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Interaction of Vitamin D Receptor Polymorphism With Inflammation and Calcification Markers, Effect on All-Cause Mortality in Women on Dialysis

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Interaction of Vitamin D Receptor Polymorphism with inflammation and calcification markers, effect on all-cause mortality in women on dialysis.

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Abstract: Polymorphism of vitamin D3 receptor (VDR), has been associated with low bone mineral density and other immune and metabolic disorders; however, its impact on mortality of female dialysis patients is not well studied. This study aimed to identify bone mass-related factors, VDR gene polymorphism, and their interaction with morbid conditions that could influence all-cause mortality. In 246 female dialysis patients, age 43±11 years on continuous ambulatory dialysis peritoneal; 48%, haemodialysis; 23% and automated peritoneal dialysis; 29%. Tscore, Ca, PO4, albumin, hs-CRP, osteoprotegerin, fetuin, osteocalcin, iPTH, PINP and β-CTx were measured. PCR products were digested with Bsml to analyze VDR polymorphisms. Patients n=229; were followed for a median of 17 (15-31) months; 42 patients died. Bsml polymorphism, bb=64% and BB+Bb=36%. Hs-CRP was the risk of death in multivariate Cox Analysis. Patients with bb and inflammation had a higher risk of death (HR 2.48, 95% CI 1.08-5.68) persisted after adjustment for age, diabetes and iPTH (HR 2.33; 95% CI, 1.01-8.33) and after further adjustment for vintage, albumin, osteoprotegerin and vitamin D therapy (HR 3.49; 95% CI, 1.20-10.9). We may conclude that the presences of the bb genotype of VDR and inflammation have additive effects on all cause-mortality in females in CAPD, APD, and HD patients.

Keywords: bone mineral markers; phosphate/calcium homeostasis; dialysis; inflammation; mortality; vitamin D receptor polymorphism; diabetes; vascular calcification; hs-CRP; CKD

1. Introduction

Mineral and bone disorders are common in patients with chronic kidney disease and are associated with bone mass loss, extra-skeletal calcifications (CKD-MBD), increased morbidity, all-cause and cardiovascular mortality, and decreased quality of life (1).

Previous studies have shown markers of inflammation (such as low serum albumin, interleukin 6 (IL-6), C-reactive protein (CRP), and of bone and mineral metabolism (such as hyperphosphatemia, hypercalcemia, increased Ca x PO4 product, secondary hyperparathyroidism, high levels of FGF23 and Klotho) independently or in combination predict adverse outcomes (2-5), significantly those linked to cardiovascular comorbidity and mortality (6-9) in end-stage renal disease (ESRD) patients.

Vitamin D exhibits immune-modulatory and anti-proliferative effects through vitamin D receptor (VDR) in chronic infections (10). Recently, VDR polymorphism variations have also been associated with the severity and susceptibility of COVID-19 (11). Some meta-analyses showed that VDR polymorphisms (FokI, Bsml, Taql, Apa) are associated with breast, skin, and prostate cancer (12-14). The Bsml genotype bb polymorphism has been reported to increase the risk of coronary heart disease (15).

VDR gene polymorphism may be a regulatory factor for losing BMD (16). We have previously shown that VDR polymorphism bb, located in 12q13-14, intron 8 (rs1544410), is associated with high

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BMD in female Mexican dialysis patients (17). Hemodialysis (HD) patients with BB lose bone mineral density faster than Bb + bb carriers (18). Whereas the link between low BMD and mineral metabolism-related proteins with vascular calcification and mortality is well-established, VDR gene polymorphism's impact on dialysis patients' morbidity and mortality remains unclear.

The present study aimed to identify bone mass-related factors, VDR gene polymorphism, and morbidity that could influence all-cause mortality of female dialysis patients.

2. Results

2.1. Clinical characteristics

A total of 246 patients were included in the study: 48% were on continuous ambulatory dialysis peritoneal (CAPD); HD; 23% HD and 29% and automated peritoneal dialysis (APD).

Demographic and clinical data are shown in Table 1

Table 1. Baseline clinical and biochemical characteristics of the total population.

