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Vascular Calcification and Oxidative DNA Damage as Nontraditional Cardiovascular Risk Factors in Chronic Renal Disease

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<http://dx.doi.org/10.5772/intechopen.73095>

Abstract

The number of CKD sufferers that require renal replacement techniques (RRTs) is increasing. The severity of cardiovascular disease (CVD) is disproportionate in these kinds of patients and contributes considerably to mortality in CKD patients. We evaluated the association between oxidative DNA damage, antioxidant activity and vascular calcification (VC) in CKD. An analytical cross-sectional study was performed. Two simple plaques were taken for each patient (pelvis-hip, and hands-wrists). The presence of VC was scored as presence (1) and absence (0). Oxidative stress was determined by activity of catalase, superoxide dismutase (SOD) and oxidative DNA damage by determination of 8-OHdG marker. Eighty-one patients were included. The RRT type was similar for hemodialysis (HD) and peritoneal dialysis (PD). Thirty-eight patients (47%) presented VC ($p < 0.01$); in 61%, the VC was severe (≥ 3 points). VC prevalence in women was significantly higher, (67%) ($p < 0.001$), and (29%) men. Sixty four percent of the patients submitted to HD presented VC and 27% to PD ($p < 0.001$). The activity of the catalase enzyme was significantly decreased in CKD vs. the healthy control (HC) ($p < 0.0001$). The oxidative DNA damage in CKD was greater vs. HC ($p < 0.0001$). In conclusion, the VC was frequent (47%) in CKD, and decreased catalase activity and greater oxidative DNA damage.

Keywords: end-stage renal disease, vascular calcification, oxidative stress, antioxidants, oxidative DNA damage

1. Introduction

1.1. Chronic kidney disease

Diabetes mellitus (DM) and systemic arterial hypertension are the predominant risk factors for chronic kidney disease (CKD). CKD has become a public health problem. In recent years there has been a progressive increase in the incidence and prevalence of CKD, as well as the number of sufferers who reach the most advanced stage and require renal replacement therapy (RRT); all of which has led to CKD being considered a real epidemic [1]. CKD has the capacity to produce cardiovascular disease (CVD), increasing the risk of hospitalization, morbidity, and mortality [2]. Renal patients are extremely vulnerable to cardiovascular pathology. The mortality is 10–30 times greater in patients with end-stage renal disease (ESRD) compared to the general population. Despite the prevalence of the traditional risk factors being very high, the broadness and severity of cardiovascular complications are clearly disproportionate to the risk profile that these patients have [3]. CVD contributes considerably to the mortality of patients with CKD, including patients with renal transplant (RT) [4]. Therefore, in the past two decades, the interest in nontraditional or emerging risk factors has increased in mineral bone disorders (MBD), inflammation, malnutrition, and oxidative stress. There is evidence that MBD in CKD plays an important role in the increase of cardiovascular morbidity and mortality by favoring the development of vascular calcifications (VC) [5]. MBD increases the risk of CKD-associated CVD with the onset of hyperphosphatemia, VC, and increased levels of fibroblast growth factor 23 [6–8]. When renal function is impaired, it increases the potential for aggravating traditional risk factors (hypertension, dyslipidemia, inflammation, and oxidative stress). The concomitant deterioration of mineral homeostasis and bone metabolism is probably the key factor leading to accelerated CVD [9]. In CKD, MBD alterations occur from the early stages of the disease (stage 2) [10]. MBD alterations favor vascular rigidity by increasing systolic blood pressure, pulse wave velocity, and left ventricular mass in patients with CKD [11]. The structural and functional abnormalities that occur in the vasculature by early CKD cause endothelial dysfunction progressing to VC.

The majority of the vascular pathologies that are present in patients with CKD are secondary to the development of VC. They are characterized as being a multifactorial pathological process with an increase in calcium phosphate deposits in a form of hydroxyapatite. The aforementioned is a result of the imbalance in mineral metabolism that kidney patients have [12]. VC is a common phenomenon in the aging of the general population; however, in CKD, the onset is rapidly accelerated, promoting the development of left ventricular hypertrophy, increasing cardiovascular risk with increased cardiac mortality in patients with CKD [13]. VC can present in the tunica media or in the tunica intima (atherosclerosis or sclerosis of Mönckeberg) of the arterial vessels. VC causes thickening of the intima-media with the formation of atherosclerotic plaques and lesions of the tunica intima, producing local inflammation, dyslipidemia, and accumulation of foam cells [14]. Atherosclerosis is restricted to medium or large arteries and is related to other risk factors such as smoking, obesity, dyslipidemia, hypertension, and aging. Calcification of the tunica media can occur in arteries of any size, is a typical process related to age, and is associated with greater arterial stiffness and reduction of

the damping function [15]. Although initial events leading to calcification of the tunica media or the tunica intima differ, both calcifications reflect an active and highly regulated process that closely resembles endochondral and/or intramembranous bone formation [16].

