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Serum vitamin D binding protein level, but not serum total, bioavailable, free vitamin D, is higher in 30-days survivors than in nonsurvivors with sepsis

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Abstract

The prognostic value of 3 types (total, bioavailable, and free) of 25-hydroxy vitamin D [25(OH)D] and vitamin D binding protein (VDBP) in patients with sepsis is unknown. The aim of this study was to evaluate the association of levels of those 3 types of 25(OH) D and VDBP with 30-day mortality in patients with sepsis. From March to December 2018, patients diagnosed with sepsis and admitted to the medical intensive care unit were enrolled, prospectively. We measured total 25(OH)D and VDBP levels, performed *GC* genotyping for the polymorphisms rs4588 and rs7041, and calculated bioavailable and free 25(OH)D levels. Total, bioavailable, and free 25(OH)D levels did not differ in 30-days nonsurvivors and survivors. Serum VDBP level was significantly higher in survivors than nonsurvivors (138.6 ug/mL vs 108.2 ug/mL, $P = .023$) and was associated with 30-day mortality in univariate but not multivariate analysis. *VDBP* polymorphisms and allele frequencies were not statistically different between the groups. Serum VDBP level was significantly higher in survivors than nonsurvivors over 30-days mortality in septic patients. However, 3 types (total, bioavailable, and free) of 25(OH)D levels did not differ between the survivors and nonsurvivors group.

Abbreviations: 25(OH)D = 25-hydroxy vitamin D, AKI = acute kidney injury, APACHE = acute physiology and chronic health evaluation, ARDS = acute respiratory distress syndrome, BMI = body mass index, CI = confidence interval, CKD = chronic kidney disease, CLD = chronic liver disease, COPD = chronic obstructive pulmonary disease, CVD = cerebrovascular disease, CRP = C-reactive protein, DM = diabetes mellitus, Hb = hemoglobin, HF = heart failure, HR = hazard ratio, ICU = intensive care unit, MV = mechanical ventilation, RRT = renal replacement therapy, SOFA = sequential organ failure assessment, SNP = single-nucleotide polymorphism, VDBP = vitamin D binding protein.

Keywords: mortality, sepsis, VDBP, vitamin D

1. Introduction

In humans, vitamin D is mainly synthesized in the skin upon exposure to sunlight, although a limited amount of vitamin D can be obtained form the diet. Two enzymatic hydroxylation reactions are required to convert vitamin D into its active form. The first hydroxylation occurs in the liver where vitamin D is converted to 25-hydroxy vitamin D [25(OH)D]. Subsequetly, 25 (OH)D is transported to the kidneys where it is converted to 1α, 25-dihydroxy vitamin D [1α, 25$(OH)_2$D], an active form of vitamin D.[1,2] The majority of circulating active vitamin D or vitamin D metabolites (85%–90%) are tightly bound to vitamin D-binding protein (VDBP). Around (10%–15%) is loosely bound to albumin and less than 1% of circulating vitamin D is present in free form.[3–5] The loosely binding fraction or the free fraction is considered bioavailable vitamin D.[3] To calculate bioavailable vitamin D, serum VDBP should be measured. The 25(OH)D generally tested in the clinical laboratory is total vitamin D, which includes all 3 forms of VDBP-bound, bioavailable, and free 25 (OH)D.

VDBP is a multifunctional protein which is synthesized in the liver and, circulates in the plasma. VDBP is an acute phase reactant, thus its serum level can change depending on various conditions.[6–9] The gene encoding VDBP (*GC*) has a high rate of polymorphism. Two single-nucleotide polymorphisms (SNPs), rs7041 and rs4588, give rise to 3 major isoforms of VDBP (GC1F, GC1S, and GC2). Their frequencies differ among ethnic

Editor: Tobias Sinnberg.

This study was supported by a grant (No. 2018R1C1B5040593) of the National Research Foundation of Korea grant funded by the Korea Government.

The authors have no conflicts of interest to disclose.

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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How to cite this article: Yoo JW, Jung YK, Ju S, Lee SJ, Cho YJ, Jeong YY, Lee JD, Cho MC. Serum vitamin D binding protein level, but not serum total, bioavailable, free vitamin D, is higher in 30-days survivors than in non-survivors with sepsis. Medicine 2020;99:25(e20756).