Clinical data:	N=246
Age (y)	43 ± 11
Time on dialysis (m)	30(1-216)
Diabetes mellitus	29%
CAPD/HD/APD %	48,23,29
Calcitriol treatment %	30.1
$CaCO_3$ %	85.6
BMI (kg/m^2)	25.3 ± 4.72
Systolic BP (mmHg)	135.4 ± 20.9
Diastolic BP (mmHg)	83.93 ± 14.29
Amenorrhea (n, Y/N)	95/89
BMD (g/cm^2)	0.42(0.16-0.87)
T-score	-1.26 ±1.06
Z-score	-1.20(-3.29 - 3.0)
% fat body mass	31 (23-37)
Biochemical data:	
Ca x PO_4	45.73 ± 17.6
cCa(alb) (mg/dL)	9.55 ± 1.01
Phosphorus (mg/dL)	5.38 ± 1.9
Albumin (g/dL)	3.6 ± 0.7
PINP (ng/mL)	321(29-2335)
β-CTx (ng/mL)	1.36(0.16-6)
Osteoprotegerin (ng/mL)	5167(893-24939)
Osteocalcin (ng/mL)	169(6-996)
iPTH (pg/mL)	124.5(41.28- 336.8)
Fetuin A (g/L)	0.47 ± 0.11
Hs-CRP (mg/dL)	0.34(0.01-1.1)

Values are mean ±SD or median (25 -75 percentile). Continuous Ambulatory Peritoneal Dialysis (CAPD), Automated Peritoneal Dialysis (APD), Haemodialysis (HD), Body Mass Index (BMI), Bone Mineral Density (BMD), Albumin-corrected calcium (cCa), Total N-terminal propeptide of type 1 (PINP), C-terminal telopeptide-β aspartic acid (β-CrossLaps or BCL), intact parathyroid hormone 1-84 (iPTH), High sensibliity Protein C reactive (Hs-CRP).

Genotype distribution was similar in patients and healthy genetic controls. Frequencies in patients of the bb, Bb, and BB genotypes were 64%, 30%, and 6%, respectively. Bsml polymorphism was in Hardy Weinberg equilibrium in controls and patients (data not shown). For analyses

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purposes, patients were classified in two groups according Bsml polymorphism. One group included patients with bb and the other group the sum BB+Bb. Sixty-four percent of patients were with bb and 36% with BB+Bb.

Patients in the bb group had lower BMI (24.77±4.1 vs 26.17±5.6, p<0.023), lower serum hs-CRP (0.77±1.2 vs 1.16±1.4, p<0.0026), and lower Tscore (-1.14±1.1 vs -1.48±0.97, p<0.023). The number of patients who had T-score in the osteopenic range (<1.0) was significantly lower (p<0.05) in bb group (56%) compared to BB+Bb (76%). In contrast, osteoporosis was not significantly different between groups 10% and 14%, respectively. But Diabetes and the other mineral bone markers were similar in bb and BB+Bb genotypes.

One hundred thirty-four (55%) patients were classified as inflamed. Hs-CRP had a correlation with BMI (r=0.213, p<0.01), Fat% (r=0.208, p<0.001), Glucose (r=0.149, p<0.029), cCa (r=0.313, p<0.001), Fetuin (r=-0.204, p<0.0004), Extracellular Water, (r=0.213, p<0.006), OPG (r=0.150, p<0.037) and BNP (r=0.125, p<0.05).

2.2. Outcome and survival analysis

Among the 246 patients, 42 died, and 17 were lost for follow-up; the clinical outcome was analyzed in 229. The cause of death was acute myocardial infarction in 4 (9%), congestive chronic heart failure in 4 (9%), arrhythmia in 5 (11%), stroke in 1 (2%), infection (except peritonitis) in 8 (18%), peritonitis in 1 (2%), other in 4 (9%) and unknown in 15 (40%) patients who died at home.

Patients were divided into two groups for survival analysis: survivors (n =187) and non-survivors (n=42). Non-survivors were older, had a higher frequency of diabetes, shorter dialysis vintage, lower albumin, creatinine and iPTH. Non-survivors also had increased hs-CRP and OPG levels. The frequency of Bsml polymorphism were not significantly different among survivor and non-survivors (Table 2).

Table 2. Clinical and biochemical characteristics according to survival status.

