Association between hematological profile and serum 25-hydroxyvitamin D levels and FokI polymorphism in individuals with cystic fibrosis

Associação do perfil hematológico com os valores séricos de 25-hidroxivitamina D e polimorfismo FokI em indivíduos com fibrose cística

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Objective
The present study aimed at investigating the association between hematological profile and serum 25-hydroxyvitamin D (25[OH]D) levels and FokI polymorphism of the vitamin D receptor gene in individuals with Cystic Fibrosis.

A B S T R A C T

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Methods
A cross-sectional study that involved 18 men and women aged 0-25 years with Cystic Fibrosis. Socio-demographic information and the factors associated with sun exposure were obtained. Weight, height, and arm circumference were also measured. Blood sample was collected for the analysis of biochemical parameters (25(OH)D, parathyroid hormone, and calcium levels and blood count) and for the validation of the presence of FokI polymorphism in the vitamin D receptor gene.

Results
Among the participants, 33.33% (n=6) had vitamin D deficiency (19.60±6.180 ng/mL), and 27.8% (n=5) presented with anemia and low weight for age. In terms of genotype, 5.6% (n=1) presented with the FF genotype, 72.3% (n=13) had the Ff genotype, and 22.2% (n=4) had the ff genotype. Serum 25(OH)D levels were associated with hemoglobin (p=0.008) and hematocrit (p=0.019) levels and leukocyte count (p=0.0114). No association was observed between 25(OH)D levels and the genotypes (FF, Ff, and ff) (p=0.2451). In addition, an association was observed between FokI polymorphism and the total leukocyte count (p=0.01).

Conclusion
An association was observed between serum 25(OH)D levels and hemoglobin and hematocrit levels and leukocyte count in individuals with Cystic Fibrosis. Moreover, FokI polymorphism was associated with total leukocyte count.

Keywords: Cystic fibrosis. Hemoglobins. Polymorphism, genetic. Vitamin D.

INTRODUCTION
Cystic Fibrosis (CF) is a fatal autosomal recessive disease that is more common in Caucasians and caused by mutations in the CF Transmembrane Conductance Regulator (CFTR) gene. The dysfunction in this channel leads to changes in the viscosity, volume, and concentration of salt in the body fluids, leading to the formation of thick secretions, which prevent
the hydration of the airways and create a favorable environment for bacterial colonization, chronic inflammation, and eventually respiratory insufficiency [1-3].

The incidence of the disease varies according to ethnicity, that is, from 1/1800 to 1/5000 live births in Caucasians in Europe, the United States, and Canada, 1/14000 in Afro-Americans, and 1/40000 in Finns. This is a rare disease among Asians and Africans. In Brazil, the incidence is approximately 1/7000. However, it is subjected to regional variations [1].

For more than 37 years, the median survival of these individuals increased from 10 years to 12 years, and one of the main contributing factors in the increase was the understanding of the role of nutrition in the general health state of these individuals [4].

Nutrition plays an important role in the treatment of CF because it influences the survival and quality of life of the patients. Approximately 90% of patients present with exocrine pancreatic insufficiency, resulting in poor digestion and absorption of nutrients, and consequently steatorrhea and poor stunted growth. Fat loss through feces carries the risk of liposoluble vitamin deficiency, including vitamin D. In addition, these patients have increased energy expenditure due to the nature of the disease and the frequency and severity of infections and chronic pulmonary inflammation. Thus, the importance of nutrition in these patients is reinforced [1-3,5].

With regard to minerals, iron deficiency is frequent in patients with CF and is multifactorial [5]. However, in adults with CF, iron deficiency is mainly functional due to chronic inflammation [6]. In children with CF, anemia can be due to malnutrition or inadequate erythropoiesis or both [2].