The oxidative stress is characterized by imbalance between the productions of reactive oxygen species (ROS) that surpasses the capacities of the antioxidants. CKD has been shown to be a prooxidant disease [17]. Atherosclerosis, CVD, and CKD are associated with oxidative stress, inflammation, and reduced availability of nitric oxide (NO). Oxidative stress and inflammation are considered as nontraditional risk factors [18]. The oxidizing compounds have physiological defense mechanisms in the organism, but when there is an imbalance in the generation of the oxidants, it results in damage to the tissues. Oxidative stress has the ability to induce endothelial dysfunction by promoting the progression of atherosclerosis and reducing the availability of NO [19]. There is growing evidence in the general population that indicates correlation between oxidative stress and VC development [20]. Macrophages, endothelial cells, and smooth muscle cells produce ROS (hydrogen peroxide (H_2O_2) and superoxide anion (O^{2-})) in response to diverse stimuli. The free radicals of (NO) are generated in the vascular endothelium from L-arginine by the NO synthase enzyme with the capacity to produce hydroxyl or peroxy radicals [21].

The most important cellular targets of ROS are DNA damage characterized by rupture of DNA strands, point mutations, and compromise of telomere integrity [22]. Shortening of telomeres and mitochondrial DNA damage is related to the rapid onset of atherosclerosis [23]. Increased oxidative stress is a prominent factor in the pathogenesis of VC, while some evidence suggests that increased DNA damage affects contractility of the vascular tunica media [24]. The ROS are capable of damaging macromolecules and DNA bases (purine, adenine, guanine, cytosine, and thymine). Other DNA bases may also be oxidized in a similar manner by the hydroxyl radical. The important consequences of oxidative damage to DNA bases are produced by mutations by the AT \leftrightarrow GC transition and by GC \leftrightarrow TA transversion. These mutations, if not repaired, can lead to changes in the gene expression of proteins [25]. Oxidative DNA damage can be measured by the 8-hydroxy-2'-deoxyguanosine (8-OHdG) marker. Antioxidants play an important role in the proper balance of oxidative stress. Monitoring the levels of antioxidants and oxidative DNA damage products in patients with CKD and the interpretation of the relationship between these markers could contribute better management of patients with CKD [26]. For the above, we set out to evaluate the association between oxidative DNA damage, antioxidant activity, and VC in patients with CKD.

2. Materials and methods

An analytical cross-sectional study was performed. Patient population > 16 years of age who were undergoing RRT and meet the selection criteria while being attended to at the Division of Nephrology-Transplants of the Subspecialty Medical Unit at the Specialties Hospital of the National Occidental Medical Centre, Mexican Social Security Institute (*División de Nefrología-Trasplantes de la Unidad Médica de Alta Especialidad (UMAE) del Hospital de Especialidades, CMNO, del Instituto Mexicano del Seguro Social (IMSS)*), in Guadalajara, Jalisco, Mexico. Patient

demographics, biochemical data, and time of RRT were recorded. Results were determined: hemoglobin (Hb), hematocrit (Hto), sodium (Na), potassium (K), calcium (Ca), phosphorus (P), magnesium (Mg), chlorine (Cl), alkaline phosphatase, intact parathyroid hormone (iPTH), creatinine (Cr), urea, albumin, vitamin D3, and specific C-reactive protein (CRP). VC was determined using simple x-ray plaques according to the method of Dr. Adragao [27]. Two simple plaques were taken for each patient (one of the pelvis and hip and one of hands and wrists); the presence or absence of VC was evaluated in the anatomical territories of the iliac-femoral and radial-digital arteries. VC was scored as 1 (presence) and 0 (absence). The highest score to obtain could be 8 and minimum 0. All plaques were checked by the same radiologist blinded to the clinical characteristics of the patients.

Oxidative stress was determined by the concentrations of antioxidants, and catalase and superoxide dismutase (SOD) and the oxidative DNA damage by determination of the 8-OHdG marker (ELISA technique). Because there were no normal parameters for the reagents, 10 mL extra blood from 20 healthy volunteers (healthy control group) (blood donors) with similar age and gender was used to determine the normal concentrations of the reagents.

2.1. Superoxide dismutase

The kit manufacturer's instructions were followed (SOD No. 706002, Cayman Chemical Company®, USA). The detection of O_2^- generated by the xanthine oxidase and hypoxanthine enzymes was through the reaction of tetrazolium salts. The serum samples were diluted in sample buffer 1:5 in sample buffer: 200 μ L of the radicals' detector, diluted 1:400, was placed, and 10 μ L of the sample was then added. After slow agitation, 20 μ L of xanthine oxidase was added to the wells. The microplate was incubated for 20 min at room temperature, and the absorbency was read at a wavelength of 440 nm.

