	0.251	
Lymphocytes (%)	39.40	7.9	38.5	7.9	40.9	7.8	0.153	
Monocytes (%)	2.10	1.0	2.0	0.9	2.1	1.1	0.698	
Platelets	211.80	54.3	209.2	49.8	217.0	60.1	0.560	

Note: The data are expressed as mean and Standard Deviation (SD) of the mean.
*25(OH)D e"75nmol/L (30 ng/mL); **25(OH)D <75 nmol/L (30 ng/mL); †*p*-values of the comparison between groups of older adults with sufficient and insufficient/deficient levels of 25(OH)D according to the independent Student's *t*-test; ††Correlation between variables with significantly different *p*-value means of groups of older adults with sufficient and insufficient/deficient levels of 25(OH)D according to Pearson's correlation coefficient.
25(OH)D: 25-hydroxy vitamin D; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; M: Mean; SD: Standard Deviation.

(Table 4). Individuals with the BB, bb, or heterozygous Bb genotypes had statistically similar values of serum vitamin D, hemoglobin, and hematocrit. These results refer both to the entire sample and to the groups SUF and INSUF/DEF.

Table 3. Association of anemia with sufficient or insufficient/deficient vitamin D level in community-dwelling older adults. *João Pessoa* (PB), Brazil, 2013.

Characteristics	Patients with anemia		Patients without anemia		$p^{\dagger}$
	n	%	n	%	
25(OH)D* INSUF/DEF	10	17.2	48	82.8	0.270
25(OH)D** SUF	8	9.2	76	90.5	

Note: *25(OH) D <75 nmol/L (30 ng/mL); **25(OH) D ≥75nmol/L (30 ng/mL); †$g\chi^2$ value.

Table 4. Relationship between the BsmI polymorphism and the blood parameters of older adults. *João Pessoa* (PB), Brazil, 2013.

Polymorphisms	BB/Bb*		Bb*		bb*		$p^{\dagger}$
General (n=126)	n=38	(28.6%)	n=42	(33.3%)	n=48	(38.1%)	
25(OH)D (ng/mL)	32.8	7.1	33.4	8.3	30.0	6.4	0.0653
Hemoglobin (g%)	13.6	1.4	13.6	1.3	13.4	1.4	0.8448
Hematocrit (%)	40.2	4.4	40.0	3.8	39.6	4.5	0.8324
*SUF 25(OH)D (n=75)***	n=23	(30.66%)	n=28	(37.33%)	n=24	(32%)	
25(OH)D (ng/mL)	36.6	5.7	37.4	7.3	34.9	4.0	0.3140
Hemoglobin (g%)	13.8	1.4	13.9	1.3	13.5	1.4	0.4862
Hematocrit (%)	40.9	4.5	40.7	4.1	39.8	4.5	0.6446
*INSUF/DEF 25(OH)D (n=51)****	n=13	(25 %)	n=14	(27%)	n=24	(47%)	
25(OH)D (ng/mL)	26.2	3.3	25.4	2.5	25.1	4.0	0.7112
Hemoglobin (g%)	13.1	1.2	13.0	1.0	13.4	1.5	0.5968
Hematocrit (%)	38.8	3.9	38.5	2.5	39.4	4.6	0.7752

Note: *Data expressed as Mean ± Standard Deviation of the mean; **25(OH) D <75 nmol/L (30 ng/mL); ***25(OH) D ≥75nmol/L (30 ng/mL); $p^{\dagger}$Analysis of variance; n= individuals number.

SUF: Sufficient; 25(OH)D: 25-hydroxy vitamin D; INSUF/DEF: Insufficient/Deficient.

DISCUSSION

The data of the present study partly confirm the hypothesis of a positive association of serum vitamin D with hemoglobin and hematocrit. However, anemia was not associated with vitamin D insufficiency/deficiency in the study sample. Additionally, the VDR gene BsmI polymorphism was not associated with hematocrit and hemoglobin levels, regardless of vitamin D level, thereby refuting our initial hypothesis.