Vitamin D is more commonly associated with poor bone formation. However, its role in health is still being investigated, particularly in terms of extra-skeletal functions. Recently, vitamin D has been associated with several diseases, including hypertension, diabetes Mellitus, cardiovascular diseases, cancer, and chronic obstructive pulmonary disease, and implicated in a wide range of biological mechanisms, including muscle strengthening, cell proliferation and differentiation, and regulation of the immune system. Its association with anemia has been suggested in recent years, indicating a role in the homeostasis of iron and erythropoiesis [3,7,8]. Vitamin D insufficiency has been associated with a higher prevalence of anemia among different age groups, that is, from children to healthy adults and elderly patients [9].

The discovery that most of the body tissues and cells contain vitamin D receptors and that several of these tissues have the enzymatic property to convert the primary form of vitamin D in the circulation to its active form has provided new insights about the function of this steroid hormone [10]. In addition, the concentration of 1,25-hydroxyvitamin D (1,25[OH]D) is a hundred times greater in the bone marrow than in the plasma. The clinical consequences of its deficit have been a topic of interest due to the new insights about the biological functions of vitamin D, and the hypotheses that there is an association between vitamin D and hemoglobin levels and that this phenomenon may be influenced by polymorphisms in the Vitamin D Receptor gene (VDR) have been reinforced [11,12]. Currently, these polymorphisms have been associated with renal insufficiency, diabetes, hypertension, and autoimmune diseases [13].

Studies have indicated the existence of several Single Nucleotide Polymorphisms (SNP) in the VDR gene. Several of these polymorphisms alter the activity of the VDR proteins [14], which include ApaI, BsmI, and TaqI (within exons 8 and 9) as well as FokI (located in exon 2) [15].

Thus, this study aimed at assessing the association between the hematological profile and serum 25(OH)D and FokI polymorphism of the VDR gene in individuals with CF who visited a referral center in northeastern Brazil.
METHODS

Characteristics of the participants and study design

This cross-sectional observational study included a non-probabilistic convenience sample of 22 men and women aged 0-25 years who were diagnosed with CF. These patients were followed-up at the Pediatric Outpatient Clinic of Hospital Universitário Lauro Wanderley da Universidade da Paraíba (HULW-UFPB, Lauro Wanderley University Hospital – Federal University of Paraíba) in the city of João Pessoa, Paraíba. Informed consent was obtained from the patients or caregivers, and patients aged 12-18 years signed an assent term. Individuals who presented with biochemical alterations that indicate liver or renal insufficiencies and/or chronic diseases that alter vitamin D metabolism were excluded from the study.

Data were collected between May 2016 and September 2016. In terms of the seasons of the study region, the four seasons of the year were not well defined, since the region has the characteristics of summer throughout the year, with some months with more rainy or cloudy days and with temperatures ranging from 23º to 34ºC.

The study was approved by the research ethics committee of the HULW-UFPB (CAAE nº 52248815.9.0000.5183). The study was in accordance with Resolution no 466/12 of the National Health Council/Ministry of Health.

Initially, all participants or guardians answered a questionnaire, which included personal data (name, date of birth, family income, and education level) as well as information on skin phototype, average time of exposure to the sun, use of pharmaceutical products, and clothing. The questionnaire was used by trained researchers.

Nutritional status was assessed using the Body Mass Index (BMI) for age and Arm Circumference (AC). BMI was calculated by dividing weight in kilograms by height in meters squared. The Arm Circumference (AC) was measured utilizing a stretchable anthropometric tape measure. The measurement was made at the midpoint between the acromion and the olecranon [16]. BMI measurements were classified according to the World Health Organization (WHO) 2006 and 2007 [17] charts for patients aged less than 5 years. For the classification of patients older than 18 years, the values proposed by the WHO [18] were used. The percentile table proposed by Frisancho was used as a reference for the classification of AC [19].

Biochemical assessment

Participants were instructed to fast for 12 hours. All analyses were performed in the clinical analysis laboratory at the HULW-UFPB in João Pessoa. Analysis of calcium levels and blood count was carried with the Wiener commercial kits using a CMD 800i analyzer (Wiener Lab Group, Argentina) or Sysmex XN1000 (Sysmex, Brazil), which was in accordance with the manufacturer’s instructions. The recommended reference values by the manufacturer were used. Parathyroid Hormone (PTH) and 25(OH)D levels were determined by chemiluminescence using the Abbott Architect i2000 apparatus.