Received: 4 March 2020 / Received in final form: 17 May 2020 / Accepted: 19 May 2020

http://dx.doi.org/10.1097/MD.0000000000020756

groups. The affinity of VDBP to vitamin D is different depending on the isoforms such as GC1F has the highest affinity for vitamin D, followed by GC1S and GC2.[10,11] Since bioavailable vitamin D concentrations vary depending on the genotype of VDBP, the analysis of genotype of VDBP may be important for critically ill patients who are in hypovitaminosis D situation.

Currently, the vitamin D status is determined by measuring the serum level of total 25(OH)D. The following criteria are used to categorize vitamin D status: less than 20 ng/mL (50 nmol/L) is considered vitamin D-deficient, and 20 to 30 ng/mL (50–75 nmol/L) is considered vitamin D-insufficient.[2,12,13] Previous studies found stronger associations of serum calcium, parathyroid hormone,[3] bone mineral density,[14] and vascular outcomes,[15] with bioavailable 25(OH)D than with total 25(OH)D, suggesting that determining bioavailable 25(OH)D level may be more clinically relevant.

The prevalence of vitamin D deficiency (25(OH)D < 20 ng/mL) exceeds 70% in critically ill patients.[16–18] Mounting evidence suggests that lower 25(OH)D levels in the intensive care unit (ICU) are associated with increased rates of infection, prolonged length of stay, excessive healthcare costs, higher in-hospital mortality, and greater mortality postdischarge from the acute care setting.[16,19,20] During critical illness, there is an increased tissue and organ demand for 1, $25(OH)_2D$.[21] Altered serum VDBP and albumin levels in the setting of inflammation, fluid shifts, capillary leaks, and renal wasting are likely to have a strong influence on the bioavailable 25(OH)D pool.[21–24] To date, most previous studies have investigated the effect of total 25(OH)D on the prognosis of critically ill patients[21] and there have been no studies analyzing 3 types of vitamin D and VDBP along with the prognosis of critically ill patients. Thus, it is not known whether bioavailable 25(OH)D or VDBP levels may be more associated with clinical outcomes than total 25(OH)D level in ICU patients. In particular, the prognostic value of total, bioavailable, free 25(OH)D or VDBP in relation to mortality of critically ill patients is still unclear.

Therefore, we prospectively assessed the prognostic value of 3 types (total, bioavailable, and free) of 25(OH)D and VDBP in ICU patients with sepsis in relation to 30-day mortality. In addition, we investigated the genotype of the VDBP and analyzed it to association to 30-day mortality in critically ill patients.

2. Materials and methods

2.1. Study subjects

From March to December 2018, patients with sepsis that were admitted to the medical ICU were prospectively enrolled. We collected clinical and laboratory data including age, gender, body mass index, underlying disease, acute physiology and chronic health evaluation (APACHE II) score, sequential organ failure assessment albumin level (SOFA) score, and albumin and total 25(OH)D levels from electronic medical records. At the time of ICU admission, blood samples were collected from which serum and leukocytes were separated and stored at −80°C. The study protocol was approved by the institutional review board of Gyeongsang National University Hospital, and written informed consent was obtained from all participants (2017-09-022).

2.2. VDBP and total 25(OH)D assays

VDBP levels were measured using an enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN) according to the manufacturer's protocol. Total 25(OH)D was measured by using the Elecsys Vitamin D Total electrochemiluminescence binding assay (Roche Diagnostics, Mannheim, Germany) and the Cobas 8000 e602 analyzer (Roche Diagnostics).

2.3. GC genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. *GC* genotyping for rs7041 and rs4588 was performed using the TaqMan SNP Genotyping Assay (Thermo Fisher Scientific, Waltham, MA) and the ABI ViiA 7 Real-Time PCR System (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Common *GC* alleles were determined as follows: *Gc1f* (c.1296T; c.1307C), *Gc1s* (c.1296G; c.1307C), and *Gc2* (c.1296T; c.1307A). The interpretation of rs7041, rs4588 for the determination of GC genotyping is summarized in Table 1.