Group	Survivors	Non-survivors	p-value
Patient n (%)	187(72)	42(24)	
Age (years)	42 ± 11	46 ± 12	0.01
Diabetes mellitus (%)	27	55	0.001
Polymorphism:			
bb n (%)	114 (75)	38 (25)	NS
BB+Bb n (%)	62(76)	20(24)	
Vintage (months)	32(16-69)	24(11-45)	0.04
Creatinine (mg/dL)	10.17 ± 3.5	8.82 ± 3.4	0.001
cCa alb (mg/dL)	9.46 ± 0.97	9.85 ± 1.15	0.040
Hs-CRP (mg/dL)	0.78 ± 1.16	1.30 ± 1.5	0.001
Albumin (g/dL)	3.78 ± 0.66	3.29 ± 0.65	0.003
iPTH (pg/mL)	154(43-349)	73.1(25-202)	0.02
OPG (ng/mL)	5710 ± 3178	7236 ± 4535	0.029

Values are mean ±SD or median (25-75 percentiles). Albumin-corrected calcium (cCa), Intact parathyroid hormone 1-84 (iPTH), Protein C reactive (Hs-CRP), Osteoprotegerin (OPG).

Seventy-three patients were treated with vitamin D therapy. 1,25 (OH)2 VitD3 (Calcitriol) was administered in doses of 0.25 to 0.75 μg/d, and CaCO3 an average of 2.5g /day. The frequency of patients treated with it in the bb group was 32%, whereas 26% were treated in the BB+Bb groups (p>0.05).

In Crude, (univariate) Cox regression analysis, treatment with vitamin D tended to be, associated with lower mortality (HR 0.53 [95% CI 0.24-1.16]; p>0.05), data not shown.

In a univariate Cox proportional hazard model (Table 3), we found that all-cause mortality was associated with high age, diabetes, low concentration of albumin, iPTH, creatinine, and high levels

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of OPG. In a multivariate Cox Proportional hazard model, only inflammation was significantly associated with mortality HR 2.36(95% CI 1.01-5.47). Only the significant variables in the previous analysis were included.

Table 3. Crude and adjusted all-cause mortality in ESRD female patients.

	Univariate		**Multivariate**	
Variable	HR (95% CI)	P	HR (95% CI)	P
Age, years	1.04 (1.01-1.07)	0.008	1.00 (0.97-1.04)	0.72
Vintage, months	0.98 (0.97-1.00)	0.05	0.99 (0.98-1.00)	0.40
Diabetes mellitus, presence	2.88 (1.57-5.31)	0.001	2.06 (0.88-4.80)	0.09
Albumin, g/Dl	0.54(0.35-0.87)	0.01	0.69(0.39-1.21)	0.18
iPTH, pg/Ml	0.65(0.41-1.03)	0.06	0.72(0.42-1.22)	0.22
OPG (per 100 ng/mL)	1.01 (1.01-1.02)	0.034	1.00 (0.99-1.01)	0.74
Creatinine, mg/Dl	0.88(0.80-0.97)	0.008	0.92(0.81-1.04)	0.21
Hs- CRP, >(0.3mg/dL)	1.45(1.22-1.72)	0.001	2.36(1.01-5.47)	0.04

Intact parathyroid hormone 1-84 (iPTH), Osteoprotegerin (OPG), Protein C reactive (Hs-CRP).

The combined impact of Bsml polymorphism and inflammation in all cause-mortality (n=42) was studied by univariate and multivariate Cox analysis with gradual adjustment. In crude analysis, the group of patients with bb and inflammation had an increased risk of death (crude HR 2.48 [95% CI 1.08-5.68]). This risk persisted HR 2.33 [95% CI 1.01-5.33] after adjustment for age (per year), diabetes mellitus, and iPTH (per pg/mL) (Table 4, Model 1). In model 2, we further adjusted for vintage and albumin (per g/dL), and there was no significant change. The adjustment for osteoprotegerin (per unit) and percentage of fat body mass, increased HR to 3.49 [95%. CI 1.20-10.9, p=0.02]

Table 4. Cox regression analysis for all-cause mortality.