Similar results have been reported in the literature. Patel *et al.*[8] determined the levels of 25(OH)D, 1.25(OH)D, and hemoglobin in 1,661 individuals with a mean age of 70 years and chronic renal failure. They found that vitamin D deficiency was independently associated with lower hemoglobin levels and anemia in this specific situation. On the other hand, Coutard *et al.*[24] investigated whether vitamin D deficiency was associated with anemia in 226 hospitalized older French aged ≥70 years and found that although 67.7% and 53.5% of the anemic and non-anemic groups had vitamin D deficiency (p<0.04), this association disappeared after adjusting for albuminemia. The authors emphasized that their finding contradicts earlier studies that had not adjusted for confounders.

Meanwhile, Sim *et al.*[25] found significant associations of vitamin D deficiency with higher risk of anemia and lower mean hemoglobin. Nonetheless, the authors pointed out that chronic renal failure was present in 65% of the 554 patients they studied, which may have confounded their results. Thus, although some pieces of evidence suggest an association between levels of 25(OH)D and hemoglobin, the literature is still divergent on the topic, so further studies are needed.

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Moreover, most studies that reported the association were conducted on population subgroups with specific clinical conditions that make them much more vulnerable to the development of anemia, such as chronic renal failure or terminal diabetics[15,] hospitalization[24], disease[26,27], HIV infection[28], and congestive heart failure[29].

Only two studies have assessed the association in older adults without known chronic disease, similar to our sample. In a cohort with 9,675 community-dwelling older adults, vitamin D deficiency was independently associated with the prevalence of anemia[7]. Nevertheless, Hirani *et al.*[16] concluded that serum 25(OH)D was not significantly associated with hemoglobin. A recent meta-analysis of 5,183 individuals concluded that vitamin D increased the risk of anemia[30]. Along with our results, pieces of evidence indicate that vitamin D deficiency may be associated with hematopoietic activity. Although the relationship between the presence of anemia and vitamin D status in the study sample was not confirmed, the small sample size may have influenced the result. Therefore, more studies are necessary to determine how much vitamin D is necessary to influence the blood profile.

Although the mechanism that associates vitamin D deficiency with anemia has not been completely elucidated, a possible justification would be that vitamin D decreases the production of proinflammatory cytokines and the antimicrobial peptide hepcidin (hormone that regulates systemic iron level)[31]. Another mechanism possibly associated would regard the VDR in certain tissues, such as the bone marrow[13,14]. Thus, the presence of polymorphisms associated with the VDR gene could influence the serum levels of this metabolite in individuals with anemia. Yet, the present study did not find a statistically significant correlation between the three alleles (BB, Bb, bb), and the blood profile of older adults from the Brazilian Northeast region.

Few studies have attempted to investigate said association with the BsmI polymorphism, such as Sezer *et al.*[18], who found that the BB genotype seemed to provide more protection against anemia. Likewise, Ertürk *et al.*[27] found that the Bb and bb variants were associated with higher levels of hemoglobin in hemodyalisis patients. Amato *et al.*[19] found that hemodyalisis patients with the BB genotype had lower hemoglobin levels. All studies mentioned above involved individuals with chronic disease, which may explain result discrepancy. Moreover, a specific polymorphism may have different patterns of association in different populations secondary to the various evolutionary lineages[32].

The present study investigated only one VDR gene polymorphism. Therefore, future studies should not only investigate the role of other polymorphisms, but also other genes associated with vitamin D metabolism and hematopoietic activity that may be associated with blood profile changes in older adults. Furthermore, an important limitation of this study should be pointed out. The small number of individuals with anemia may have influenced data analysis. Although the study prevalence of anemia was similar to that reported in the literature (12.6%), studies with larger samples of individuals with anemia or with larger samples in general should be conducted to reduce the error margin and determine the influence of vitamin D on blood profile.

CONCLUSION

The study older adults had a high prevalence of vitamin D insufficiency/deficiency. In addition, serum vitamin D level was associated with hematocrit and hemoglobin levels. Even so, this association disappeared when individuals were categorized according to anemia status. What is more, the VDR gene BsmI polymorphism was associated with the hematocrit and hemoglobin levels of older adults, regardless of vitamin D status.

CONTRIBUTORS

All authors contributed to the conception and design of the study, data analysis and final editing.

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