2.4. Calculation of bioavailable 25(OH)D

Based on total 25(OH)D, VDBP, and albumin level, bioavailable 25(OH)D levels were calculated using the following equations.[25]

$$\begin{aligned}&\text{Bioavailable } 25(\text{OH})\text{D}\\&= \text{albumin} - \text{bound } 25(\text{OH})\text{D} + \text{free } 25(\text{OH})\text{D}\\&= (\text{albumin} \times \text{Kalbumin} + 1) \times \text{free } 25(\text{OH})\text{D}\end{aligned}$$

$$\text{Free } 25(\text{OH})\text{D} = \frac{-b + \sqrt{b^2 - 4ac}}{2a}$$

$$\text{a} = K_{VDBP} \times \text{K}_{\text{albumin} \times \text{albumin}} + \text{K}_{\text{VDBP}}$$

$$\text{b} = K_{\text{VDBP}} \times \text{VDBP} - K_{VDBP} \times \text{total } 25(\text{OH})\text{D} + K_{albumin} \times \text{albumin} + 1$$

$$\text{c} = -\text{Total } 25(\text{OH})\text{D}$$

$$K_{albumin} = 6 \times 10^5 \text{M}^{-1}$$

K_{VDBP} [for *Gc1f*] $= 1.12 \times 10^9\ \text{M}^{-1}$, K_{VDBP} [for *Gc1s*] $= 0.6 \times 10^9\ \text{M}^{-1}$, K_{VDBP} [for *Gc2*] $= 0.36 \times 10^9\ \text{M}^{-1}$.[10]

For heterozygous genotypes, the mean affinity for the 2 homozygotes was used ($K_{VDBP1f/1s}$, $0.86 \times 10^9\ \text{M}^{-1}$; $K_{VDBP1f/2}$, $0.74 \times 10^9\ \text{M}^{-1}$; $K_{VDBP1s/2}$, $0.48 \times 10^9\ \text{M}^{-1}$).[26]

2.5. Statistical analysis

Categorical variables are expressed as numbers and percentage and compared with χ^2or Fisher exact tests. Continuous variables

Table 1

The interpretation of rs7041, rs4588 for the determination of GC genotyping.

		DNA base	
		rs7041 (c.1296)	rs4588 (c.1307)
GC allele	*Gc1f*	T	C
	Gc1s	G	C
	Gc2	T	A

are presented as median and interquartile range or as mean ± standard deviation, unless indicated otherwise, and compared with Mann–Whitney *U* or Student *t* tests. Factors associated with mortality were evaluated with Cox proportional hazards regression analysis. All tests of significance were 2-tailed. A *P* value of.05 was considered statistically significant. All data were analyzed with SPSS software version 18.0 (SPSS Inc, Chicago, IL).

3. Results

3.1. Baseline and clinical characteristics of the patients

The baseline and clinical characteristics are shown and compared between survivors and nonsurvivors at day 30 in Table 2. In total, 98 patients were enrolled for this study. The median age was 73.5 years old. 66.3% of the patients were men. At day 30, 54 patients survived whereas 44 patients (44.9%) died. The proportion of men was higher in nonsurvivors than in survivors (79.5% vs 55.6%, $P=.012$). Except for gender proportions, there was no significant difference in the baseline characteristics between survivors and nonsurvivors.

The most common infection site was the lung accounting for 70% of total patients. The APACHE II and SOFA scores were higher in nonsurvivors than survivors ($P<.001$). The proportion of septic shock was a higher trend toward nonsurvivors ($P=.061$). Nonsurvivors had a higher frequency of acute kidney injury and renal replacement therapy more.

There were significantly shorter duration in nonsurvivors than in survivors in terms of ICU [7 days (interquartile range (IQR), 2.25–14 days) vs 9 days (IQR, 4.8–20.3 days), $P=.022$] and hospital period [8 days (IQR, 2.3–14.8 days) vs 24 days (IQR, 14–30 days), $P<.001$] until 30 days.

Table 2

Baseline and clinical characteristics.