Bsml	**n**	**Deaths, n (%)**	**Model 1**	**P-value**	**Model 2**	**P-value**	**Model 3**	**P-value**
bb & non-inflamed	73	8 (19%)	1.00		1.00		1.00	
BB+Bb & non-inflamed	30	3 (7%)	0.84(0.22-3.19)	0.80	0.82(0.21-3.13)	0.77	1.11(0.26-4.77)	0.87
bb & inflamed	75	19 (45%)	**2.33 (1.01-5.33)**	**0.04**	**2.38(1.03-5.50)**	**0.04**	**3.49(1.20-10.9)**	**0.02**
BB+Bb & inflamed	51	12 (29%)	1.96 (0.78-4.94)	0.15	1.90 (0.75-4.85)	0.17	2.76 (0.88-8.61)	0.07

Hazard ratios, their 95% confidence interval, and significance level are indicated. Model 1 includes Bsml genotype versus inflammation, age, DM, and iPTH; model 2 further adjusted for vintage, serum albumin; model 3 further adjusted for OPG and percentage of fat body mass and Vitamin D therapy.

3. Discussion

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To our knowledge, this is the first study in female Mexican dialysis patients in whom the interaction between Bsml polymorphism, inflammation, and its influence on mortality was analyzed. The current study showed that Bsml polymorphism does not significantly influence all-cause mortality in female patients. However, its interaction with inflammation may influence the risk, as inflamed patients with bb genotype had higher mortality than those with the BB+Bb genotype.

Vitamin D regulation of the expression of genes involved in the skeleton, renal, and cardiovascular requires the activation of its nuclear receptor (VDR) through the endocrine/paracrine and autocrine pathways. Vitamin D plays a role in regulating proliferation, immune response modulation, and differentiation of cells, in addition to its classic function in mineral homeostasis (19).

Regarding the VDR, it is already known that it suppresses the expression of PTH, and in turn, it is suppressed by an excess of phosphorus in the diet, and it is stimulated by the FGF 23 of the osteocytes (7).

However, the influence of VDR gene polymorphisms on VDR protein function remains unclear. As Bsml polymorphism is probably nonfunctional, linkage disequilibrium with one or more genuinely functional polymorphisms elsewhere in the VDR gene is assumed to explain the associations observed. It is known that the human VDR is post-transcriptionally regulated by miR-125b (20). In CKD, transcription of several genes, including Bsml polymorphism of VDR, has been implicated in the BMD(17, 18) and iPTH (21). The present study found a bb VDR allele in 64% of patients. Morrison et al; (22) showed that the BB genotype had lower BMD than the bb genotype in normal healthy Caucasian twins. In accordance with the latter, we found that patients with the bb genotype had higher T-score, lower BMI, and hs-CRP than BB+Bb at baseline. iPTH concentration was higher than 250 pg/mL in 38% of bb patients and in 44% of BB+Bb genotype patients, although median iPTH levels were similar in both genotypes (data not shown). Our results contradict those of Torres et al. (23), who found a low PTH in the bb genotype. Notwithstanding, other groups have reported low PTH levels in patients carrying the BB genotype. (24, 25)

The bb genotype has been found to be associated with a higher frequency of diabetes and inflammation (26, 27). For this reason, bb associated with inflammation and diabetes were analyzed. Our findings that age, diabetes mellitus, and the so-called novel risk factors for CVD (such as inflammation, corrected calcium, and albumin) are associated with survival are in concordance with other studies (28, 29). We extended these observations to include levels of serum OPG, as in other studies, OPG was associated with rapid progression of vascular calcification and was a predictor of all-cause mortality in patients on hemodialysis and PD (30). How OPG may operate in vascular pathophysiology has yet to be discovered precisely. However, several clues from clinical studies suggest the role of OPG in vascular calcification (31).

Nevertheless, when we did crude Cox proportional hazards, our results showed no association of serum calcium, phosphorus, calcium-phosphorous product, dialysate calcium concentration, and polymorphism with all-cause mortality. Contrary, as noted in other studies, these variables were associated with all-cause mortality and cardiovascular mortality risk (1).

CKD is associated with enhanced inflammatory activity and an impaired immune response. The development of endothelial damage is widely recognized in CKD patients. The endothelial damage results from sustained toxic inflammatory conditions and contributes to the immune dysfunction developing in these patients. (19)

In multivariate Cox proportional, we found that inflammation was the only factor significantly associated with high mortality when we adjusted for age, iPTH, creatinine, osteoprotegerin, albumin, and diabetes mellitus. The occurrence of inflammation in ESRD patients is estimated at 20% to 70% (32). Our patients showed a high prevalence of inflammation (55%) (33).