The serum concentration of 25(OH)D was classified based on the recommendation by the Endocrine Society. Serum concentrations between 20ng/mL and 29ng/mL (50-75nmol/L) were considered within normal limits, and those below 20ng/mL (50nmol/L) were considered below the normal range [20]. The reference values stipulated for calcium were 8.8-11.0mg/dL, and those for PTH were 15.0-68.3pg/mL. The cut-off hemoglobin level that was used for the diagnosis of anemia was 12ng/dL.

Identification of the VDR FokI genotype

DNA was extracted from 4mL of whole blood obtained through venous puncture using
sterile tubes containing 7.2mg of K3EDTA. The extraction of genomic DNA was performed according to a previously described methodology [21].

The genotypes were determined using PCR-RFLP. The primers used were 5’-AGCTGGC CCTGGCACGTCTGCTCT-3’ (sense) and 5’-TGAAACACCTTGCTTCTCCTCCCTG-3’ (antisense), and amplification was carried out under the following conditions: initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation (30 seconds at 94°C), annealing (30 seconds at 60°C), and extension (30 seconds at 72°C) with an extra final extension step of 10 minutes. The 265-pb product was digested with FokI, which recognizes and cleaves the polymorphic allele f, generating two fragments (196bp and 69bp), whereas the ancestral allele F remains at 265pb. The genotypes were analyzed via electrophoresis using 10% polyacrylamide gel and staining with 0.5% silver nitrate [22].

**Statistical analysis**

Data were expressed in percentage, mean, and standard deviation. All data were tested for normality and homogeneity using the Shapiro-Wilk test and Levene’s test, respectively. Differences between the groups were tested using the independent Student’s t-test or Mann-Whitney U test. Bivariate analysis was carried out to evaluate the statistical associations between the variables and serum 25(OH)D levels in the insufficient/deficient and sufficient groups using the Spearman chi-squared test and Pearson’s correlation test. The Spearman chi-squared test was used to verify the influence of the dominant allele (F). All statistical analyses were performed using Instat and SPSS version 21 (Statistical Package for the Social Sciences, released 2012 IBM SPSS statistics for windows, version 21, Armonk, New York, IBM Corp.) using a significance level of 5%.

**RESULTS**

Among the 22 participants, 18 were finally included in the analysis due to the loss of 18.2% (n=4) of the sample due to problems related to data collection or refusal to participate in the research. No biochemical changes that indicated hepatic or renal insufficiency were observed in the participants. In addition, the presence of chronic diseases that alter the metabolism of vitamin D was not observed.

The sample included individuals with an average age of 10.61±6.57 years, of which 55.60% (n=10) were men and 44.40% (n=8) were women (Table 1). Table 2 shows the mean serum 25(OH)D levels of the general population, which are within normal range (mean: 34.63±13.768ng/dL), and 33.33% (n=6) had a serum 25-(OH)D level of 30ng/dL. In relation to the genotypes, 5.6% (n=1) presented with the FF genotype, 72.30% (n=13) presented with the Ff genotype, and 22.20% (n=4) presented with ff genotype. In addition, the prevalence rate for anemia and low weight for age was 27.80% (n=5). Data are not presented.

A lower level of serum hemoglobin (p=0.008) and hematocrit (p=0.019) was observed in the deficient group, and these values were positively correlated with the levels of 25(OH)D (0.592ng/dL and 0.512ng/dL, respectively). In addition, these individuals had higher leukocyte count (p=0.0114) (Table 2).

Based on the analysis, no association was observed between the genotypes (FF, Ff, and ff) and 25-(OH)D levels (p=0.2451) (Table 3). An association was observed between the genotype FF/Ff and total leukocyte count (p=0.01) (Table 4).