2.2. Catalase-520

The determination of the antioxidant activity of catalase was performed according to the manufacturer's commercial kit (Bioxytech® Catalase-520™, USA). The spectrophotometric assay was performed by adding 30 μ L of the diluted standards or samples into the corresponding tubes. Five hundred microliter of the substrate (10 mM H_2O_2) was added to each tube and then incubated for 1 min at room temperature, and 500 μ L of the stop reagent was added to each tube. The tubes were capped and mixed by inversion, and 20 μ L of the mixture was added. Two milliliter of the HRP chromogenic reagent was added to each tube, mixed inversely, and incubated for 10 min at room temperature. The absorbance was obtained at 520 nm of optical density.

2.3. 8-Hydroxy-2'-deoxyguanosine

Instructions for the ELISA kit were followed (8-hydroxy-2-deoxyguanosine No. ab10124 Abcam®, Cambridge, United Kingdom). The plasma sample, the EIA buffer, the standards, and the 8-OHdG-AChE tracer were added to all the wells except the blank. The monoclonal antibody 8-OHdG was added, and the plate was incubated for 18 h at 4°C and washed with buffer for the recommended times, and 200 μ L of Ellman's reagent was added to each well. The optical density was read at 405 nm.

2.4. Statistical analysis

The data were analyzed using SPSS software (Statistical Package for the Social Sciences, v. 20, SPSS Inc., Chicago, IL). The Kolmogorov–Smirnov test was used to determine the distribution of the study variables. The quantitative variables are expressed in mean \pm standard deviation/error or median (percentile 25–75%) and Spearman's correlation test. The qualitative variables are expressed in frequencies and percentages and were analyzed with χ^2 or Fisher's exact test for the intragroup analysis. A value of $p \leq 0.05$ was considered statistically significant, with a confidence interval of 95%.

2.5. Ethical considerations

The study was carried out according to the General Law of Health of Mexico in the Field of Research for Health. The study was classified as category III, that is to say, research with a risk greater than the minimum, for which it was necessary to sign the consent under information. Confidentiality of data and patient outcomes was respected. The project was evaluated and accepted by the Local Committee of Research and Ethics in Health of the UMAE, HE, CMNO, Guadalajara, Jalisco, with folio R-2016-1301-97.

3. Results

We included 81 patients with CKD candidates for TR who had previously received dialysis. The patients were young adults, barely older than 30 years of age. The male–female ratio was 1:1. The RRT type was similar for hemodialysis (HD) and peritoneal dialysis (PD). Seventy-five of patients had at least 1.5 years on dialysis. In general, patients had regular control of azoles, serum electrolytes, lipid profile, and albumin. PCR and iPTH were elevated without clinical evidence of infection. Vitamin D3 levels were found to be decreased (**Table 1**).

3.1. Vascular calcification

Of the 81 patients evaluated, 38 (47%) had VC, and in 61% of them VC was severe (≥ 3 points). Women had a significantly higher prevalence of VC: 39 women (67%) had VC, while 42 (29%) of male had VC, although age, dialysis time, lipid profile, urea, creatinine, and albumin were similar in patients with and without VC. iPTH, P, and alkaline phosphatase were significantly higher in patients with VC. Vitamin D3 and calcium levels showed no significant difference (**Table 2**). Patients were compared according to the type of RRT to which they were subjected since the onset of CKD. Sixty-four percent of patients submitted to HD presented VC, and 27% of those submitted to PD. The HD patients were older and were predominantly women, had better control of azoles, and had higher levels of vitamin D3.

3.2. The activity of the antioxidant enzyme

Catalase was found to be significantly decreased ($p < 0.0001$) in CKD. The SOD activity was not found to be decreased in CKD ($p = 0.58$). Regarding the type of RRT and oxidative

	n-81
Age (years)	30.6 ± 9.7
Gender (%)	
Female	39 (48)
Male	42 (52)
Time of dialysis (months)	19 (15–30)
Type of dialysis (n (%))	
Hemodialysis	44 (54)
Peritoneal dialysis	37 (46)
Urea (mg/dL)	121 ± 45
Creatinine (mg/dL)	13 ± 4.1
Phosphorus (mg/dL)	3.5 ± 2.2
Calcium (mg/dL)	9.1 ± 1.1
Sodium (mg/dL)	139 ± 4.0
Total cholesterol (mg/dL)	160 ± 39
Triglycerides (mg/dL)	149 ± 86
LDL (mg/dL)	85 ± 35
Albumin (g/dL)	3.5 ± 0.7
Alkaline phosphatase (U/I)	125 (91–240)
iPTH (pg/mL)	557 (173–911)
Vitamin D3 (ng/mL)	21.1 (16.2–27.4)
PCR (mg/mL)	3.5 (3.0–9.6)

Mean ± SD, median (percentile 25–75%).