Few studies have analyzed the possible association between Bsml polymorphism and outcomes of dialysis patients. Marco et al. (34) showed that Bsml polymorphism strongly influenced clinical outcomes. They showed that bb genotype was over-represented among survivors (46%) compared with non-survivors (22%); in contrast, we found that the percentage of bb was similar in survivors (75vs74%) and non-survivor (25vs24%). Testa et al. (35) found that the number of BB alleles was associated with the left ventricular mass index in ESRD patients in crude and fully adjusted analyses.

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However, they did not explore survival. This study agrees with Santoro et al. (36), who found a higher incidence of left ventricular hypertrophy in the subgroup of patients with the BB or Bb genotype. We did not measure LVH, but higher mortality was found with bb added with hs-CPR.

Results of the present study showed that Bsml polymorphism had no significant independent influence on mortality. However, its interaction with inflammation was striking: inflamed patients with the bb genotype had higher mortality than other patient groups. We assume that in the risk of mortality from all causes, the inflammation had more weight than the VDR polymorphism. This risk persisted after adjustment for age, diabetes mellitus, and iPTH (Table 4, Model 2); there was no modification after a further adjustment for dialysis vintage and albumin. The HR increased by adjustment for OPG and percentage of body fat mass.

These data support the interaction between the VDR genotype and inflammation; this may indicate a close relationship between VDR gene polymorphism bb and the immunological action. Since more than 60 types of cells possess VDR and more than 200 genes represent the target for VDR activation. The principle of this biological effect of vitamin D is gene control. VDR activation generally inhibits cell differentiation and proliferation while promoting cell maturation (37). The involvement of Vit D3 and the VDR system in chronic inflammatory processes has been shown in-vitro experiments; Interleukin 12 (IL-12), produced by myelomonocytic cells, plays a pivotal role in the development of T helper 1 (Th1) cells, which are involved in the pathogenesis of chronic inflammatory autoimmune disorders. Vit D3 inhibits mRNA expression for both IL-12 p35 and p40 gene subunits, acting at the transcriptional level by activated macrophages and dendritic cells, thus providing a novel interpretation of the immunosuppressive properties of Vit D3 (38). On the other hand, an inverse association between Vit D3 and inflammation has been shown in vitro in a study with incubation of human peritoneal macrophages with Vit D3 of CAPD patients, inhibited expression of TNF-alpha at both mRNA and protein levels (39).

The present study has several strengths and limitations that merit discussion; one strength is the relatively large sample size and uniformity of ESRD etiologically distribution. A limitation is that continuous variables reduce residual confounding in our analysis. However, we cannot exclude the possibility of other unknown confounders.

Patients with polymorphism bb during following-up had probably diminished VDR, and their complex with Vit D3, their capacity for suppressing T-cells was insufficient and let the increased inflammation, so the few inflamed bb patients have a greater risk of mortality. With this in mind, inflammation, in combination with Bsml polymorphism of VDR, may be the most important predictor for all-cause mortality in female dialysis patients.

4. Materials and Methods

The template details the sections that can be used in a manuscript. Note that each section has a corresponding style, which can be found in the “Styles” menu of Word. Sections that are not mandatory are listed as such. The section titles given are for articles. Review papers and other article types have a more flexible structure.

4.1. Study populations

A prospective cohort study was performed on 246 prevalent female dialysis patients recruited from five hospitals in Mexico City, with chronic dialysis programs belonging to the Instituto Mexicano Del Seguro Social. The Research and Ethics Committee approved the study protocol from each hospital, and informed consent was obtained from each patient.

All patients were clinically stable and free from infections or acute complications one month before the investigation. We excluded patients with Hepatitis B and HIV seropositivity, cancer, immunosuppressive therapy, parathyroidectomy, and steroid therapy. The causes of ESRD were diabetic nephropathy in 66 patients (27%), arterial hypertension in 31 patients (13%), polycystic kidney disease in 20 (8%), glomerulonephritis in 14 patients (6%), lupus in 13 (5%), pyelonephritis in 4 (2%), obstructive uropathy in 4 patients (2%), and other or unknown causes in 94 patients (37%).

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Patients received dialysis treatment with hemodialysis (HD; n=57, 23%), continuous ambulatory dialysis peritoneal (CAPD; n=118, 48%), or automated peritoneal dialysis (APD; n=71, 29%). HD patients were on a 4-hours, three-times-a-week dialysis schedule with bicarbonate buffered dialysis solutions containing 3.0 mEq/L of calcium (Gambro). CAPD patients received four exchanges with 2 L bags of conventional dialysis solution containing 3.5 mEq/L of calcium (Dianeal, Baxter, Mexico). APD patients also received conventional therapy with dextrose dialysis solutions containing 3.5 mEq/L of calcium (Baxter, Mexico). Demographic and clinical data were recorded for each patient during the investigation.