**DISCUSSION**

Studies on the association between hematological profile and serum 25-(OH)D levels and the FokI polymorphism of the VDR gene in individuals with CF. Thus, this is the first study
Table 1. Characteristics of the participants and association between serum 25-hydroxyvitamin D level and the sociodemographic data of individuals with cystic fibrosis. João Pessoa (PB), Brazil, 2016.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Participants (n=18)</th>
<th>Individuals with normal 25(OH) D* levels (n=12)</th>
<th>Individuals with low 25(OH) D** levels (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence</td>
<td>Prevalence</td>
<td>Prevalence</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>55.6%</td>
<td>75%</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>44.4%</td>
<td>25%</td>
</tr>
<tr>
<td>Level of education</td>
<td>Illiterate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1st degree</td>
<td>44.45%</td>
<td>58.33%</td>
</tr>
<tr>
<td></td>
<td>2nd degree</td>
<td>44.4%</td>
<td>41.67%</td>
</tr>
<tr>
<td></td>
<td>Graduate</td>
<td>11.10%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Post-graduate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Family income</td>
<td>1–5 minimum wages</td>
<td>88.89%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>5–10 minimum wages</td>
<td>5.56%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>&gt;10 minimum wages</td>
<td>5.56%</td>
<td>-</td>
</tr>
<tr>
<td>Skin color</td>
<td>Very pale</td>
<td>16.67%</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fair</td>
<td>22.22%</td>
<td>16.67%</td>
</tr>
<tr>
<td></td>
<td>Less fair</td>
<td>16.67%</td>
<td>16.67%</td>
</tr>
<tr>
<td></td>
<td>Light brown</td>
<td>16.67%</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>Dark brown</td>
<td>22.22%</td>
<td>33.33%</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>5.55%</td>
<td>8.33%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>p† value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>10.61±6.57</td>
<td>11.03±4.400</td>
<td>9.66±10.13</td>
<td>0.6797</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.10±2.13</td>
<td>15.38±2.541</td>
<td>14.63±0.93</td>
<td>0.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure to sunlight (minutes)</td>
<td>20±19.92</td>
<td>24.54±23.71</td>
<td>11.66±4.02</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *25(OH)D ≥75nmol/L (30ng/mL). **25(OH)D<75nmol/L (30ng/mL). †p***Values between the groups, according to the independent Student t-test.
Data are expressed as mean and Standard Deviation (SD); 25(OH)D: 25-hydroxyvitamin D; BMI: Body Mass Index; PTH: Parathyroid Hormone.

that evaluated this association. In addition, because CF is a rare disease, a sample of 18 patients is representative, and the results found are relevant.

Based on the data obtained in the present study, an association was observed between serum 25-(OH)D levels and hemoglobin (p=0.008) and hematocrit (p=0.019) levels and total leukocyte count (p=0.0114). Individuals with 25-(OH)D deficiency had lower hemoglobin and hematocrit levels and increased total leukocyte count than those with normal hemoglobin and hematocrit levels and total leukocyte count. In addition, no correlation was observed between the genotypes (FF, FF, and ff) and the serum 25-(OH)D levels (p=0.2451).

The results of the present study were in accordance with those of previous studies [23]. When examining the interrelationships between iron and vitamin D and the hormones in pregnant adolescents, Thomas et al. [24] have observed that maternal 25-(OH)D level was positively associated with maternal hemoglobin level during the start of gestation as well as during delivery (p<0.01.
Table 2. Biochemical variables according to serum 25-hydroxyvitamin D levels in individuals with cystic fibrosis. João Pessoa (PB), Brazil 2016.