Table 1. Demographic and biochemical characteristics of the patients.

stress, we evidenced significantly higher levels of the marker of oxidative damage to DNA (8-OHdG) in patients submitted to PD and more consumed SOD activity ($p < 0.0001$). Serum catalase concentrations were similar between HD and DP (**Table 3**). However, when comparing the results between the antioxidants and the enzymes, we found to decrease significantly the activity of the catalase in CKD vs. the healthy controls ($p < 0.0001$). The activity of SOD was similar between CKD and healthy controls. We found greater oxidative damage to DNA in CKD vs. healthy controls ($p < 0.0001$) (**Table 4**).

To determine the association between oxidative stress data with the MBD results, we used the Spearman correlation test. Catalase showed a negative trend with alkaline phosphatase levels. SOD correlated negatively with the marker of oxidative damage to DNA (8-OHdG),

	CV n-38	No CV n-43	<i>p</i>
Age (years)	29.9 ± 9.2	31.3 ± 10.2	0.51
Gender	12 (29)	30 (71)	
Male (n (%))			
Female (n (%))	26 (67)	13 (33)	0.001
Time in dialysis (months)	18.5 (14.8–30)	22 (13–34)	0.76
BMI (Kg/m ²)	24.2 ± 3.7	23.5 ± 3.0	0.50
Urea (mg/dL)	124 ± 47	120 ± 45	0.73
Creatinine (mg/dL)	12.8 ± 4.2	13.2 ± 4.2	0.70
Albumin (g/dL)	3.7 ± 0.6	3.8 ± 0.7	0.79
PCR (mg/mL)	3.8 (3.0–9.8)	3.0 (3.0–13.9)	0.66
Total cholesterol (mg/dL)	160 ± 43	159 ± 35	0.95
Triglyceride (mg/dL)	160 ± 100	139 ± 69	0.29
LDL (mg/dL)	86 ± 36	85 ± 33	0.94
MBD in CKD			
Vitamin D3 (ng/mL)	21.0 (16.2–26.8)	21.4 (16.2–29.2)	0.91
iPTH (pg/mL)	675 (237–980)	428 (114–650)	0.04
Phosphorus (P) (mg/dL)	4.2 ± 2.3	2.7 ± 1.7	0.001
Calcium (mg/dL)	9.0 ± 1.0	9.2 ± 1.1	0.53
Alkaline phosphatase (UI/L)	141 (106–317)	100 (77–152)	0.01

BMI, body mass index; LDL, low-density lipoproteins; ALP, alkaline phosphatase; iPTH, intact parathormone (data shown as average ± SD or median (percentile 25–75%)).

Table 2. Demographic and biochemical data with and without CV.

and VC score showed a positive correlation with alkaline phosphatase and P. iPTH correlated positively marginally. 8-OHdG was negatively correlated with VC, alkaline phosphatase, and P (**Table 5**). The strength of association between VC and oxidative stress was performed through uni- and multivariate logistic regression analysis. Being female and being ≥30 years of age confer greater risk for VC (**Table 6**).

Demographic, biochemical, and gender-specific differences in MBD in CKD are shown in age (**Table 7**). Women had significantly higher VC frequency (67%) and were predominantly found in HD, although they had significantly lower levels of creatinine and higher concentrations of P compared to men. Age was similar in patients with and without VC. Significantly, RRT (HD) predominated in patients aged ≥30 years with less time in dialysis.

	HD n-44	DP n-37	<i>p</i>
Vascular calcification (n (%))	28 (64)	10 (27)	0.001
Age (years)	30.0 (25.0–37.0)	26.0 (23.3–30.8)	0.05
Gender (male) (n (%))	12 (27%)	30 (82%)	<0.0001
BMI (Kg/m²)	22.3 (21.4–24.3)	23.6 (21.6–26.6)	0.29
Time in dialysis (months)	17.0 (6.8–23.5)	21.5 (15.3–33.0)	0.30
Urea (mg/dL)	108 (78–120)	120 (102–157)	0.03
Creatinine (mg/dL)	11.0 (9.0–12.1)	13.9 (10.1–16.8)	0.005
Albumin (g/dL)	3.9 (3.4–4.2)	3.6 (3.0–4.1)	0.18
Total cholesterol (mg/dL)	149 (128–174)	160 (142–198)	0.07
Triglycerides (mg/dL)	130 (98–179)	134 (82–190)	0.84
LDL (mg/dL)	72 (57–87)	92 (73–120)	0.002
MBD and CKD			
iPTH (pg/mL)	593 (173–911)	509 (126–918)	0.66
Antioxidants			
Catalase (U/mg)	13.5 (8.5–19.0)	18.7 (9.5–25.7)	0.19
SOD (UI/mL)	7.9 (4.3–13.3)	3.3 (2.3–4.6)	<0.0001
Oxidative DNA damage			
8-OHdG (ng/mL)	4.4 (3.1–6.4)	9.4 (7.3–10.8)	<0.0001

MBD, metabolic body disease; iPTH, intact parato-hormone; SOD, superoxide dismutase; 8-OHdG, 8-hydroxi-2'-deoxiguanosina; mean \pm SD or median (percentile 25–75%).