Variables	Total N = 98	Survivors N = 54	Nonsurvivors N = 44	*P* value
Age, years old	73.5 (59.8–80)	72.5 (59–79.3)	74 (61.3–80.8)	.449
Gender, male	65 (66.3%)	30 (55.6%)	35 (79.5%)	**.012**
BMI	21.9 (19.5–24.9)	22.2 (19.9–25)	21.5 (18.7–24.8)	.256
Underlying disease				
DM	22 (22.4%)	12 (22.2%)	10 (22.7%)	.952
CKD	12 (12.2%)	6 (11.1%)	6 (13.6%)	.704
CLD	7 (7.1%)	2 (3.7%)	5 (11.4%)	.238
CVD	16 (16.3%)	7 (13%)	9 (20.5%)	.318
COPD	27 (27.6%)	17 (31.5%)	10 (22.7%)	.335
APACHE II	24.5 (21–30.3)	23 (16–25.3)	30.5 (24–34.8)	**<.001**
SOFA	11 (8–14)	10 (6.8–12)	12.5 (10–116)	**<.001**
Infection site				.185
Pulmonary	69 (70.4%)	41 (75.9%)	28 (63.6%)	
Extrapulmonary	29 (29.6%)	13 (24.1%)	16 (36.4%)	
Septic shock	59 (60.2%)	28 (51.9%)	31 (70.5%)	.061
ARDS	28 (28.6%)	14 (25.9%)	14 (31.8%)	.521
AKI	47 (48%)	15 (27.8%)	32 (72.7%)	**<.001**
RRT	31 (31.6%)	8 (14.8%)	23 (52.3%)	**<.001**
Steroid	53 (54.6%)	30 (56.6%)	23 (52.3%)	.670
Invasive MV	94 (95.9%)	53 (98.1%)	41 (93.2%)	.323

AKI = acute kidney injury, APACHE = acute physiology and chronic health evaluation, ARDS = acute respiratory distress syndrome, BMI = body mass index, CKD = chronic kidney disease, CLD = chronic liver disease, COPD = chronic obstructive pulmonary disease, CVD = cerebrovascular disease, DM = diabetes mellitus, HF = heart failure, MV = mechanical ventilation, RRT = renal replacement therapy, SOFA = sequential organ failure assessment.

Bold values: $P<.05$.

3.2. Laboratory findings and vitamin D and VDBP levels

Laboratory parameters, 3 types (total, bioavailable, and free) of 25(OH)D and VDBP levels are shown in Table 3. Platelet and albumin levels were significantly lower in non-survivors at ICU admission ($P=.016$ and $P=.003$, respectively). The level of blood lactate was higher in nonsurvivors than in survivors. Total 25 (OH)D level was a lower trend in nonsurvivors compared with survivors ($P=.088$). Both bioavailable and free 25(OH)D levels were not significantly different between survivors and non-survivors. Serum VDBP levels were significantly higher in survivors than in nonsurvivors ($P=.023$). While the frequency of vitamin D deficiency (<20 ng/mL) among all patients was 67.3% (66/98), there was a higher trend of the rate of vitamin D deficiency toward in nonsurvivors than survivors [77.3% (34/44) in nonsurvivors vs 59.3% (32/54) in survivors, $P=.059$].

3.3. Factors associated with 30-day mortality

Factors associated with 30-day mortality were evaluated by univariate and multivariate analyses and are shown in Table 4. Male gender, APACHE II score, acute kidney injury, and low VDBP levels were associated with 30-day mortality in the univariate analysis. Multivariate analysis revealed an association of acute kidney injury and APACHE II score, but not of VDBP levels.

3.4. GC genotype and allele frequencies in survivor and nonsurvivors

GC genotype and allele frequencies in total patients, survivor and nonsurvivors at day 30 are summarized in Table 5. The most common genotype in all patients was *Gc1f/Gc2* (33.7%), followed by *Gc1f/Gc1s* (28.6%), *Gc1f/Gc1f* (15.3%), *Gc1s/Gc2 (*12.2%), *Gc1s/Gc1s* (5.1%), and *Gc2/Gc2* (5.1%). *Gc1f/Gc2* was the most common genotype in survivors (40.7%) whereas *Gc1f/Gc1s* was the most common genotype in non-survivors (36.4%). VDBP genotype frequencies are not statistically different between survivors and nonsurvivors. In the survivors and nonsurvivors groups combined, frequencies of *Gc1f*, *Gc1s*, and *Gc2* allele were 46.4%, 25.5%, and 28.1%, respectively. *Gc1f* was the most common allele in both survivors (46.3%) and nonsurvivors (46.6%). VDBP allele frequencies did not statistically differ between survivors and nonsurvivors.