<table>
<thead>
<tr>
<th>Variables</th>
<th>General (n=18)</th>
<th>Individuals with normal 25(OH)D* levels (n=12)</th>
<th>95% CI</th>
<th>Individuals with low 25(OH)D** levels (n=6)</th>
<th>95% CI</th>
<th>Student t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D² level</td>
<td>34.63±13.768</td>
<td>42.15±9.53</td>
<td>36.09–48.02</td>
<td>19.60±6.18</td>
<td>13.11–26.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTH¹ level</td>
<td>41.1±17.717</td>
<td>42.38±18.25</td>
<td>30.78–53.98</td>
<td>38.53±17.96</td>
<td>19.68–57.38</td>
<td>0.6772</td>
</tr>
<tr>
<td>Calcium¹ level</td>
<td>9,960</td>
<td>10.020</td>
<td>9.87–10.29</td>
<td>9.800</td>
<td>19.68–57.38</td>
<td>0.2161</td>
</tr>
<tr>
<td>HMG¹ level</td>
<td>12.91±1.602</td>
<td>13.56±1.26</td>
<td>12.76–14.36</td>
<td>11.53±1.44</td>
<td>10.06–13.09</td>
<td>0.008</td>
</tr>
<tr>
<td>HMT¹ level</td>
<td>39.09±4.700</td>
<td>40.85±3.84</td>
<td>38.40–43.29</td>
<td>35.56±4.49</td>
<td>30.84–40.28</td>
<td>0.019</td>
</tr>
<tr>
<td>Leukocyte¹ count</td>
<td>10,286.1±3,847.8</td>
<td>8,790.8±2,556.2</td>
<td>7,166.7–10,415</td>
<td>13,277±4,457.1</td>
<td>8,598.4–17,955</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Note: Data are expressed as mean and Standard Deviation (SD). *25(OH)D ≥75nmol/L (30ng/mL); **25(OH)D <75nmol/L (30ng/mL). Parametric data according to normality test, comparison between the groups according to the independent Student t-test. Non-parametric data, comparison between groups according to the Mann-Whitney test presented as medians.


Table 3. Association between genotypes and serum 25-hydroxyvitamin D levels in individuals with cystic fibrosis. João Pessoa (PB), Brazil, 2016.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Sufficient* (n=12)</th>
<th>Insufficient/deficient** (n=6)</th>
<th>Number of participants (18)</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF/FF genotype</td>
<td>8</td>
<td>6</td>
<td>14</td>
<td>0.1453</td>
<td>0.0065–3.212</td>
<td>0.2451</td>
</tr>
<tr>
<td>ff genotype</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *25(OH)D ≥75 nmol/L (30 ng/mL); **25(OH)D <75nmol/L (30ng/mL). OR: Odds Ratio; 95% CI: 95% Confidence Interval; p: p-value according to the chi-squared test. 25(OH)D: 25-hydroxyvitamin D.

Table 4. Biochemical variables in accordance with the allelic distribution of the VDR FokI polymorphism in individuals with cystic fibrosis. João Pessoa (PB), Brazil, 2016.

<table>
<thead>
<tr>
<th>Variables</th>
<th>FF/Ff genotype (n=14)</th>
<th>95% CI</th>
<th>ff genotype (n=4)</th>
<th>95% CI</th>
<th>Student t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D³ level (mg/dL)</td>
<td>32.35±14.02</td>
<td>24.26–40.45</td>
<td>42.60±10.62</td>
<td>25.71–59.49</td>
<td>0.19</td>
</tr>
<tr>
<td>PTH¹ level</td>
<td>37.60±13.10</td>
<td>30.03–45.12</td>
<td>53.35±27.80</td>
<td>8.99–97.72</td>
<td>0.11</td>
</tr>
<tr>
<td>Calcium¹ level</td>
<td>9.925</td>
<td>9.42–10.13</td>
<td>10.16</td>
<td>9.55–10.83</td>
<td>0.272</td>
</tr>
<tr>
<td>Hemoglobin¹ level</td>
<td>12.59±1.30</td>
<td>11.82–13.33</td>
<td>14.05±2.21</td>
<td>10.53–17.57</td>
<td>0.10</td>
</tr>
<tr>
<td>Hematocrit¹ level</td>
<td>38.67±4.11</td>
<td>36.16–40.91</td>
<td>41.00±6.75</td>
<td>30.24–51.75</td>
<td>0.37</td>
</tr>
</tbody>
</table>

Note: Data are expressed as mean and Standard Deviation (SD). *25(OH)D ≥75nmol/L (30ng/mL); **25(OH)D <75nmol/L (30ng/mL). Parametric data according to normality test, comparison between the groups according to the independent Student t-test. Non-parametric data, comparison between the groups according to the Mann-Whitney test presented as medians. 25(OH)D: 25-hydroxyvitamin D; PTH: Parathyroid Hormone.
for both). In addition, maternal 25-(OH)D level was inversely associated with erythropoietin level during the onset of gestation (p<0.05) and during delivery (p<0.001).