Table 3. Type of dialysis with and without vascular calcification.

	CKD	Healthy control	<i>p</i>
Antioxidants			
Catalase (U/mg)	17.7 \pm 9.0	94.17 \pm 1.58	<0.0001
SOD (UI/mL)	8.2 \pm 7.7	10.2 \pm 1.9	0.58
Oxidative DNA damage			
8-OHdG (ng/mL)	9.4 \pm 11.1	4.7 \pm 1.0	<0.0001

SOD, superoxide dismutase; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; mean \pm SD or median (percentile 25–75%).

Table 4. Oxidative stress in CKD vs. healthy controls.

	r	p
Catalase (U/mg)	-0.29	0.08
Alkaline phosphatase (mg/dL)		
SOD (UI/mL)	-0.40	0.002
8-OHdG (ng/mL)	-0.27	0.03
Score of VC	0.24	0.06
iPTH (pg/mL)	0.30	0.02
Alkaline phosphatase (mg/dL)	0.50	<0.0001
Phosphorus (P) (mg/dL)		
8-OHdG (ng/mL)	-0.52	<0.0001
Score of VC	-0.37	0.003
Alkaline phosphatase (mg/dL)	-0.55	<0.001
Phosphorus (P) (mg/dL)		

SOD, superoxide dismutase; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; iPTH, intact parato-hormone.

Table 5. Correlation between MBD and CKD.

	OR	IC 95%	p
SOD (UI/mL)	1.08	0.99–1.20	0.09
Age (≥30 years)	1.1	1.02–1.20	0.02
Gender (female)	20.8	2.5–173.70	0.00

SOD, superoxide dismutase. Oxidative DNA damage markers: 8-OHdG, PCR, vitamin D3, iPTH, age, gender, phosphorus, LDL, albumin, alkaline phosphatase.

Table 6. Predictors of VC ($\chi^2 = 13.38$; $p = 0.004$).

	Male (n-42)	Female (n-39)	p
VC (n (%))	12 (29%)	26 (67%)	0.001
Age (years)	26.0 (23.5–31.5)	30.0 (25.0–38.0)	0.13
RRT (HD/DP (%))	12/30 (29/71)	32/7 (82/18)	<0.0001
Time in dialysis (months)	21.5 (15.3–30.0)	15.0 (4.5–67.5)	0.37
BMI (Kg/m²)	22.9 (21.9–25.2)	23.6 (20.7–26.9)	0.96
Urea (mg/dL)	114 (96–144)	105 (79–165)	0.54
Creatinine (mg/dL)	13.4 (11.3–16.7)	9.7 (7.3–10.9)	0.001
Total cholesterol (mg/dL)	149 (129–184)	155 (131–183)	0.9
Triglycerides (mg/dL)	134 (81–194)	130 (98–179)	0.76

	Male (n-42)	Female (n-39)	<i>p</i>
LDL (mg/dL)	79 (62–112)	81 (68–88)	0.43
PCR (mg/mL)	3.1 (3.0–17.3)	3.9 (3.0–8.9)	0.94
Vitamin D3 (ng/mL)	21.2 (16.2–31.1)	21.0 (15.9–26.4)	0.51
Calcium (mg/dL)	9.2 (8.5–9.9)	8.9 (8.4–9.4)	0.33
Phosphorus (P) (mg/dL)	2.1 (1.6–2.5)	5.3 (3.2–6.5)	<0.0001
iPTH (pg/mL)	369 (137–615)	650 (203–950)	0.09
Alkaline phosphatase (U/I)	106 (78–138)	150 (95–329)	0.02
Demographic, biochemicals, and MBD in CKD by age data			
	<30 years (n-48)	≥30 years (n-33)	<i>p</i>
VC (n (%))	24 (50)	14 (42)	0.45
RRT (HD/DP (%))	21/27 (45/55)	23/10 (70/30)	0.03
Time in dialysis (months)	24 (17–32)	12 (2–15)	0.02
BMI (Kg/m ²)	23.2 (21.5–25.2)	22.6 (21.6–28.0)	0.44
Urea (mg/dL)	108 (93–144)	123 (94–144)	0.46
Creatinine (mg/dL)	13.4 (10.8–15.8)	12.0 (9.2–15.7)	0.15
Total cholesterol (mg/dL)	153 (129–187)	152 (133–182)	0.86
Triglycerides (mg/dL)	131 (98–187)	127 (88–176)	0.45
LDL (mg/dL)	81 (68–98)	74 (60–102)	0.52
PCR (mg/mL)	4.3 (3.0–13.6)	3.0 (3.0–7.2)	0.12
Vitamin D3 (ng/mL)	20.5 (15.7–28.0)	21.7 (16.4–27.2)	0.73
Calcium (mg/dL)	9.0 (8.4–9.8)	9.2 (8.6–9.8)	0.37
Phosphorus (P) (mg/dL)	2.3 (1.8–4.1)	3.7 (2.1–5.9)	0.06
iPTH (pg/mL)	557 (222–770)	526 (125–955)	0.64
Alkaline phosphatase (U/I)	117 (89–179)	145 (94–320)	0.33
VC, vascular calcification; RRT, renal replacement therapy; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PCR, C-reactive protein; iPTH, intact parathyroid hormone.			