4. Discussion

The present study revealed that 70% of patients with sepsis were deficient in vitamin D at ICU admission. Total, bioavailable, and free vitamin D concentrations did not differ between 30-day survivors and nonsurvivors. Serum VDBP level is higher survivors than in nonsurvivors with sepsis. In addition, VDBP was associated with 30-day mortality in the univariate, but not the multivariate analysis.

Previous studies on serum vitamin D levels and the prognosis of critically ill patients have shown controversial results.[27–31] Contradictions may be a result of different characteristics of the enrolled patients and the timing of the prognostic analysis. Most previous studies focused on the prognostic value of total 25(OH) D level in critically ill patients; total 25(OH)D is also commonly used to assess vitamin D status in healthy subjects. However, 25 (OH)D bound to VDBP is typically involved in the regulation of gene expression, requiring intracellular enzymatic cleavage of the

Table 3

Laboratory findings including 3 types (total, bioavailable, and free) of 25(OH)D and VDBP levels of total patients, survivor, and nonsurvivors at 30 d.

Variables	Total N = 98	Survivor N = 54	Nonsurvivors N = 44	*P* value
WBC, $\times 1000/mm^3$	13.2 (9.8–21.6)	12.8 (10.2–23)	13.8 (8–20.4)	.405
Hb, g/dL	10.9 (9.5–12.9)	11.6 (9.7–13.3)	10.6 (9.3–12.4)	.148
Platelet, $\times 1000/mm^3$	167 (93.2–236)	196 (134.3–246.3)	106 (62.8–231.8)	**.016**
Albumin, g/dL	2.8 (2.2–3.4)	3.1 (2.6–3.3.7)	2.6 (2.2–3)	**.003**
CRP, mg/dL	12 (5.5–23.1)	12.6 (3.8–25)	11.5 (6.6–23)	.786
Lactate, mmol/L	3 (1.5–6.5)	2.4 (1.3–4.1)	4.7 (2–9.6)	**.004**
Total 25(OH)D, ng/mL	14 (8–23.2)	15.5 (8.3–25.4)	12.4 (8–19.8)	.088
Bioavailable 25 (OH)D, ng/mL	2 (1.1–3.5)	2.4 (1.2–3.7)	1.7 (0.7–3.3)	.178
Free 25 (OH), pg/mL	7.9 (4.4–12.5)	8.5 (5.2–12.9)	6.1 (3.7–12.1)	.281
VDBP, ug/mL	129.8 (92.4–163.3)	138.6 (103.4–169.3)	108.2 (80.9–153.9)	**.023**

25(OH)D = 25 hydroxy-vitamin D, CRP = C-reactive protein, Hb = hemoglobin, VDBP = vitamin D binding protein, WBC = wthite blood cells.
Bold values: $P < .05$.

Table 4

Univariate and multivariate analysis for factor associated with 30-d mortality.

	Univariate			Multivariate		
Variable	HR	95% CI	*P* value	HR	95% CI	*P* value
Age	1.017	0.993–1.041	.173	1.020	0.990–1.050	.195
Male gender	2.216	1.063–4.617	.034	2.003	0.940–4.268	.072
APACHE II	1.143	1.088–1.200	<.001	1.095	1.035–1.158	**.002**
AKI	4.321	2.217–8.420	<.001	2.440	1.133–5.254	**.023**
VDBP	0.993	0.987–0.999	.024	0.988	0.992–1.005	.642

AKI = acute kidney injury, APACHE = acute physiology and chronic health evaluation, CI = confidence interval, HR = hazard ratio, VDBP = vitamin D binding protein.
Bold values: $P < .05$.