Recently, Silva et al. [25] investigated the association between hematological profile and serum 25-(OH)D levels and the BsmI polymorphism of the VDR gene in non-institutionalized elderly patients, thus showing that vitamin D deficiency is associated with low hemoglobin levels. The influence of nutritional status on anemia was assessed in this group, and no association was observed. Thus, this result was in accordance with that of previous studies showing that serum vitamin D levels is associated with hemoglobin levels.

In recent years, the associations between vitamin D and multiple chronic and degenerative diseases have been a topic of interest. These effects have been attributed mainly to the immunomodulatory and anti-inflammatory activity of vitamin D. The cells involved in these processes express the VDR gene and can produce and respond to its active form [26].

When assessing the association between serum 25-(OH)D levels and the biomarkers of oxidative stress, inflammation, and endothelial activation in obese children, researchers found insufficient serum 25-(OH)D levels (<20ng/mL) in 5% of individuals in the control group and in 30% of obese children. In addition, in obese children with vitamin D insufficiency, the concentrations of inflammatory markers were substantially higher [27].

Studies have shown that polymorphisms of the VDR gene affect the activity of its encoding protein and, consequently, the metabolic effects mediated by vitamin D. The FokI polymorphism has been associated with increased susceptibility to gastric, prostate, liver, and breast cancer [28-32]. It has also been associated with bone mass and the components of metabolic syndrome [33,14].

Bhanushali et al. [34] have found a correlation between serum 25(OH)D levels (p<0.05) and TaqI but not with FokI (p>0.05) in healthy individuals in India.

On the other hand, in contrast to the results of a previous study mentioned above, Mackawy & Badawi [14] have investigated the role of vitamin D in chronic inflammation and insulin resistance in Egyptians with Type 2 Diabetes (T2DM) and those with or without Metabolic Syndrome (METS). Moreover, they have found out that FokI polymorphism is associated with serum 25(OH)D levels in individuals with T2DM and those with or without SM.

Previous studies on the association between the VDR FokI polymorphism and inflammatory conditions in individuals have not been carried out. However, in the present study, results showed an increased prevalence of inflammatory diseases in individuals with the FokI polymorphism, as reflected in the increase in total leukocyte count.

Moreover, only one polymorphism of the VDR gene was analyzed. Thus, other studies must be conducted to investigate the influence of other polymorphisms on the hematological profile and the metabolism of 25(OH)D in this population. In addition, as this is a cross-sectional study, a causal relationship between FokI polymorphism and total leukocyte count or serum 25(OH)D levels and hemoglobin and hematocrit levels and total leukocyte count cannot be established.

Although this study provided relevant data, it has some limitations. First, its sample size was reduced. Nevertheless, this study first evaluated the proposed associations, suggesting that it is reproducible in samples with larger sample sizes of patients with CF.

**CONCLUSION**

Results showed that vitamin D plays a role in regulating the immune response. In the present study, 25-(OH)D insufficiency was associated with hemoglobin and hematocrit
levels and total leukocyte count in individuals with CF. In addition, an association was observed between the FokI polymorphism and total leukocyte count.

CONTRIBUTORS

MLC ASSIS: Conception and design of the study, data acquisition, analysis and interpretation of data, drafting of the article; CGB CARTAXO: Conception and design of the study, analysis and interpretation of data, drafting of the article, review and approval of the final version of the article for submission; MJC COSTA: Conception and design of the study, analysis and interpretation of data, drafting of the article, review and approval of the final version of the article for submission; DJM QUEIROZ: Data acquisition, analysis and interpretation of data, drafting of the article, review and approval of the final version of the article for submission; DC PERSUHN: Conception and design of the study, analysis and interpretation of data, drafting of the article, review and approval of the final version of the article for submission.

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