Table 7. Demographic, biochemicals, MBD, and CKD data.

4. Discussion

VC appears as an independent cardiovascular risk factor of the state of hypercholesterolemia and atherosclerosis that present the patient [28]. In the general population, this previously described the protective effect of estrogens against VC [29]. The estrogen-related receptors (ERR) are closely related to estrogen receptors, sharing high homology in the DNA-binding domain, although they do not bind to estrogen [30]. The ERR subfamily consists of three members, ERR α ,

ERR β , and ERR γ (NR3B1–NR3B3), which bind to the classical estrogen response elements and to the extended middle site sequences (ERNA TNAAGGTCA; ERR) as monomers or dimers [31]. ERR α is strongly expressed throughout the differentiation of osteoblasts and regulates the expression of osteopontin through a noncanonical ERR α response element [32]. ERR γ is expressed in osteoblast progenitors and negatively regulates osteoblast differentiation induced by BMP2 and bone formation [33]. ERR γ plays a key role in VC through the positive regulation of the BMP2 signaling pathway, suggesting that inhibition of ERR γ could be a potential therapeutic strategy for VC prevention [34]. Previously, the influence of hormonal *status* in patients with ESRD prevalent in HD was evidenced by the association between follicle-stimulating hormone and MBD in CKD, particularly with bone mineral density. This association could also reflect alterations in the appearance of VC [35]. The majority of patients who suffer VC tend to be of advanced ages and have multiple metabolic, renal, and inflammatory complications. Although in recent years knowledge of the pathophysiology of arteriosclerosis and its close relationship to the mechanisms of oxidative stress has increased considerably, there is still little known on the influence these inflammatory processes have on the development of VC, but it is thought that there is a close link between them [36].

The secondary hyperparathyroidism, hyperphosphatemia, hypercalcemia, and other inherent factors of ESRD, like the state of uremia, inflammation, and the oxidative stress, all of them play a relevant role in the pathophysiology of these vascular alterations. Patients with ESRD present with a chronic inflammatory state that is associated with VC, increased morbidity, and cardiovascular mortality. As well as promoting simultaneous changes in mineral metabolism, the inflammation favors vascular damage that in the long term could lead to the development of VC [37].

Over time, VC has been considered a passive or degenerative illness where minerals spontaneously deposited in the vascular tissue. However, a large number of studies in recent years have contributed to the understanding of the underlying mechanisms of VC and have demonstrated that they form part of a regulated process that carries with it the phenotypical transformation of the vascular smooth muscle cells in osteogenic cells, in response to diverse calcifying stimuli. Thus, it is thought that VC in renal patients is not only due to disturbances in mineral metabolism but that there are other risk factors involved like oxidative stress [38]. The inflammatory mediators must be considered because they are often increased with capacity to activate the renin-angiotensin system in CKD, which probably contributes to increase ROS production and accelerates atherosclerosis. Therefore, promoters of VC increase and inhibitors are reduced, which favors metastatic VC in ESRD. Accelerated atherosclerosis will lead to a higher prevalence of coronary artery disease, heart failure, stroke, and peripheral arterial disease. Consequently, subjects with CKD are exposed to increased morbidity and mortality as a result of CVD [39].

In our study, the predictors of VC were significantly the woman being aged ≥ 30 years. Patients in HD had a higher age and a higher percentage of VC compared to patients undergoing PD, which to some extent was expected due to the higher degree of atherosclerosis and secondary vascular damage that occurs in patients with HD. On the other hand, MBD variables in CKD were evaluated in patients with and without VC; iPTH and alkaline phosphatase levels were elevated in patients with VC (no significant difference) as expected.

Vitamin D 3 levels were slightly decreased in patients with VC. This behavior reflects changes of secondary hyperparathyroidism in CKD. The VC is a complex phenomenon, with many causal associations yet to be fully elucidated, despite the fact that current therapeutic strategies still fail to improve the impact on cardiovascular morbidity and mortality in patients with CKD [40].