25(OH)D, and thus, it is thought to have limited biological activity during acute stress such as septic condition.[24] Thus, it has been hypothesized that assessment of the bioavailable forms of vitamin D may have greater predictive value for important ICU-related outcomes when compared with total serum 25(OH)D.[24,31,32]

Studies on serum bioavailable vitamin D levels and the prognosis of critically patients are very limited. A previous study that analyzed the association of vitamin D status and 90-day mortality in surgical ICU patients reported that total 25(OH)D, bioavailable 25(OH)D, total $1,25(OH)_2D$, and bioavailable $1,25(OH)_2D$ levels were all predictive of clinically important outcomes.[31] In contrast, in the current study, total, bioavailable, and free 25(OH)D were not associated with 30-day mortality. These conflicting outcomes may be due to different clinical characteristics of patient (surgical patients vs sepsis patients) and the different timing of mortality analysis (90-day mortality vs 30-day mortality).

Table 5

Major *GC* genotype and allele frequencies in total patients, survivor, and nonsurvivors at 30 d.

	Total (N = 98)	Survivors (N = 54)	Nonsurvivors (N = 44)	*P* value
Genotype frequencies				
Gc1f/Gc1f	15 (15.3%)	8 (14.8%)	7 (15.9%)	.898
Gc1f/Gc1s	28 (28.6%)	12 (22.2%)	16 (36.4%)	.253
Gc1f/Gc2	33 (33.7%)	22 (40.7%)	11 (25.0%)	.244
Gc1s/Gc1s	5 (5.1%)	2 (3.7%)	3 (6.8%)	.508
Gc1s/Gc2	12 (12.2%)	6 (11.1%)	6 (13.6%)	.738
Gc2/Gc2	5 (5.1%)	4 (7.4%)	1 (2.3%)	.274
Allele frequencies				
Gc1f	91 (46.4%)	50 (46.3%)	41 (46.6%)	.983
Gc1s	50 (25.5%)	22 (20.4%)	28 (31.8%)	.201
Gc2	55 (28.1%)	36 (33.3%)	19 (21.6%)	.212

In the present study, the 3 types of serum vitamin D levels were measured to evaluate the association with 30-day mortality in patients with sepsis who admitted MICU. In addition, serum VDBP level and genotype were analyzed. While vitamin D levels including 3 types of 25(OH)D did not differ between survivors and nonsurvivors in the present study, VDBP was significantly higher in the survivors group than in the nonsurvivors group with sepsis at 30 days. The current finding is consistent with a previous 1 study, which reported VDBP, but not vitamin D or vitamin D-related peptides, are associated with septic shock mortality.[33] In addition to transporting vitamin D, VDBP has multifunctional properties including immune modulation such as macrophage activation, enhancement of the leukocyte chemotactic activity of activated complement peptides, influencing of macrophage chemotaxis, and may also act through excessive globular actin scavenging.[34–36] In critically-ill trauma patients, low serum VDBP level was associated with a higher risk of respiratory failure and sepsis development.[37] Similarly, a previous study compared a group of critically-ill subjects admitted to ICU with and without sepsis, and healthy controls. Serum VDBP levels were significantly lower in subjects with sepsis compared with those without sepsis.[38] The results of our study and those of previous studies indicate that serum VDBP level may play an important role in critically ill states such as sepsis. This suggests that serum VDBP level could potentially be used as a biomarker to predict clinical outcomes in patients with sepsis. A study

investigating a recombinant VDBP supplementation is required to evaluate the therapeutic potential of VDBP.

In the present study, we also investigated the association of VDBP genotype and allele frequency with the prognosis of critically ill patients. The genotype and allele frequencies of the *GC* gene encoding VDBP were similar to those identified in previous studies.[39,40] No statistically significant association between VDBP genotype, allele frequency, and prognosis was found in this study. However, the number of patients enrolled in our study was warranting further investigation of a potential prognostic relationship between VDBP genotype and mortality in critically ill patients using a large sample size.

Our study has several limitations. First, having a small cohort from a single center limits the generalizability of the results. Second, we did not survey additional information that may influence vitamin D or VDBP levels, such as vitamin D supplementation or the duration of sunlight exposure. Third, the serial measurement of serum vitamin D and VDBP levels of patients during hospitalization were not performed.

In the present study, serum VDBP levels were significantly higher in patients with sepsis who were admitted to the ICU that survived over 30 days, than in those who did not survive. However, total, bioavailable, and free 25(OH)D concentrations and the genotype and allele frequency of VDBP did not differ.

Author contributions

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