The patients received medications typical for dialysis patients, such as antihypertensive, calcium-containing phosphate binder (CaCO3) and active vitamin D, as appropriate; 1,25(OH)D3, Vit D3, (calcitriol) was administered in doses of 0.25 to 0.75 μg/d, and CaCO3 an average of 2.5g /day. Patients were considered to have calcitriol treatment if such treatment had been continued for over a month.

Two-hundred twenty-nine patients were followed for a median of 17 months (15-31), and 17 patients were lost for follow-up. The primary cause of death was obtained from the death certificate, and patient files were recorded.

4.2. Biochemical measurements.

After an overnight fast, a venous blood sample was drawn for biochemical analyses. Albumin (g/dL), phosphorus (PO4; g/dL), and total calcium (tCa, g/dL) were measured by conventional spectrophotometry assays and high sensitivity C-reactive protein (mg/dL, hs-CRP) by the Tina-quant immunoturbidometric assay on a Roche/Hitachi 902 (Tokyo, Japan). Intact parathyroid hormone (iPTH,1-84), total pro-collagen type1-amino-terminal propeptide (PINP) in ng/mL and C-terminal telopeptide-β aspartic acid (β-Cross Laps, or β-CTx) in ng/mL were measured by electrochemiluminescence immunoassay (Roche/Elecsys 1010/2010 Roche Manheim, Germany). Osteoprotegerin (OPG; ng/mL) and fetuin (g/L) were determined by ELISA (R&D System Inc, Minneapolis MS, USA) and Epitope Diagnostic Inc (San Diego CA. USA), respectively. The present study defined inflammation as hs-CRP> 0.30 mg/dL.

4.3. Bone mineral density.

The bone mineral density of the calcaneus was determined by quantitative ultrasound (QUS) on a Sahara Clinical Bone Sonometer (Hologic, Waltham, MA, USA). The method has been described previously.

The cut-off point for osteopenia was considered to be a T-score ≤-1.0, and for osteoporosis, a T-score ≤ -2.5 in the calcaneus of healthy women (40).

4.4. Genotyping

DNA was extracted from peripheral blood. PCR products were digested with Bsml to analyze the VDR alleles bb, Bb, and BB, located in 12q13.11, and it is focused on G/A change (rs1544410) in intron 8, as described previously (17). Ninety-four healthy Mexican women served as genetic control.

4.5. Statistical analysis

Unless otherwise indicated, all variables are expressed as mean ± SD or median (25th and 75th percentile). Statistical significance was set at the level of $p<0.05$. Comparisons between the two groups were assessed with the $\chi 2$test. Spearman's rank correlation (p) was used to determine correlations between variables. Hardy-Weinberg equilibrium was tested by comparing expected and observed genotype frequencies using the $\chi 2$ test. Cox regression analyses were performed since the assumption of the proportionality of the hazards was met for all covariates. These models selected confounders based on presumed pathophysiological pathways, and all covariates satisfied the proportionality assumption. Since p values are not adjusted for multiple testing, they must be considered descriptive.

All statistical analyses were performed using the statistical software Stata version 11.2 (Stata Corp, College Station, TX, USA).

5. Conclusions

Presences of bb genotype of VDR had no independent influence on mortality. However, its interaction with inflammation had high risk mortality on all cause-mortality in females in continuous ambulatory peritoneal, automated peritoneal dialysis and Hemodialysis patients.

Author Contributions: Conceptualization, Marcela Avila; Formal analysis, Abdul Rashid Qureshi and Bengt Lindholm; Investigation, María-del Carmen Prado-Uribe and Carmen Mora; Resources, Alfonso Cueto-Manzano; Visualization, Ramón Paniagua; Writing – original draft, Marcela Avila; Writing – review & editing, Marcela Avila, Abdul Rashid Qureshi, Sofia Bernal-Amador and Ramón Paniagua.

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Institutional Review Board Statement: The Research and Ethics Committee approved the study protocol from each hospital.

Informed Consent Statement: Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The database used in the current study is not available in a public repository, but they are available from the corresponding author on reasonable request.

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