Alkaline phosphatase and P were significantly increased in patients with HD. These results agree with previous studies reporting a lower prevalence of VC in patients submitted to PD, which could be a reflection of factors such as lower age, lower anterior vascular damage, and better residual renal function [35]. The prooxidant status of the ESRD was evaluated; our results were consistent with what was previously reported by other authors [41]. The marker levels of oxidative DNA damage (8-OHdG) were significantly increased in patients with ESRD compared to healthy controls. The antioxidant catalase was found to be significantly decreased in patients with ESRD. We cannot explain why the levels of SOD enzyme activity showed no significant difference, since like catalase reflects the activity of the antioxidant system. This could be a result of the magnitude of the defense system; that is to say that large amounts of SOD are required and therefore small changes are not enough showing mathematical differences. We also do not know if the members of the antioxidant defense system operate jointly or independently in CKD, which could explain such results. However, more specific studies are needed to elucidate such findings. Theoretically, there is a link between oxidative stress and VC in ESRD, since both conditions converge in the uremic environment; however, so far there is insufficient evidence in the available literature in human beings about this association. Among their main experimental theories in the study, the possible role of ROS as promoters of VC in patients with ESRD is postulated [42].

With the obtained information, we cannot explain these results, and we have some questions: The levels of the 8-OHdG marker appear to be a compensatory consequence of the SOD activity in the presence of VC. These findings could be due to the limitation of the type of study performed, because no causal association can be determined. However, it would be logical to think that in those patients with VC that showed better antioxidant activity (SOD); its effect with 8-OHdG levels is reflected, although there are no cellular and/or tissue evaluations to prove it. In addition, we cannot know if those patients with VC and 8-OHdG levels previously had them before the presence of VC. In addition, we wonder if there is another associated determinant in patients with VC, which influences such findings. Regarding this, one of the most studied theories is the presence of repair systems of oxidative damage to DNA. Of these systems, it is known that eukaryotic cells can activate mechanisms to repair damage, modify transcriptional activity, and stop the cell cycle [43]. These mechanisms may act independently but frequently act together. If the level of damage exceeds the mechanisms intended to repair DNA, the cell can activate mechanisms of cell death by apoptosis. Among the major DNA repair enzymes are endonucleases, exonucleases, ligases, and DNA glycosylase. In mammals, an important mechanism of DNA repair is the base cleavage repair system which operates as follows: a DNA glycosylase removes the damaged base, generating an apurinic/apirimidic site or abasic site. Subsequently, the sugar/phosphate residue remaining in the abasic site is eliminated by the activity of the APE1 enzyme. Through the activity of DNA polymerase β and ligase III, the correct nucleotide is incorporated and the 3' to 5' ends of the DNA strand is

repaired [44]. If the abasic sites are not repaired, they determine breaks in the DNA strands, and the induction of cellular apoptosis occurs [45].

When performing the correlation tests between oxidative stress and MBD results in patients with CKD, we found that SOD were correlated negatively with the 8-OHdG marker and the VC score and the oxidative stress correlated positively with alkaline phosphatase and phosphorus. The 8-OHdG correlated negatively with VC, alkaline phosphatase, and P. Based on the findings, the importance of the association between oxidative stress and VC in patients with ESRD, and the recent studies evidencing the persistence of alterations in oxidative status even after TR, it is necessary to evaluate the usefulness of antioxidant therapeutic strategies in this population of patients [46]. The results of this study, being the first in our country to describe the association between oxidative stress behavior and VC, contribute to the future evaluation of the oxidative status of the ESRD for prognostic and therapeutic purposes.

5. Conclusions

The VC frequency was 47%. Compared with healthy subjects, patients with ESRD had decreased catalase activity and increased marker levels of oxidative DNA damage (8-OHdG). The correlations of the oxidative stress markers were significant weak; in the case of SOD, it had negative significance with the VC score and the 8-OHdG score. There was a positive correlation with alkaline phosphatase and P. The 8-OHdG marker was negatively correlated with VC, alkaline phosphatase, and P. The significant predictors of VC were being female aging ≥ 30 years.

Conflict of interest

No conflict of interest.

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References

- [1] Brück K, Stel VS, Gambaro G, Hallan S, Völzke H, Ärnlöv J, Katarinen M, Guessous I, Vinhas J, Stengel B, Brenner H, Chudek J, Romundstad S, Tomson C, Gonzalez AO, Bello AK, Ferrieres J, Palmieri L, Browne G, Capuano V, Van Biesen W, Zoccali C, Gansevoort R, Navis G, Rothenbacher D, Ferraro PM, Nitsch D, Wanner C, Jager KJ, European CKD Burden Consortium. CKD prevalence varies across the European general population. *Journal of the American Society of Nephrology*. 2016;**27**(7):2135-2147
- [2] Chien KL, Lin HJ, Lee BC, Hsu HC, Lee YT, Chen MF. A prediction model for the risk of incident chronic kidney disease. *The American Journal of Medicine*. 2010;**123**:836-846
- [3] Liu M, Li XC, Lu L, Cao Y, Sun RR, Chen S, Zhang PY. Cardiovascular disease and its relationship with chronic kidney disease. *European Review for Medical and Pharmacological Sciences*. 2014;**18**(19):2918-2926
- [4] Fliser D, Wiecek A, Suleymanlar G, Ortiz A, Massy Z, Lindholm B, Martinez-Castelao A, Agarwal R, Jager KJ, Dekker FW, Blankestijn PJ, Goldsmith D, Covic A, London G, Zoccali C, for European Renal and Cardiovascular Medicine working group of the European Renal Association–European Dialysis and Transplant Association (ERA–EDTA). The dysfunctional endothelium in CKD and in cardiovascular disease: Mapping the origin(s) of cardiovascular problems in CKD and of kidney disease in cardiovascular condition for a research agenda. *Kidney International. Supplement*. 2011;**1**(1):6-9
- [5] Staude H, Jeske S, Schmitz K, Warncke G, Fischer DC. Cardiovascular risk and mineral bone disorder in patients with chronic kidney disease. *Kidney & Blood Pressure Research*. 2013;**37**(1):68-83
- [6] Moe S, Drüeke T, Cunningham J, Goodman W, Martin K, Olgaard K, Ott S, Sprague S, Lameire N, Eknoyan G; kidney disease: Improving global outcomes(KDIGO). Definition, evaluation, and classification of renal osteodystrophy: A position statement from kidney disease: Improving global outcomes (KDIGO). *Kidney International*. 2006;**69**:1945-1953
- [7] Gutiérrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, Smith K, Lee H, Thadhani R, Jüppner H, Wolf M. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *New England Journal of Medicine*. 2008;**359**:584-592
- [8] Matsushita K, Sang Y, Ballew SH, Shlipak M, Katz R, Rosas SE, Peralta CA, Woodward M, Kramer HJ, Jacobs DR, Sarnak MJ, Coresh J. Subclinical atherosclerosis measures for cardiovascular prediction in CKD. *Journal of the American Society of Nephrology*. 2014;**26**:439-447
- [9] Drüeke TB, Massy ZA. Atherosclerosis in CKD: Differences from the general population. *Nature Reviews. Nephrology*. 2010;**6**:723-735
- [10] Fang Y, Ginsberg C, Sugatani T, Monier-Faugere MC, Malluche H, Hruska KA. Early chronic kidney disease-mineral bone disorder stimulates vascular calcification. *Kidney International*. 2014;**85**:142-150

- [11] Ix JH, Katz R, De Boer IH, Kestenbaum BR, Allison MA, Siscovick DS, Newman AB, Sarnak MJ, Shlipak MG, Criqui MH. Association of chronic kidney disease with the spectrum of ankle brachial index: The cardiovascular health study. *Journal of the American College of Cardiology*. 2009;**54**:1176-1184
- [12] Lu KC, Wu CC, Yen JF, Liu WC. Vascular calcification and renal bone disorders. *The Scientific World Journal*. 2014;**2014**(637065)
- [13] Hruska KA, Seifert M, Sugatani T. Pathophysiology of the chronic kidney disease-mineral bone disorder. *Current Opinion in Nephrology and Hypertension*. 2015;**24**(4):303-309
- [14] Ross R. Atherosclerosis-an inflammatory disease. *The New England Journal of Medicine*. 1999;**340**:115-126
- [15] O'Neill WC, Lomashvili KA. Recent progress in the treatment of vascular calcification. *Kidney International*. 2010;**78**:1232-1239
- [16] Persy V, D'Haese P. Vascular calcification and bone disease: The calcification paradox. *Trends in Molecular Medicine*. 2009;**15**:405-416
- [17] Bonomini F, Rodella LF, Rezzani R. Metabolic syndrome, aging and involvement of oxidative stress. *Aging Diseases*. 2015;**6**:109-120
- [18] Himmelfarb J. Linking oxidative stress and inflammation in kidney disease: Which is the chicken and which is the egg. *Seminars in Dialysis*. 2004;**17**:449-454
- [19] Locatelli F, Canaud B, Eckardt KU, Stenvinkel P, Wanner C, Zoccali C. Oxidative stress in end-stage renal disease: An emerging threat to patient outcome. *Nephrology, Dialysis, Transplantation*. 2003;**18**:1272-1280
- [20] You H, Yang H, Zhu Q, Li M, Xue J, Gu Y, Lin S, Ding F. Advanced oxidation protein products induce vascular calcification by promoting osteoblastic trans-differentiation of smooth muscle cells via oxidative stress and ERK pathway. *Renal Failure*. 2009;**31**(4):313-319
- [21] Byon CH, Javed A, Dai Q, Kappes JC, Clemens TL, Darley-Usmar VM, JM MD, Chen Y. Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. *The Journal of Biological Chemistry*. 2008;**283**(22):15319-15327
- [22] Fyhrquist F, Saijonmaa O, Strandberg T. The roles of senescence and telomere shortening in cardiovascular disease. *Nature Reviews. Cardiology*. 2013;**10**:274-283
- [23] Salpea KD, Humphries SE. Telomere length in atherosclerosis and diabetes. *Atherosclerosis*. 2010;**209**:35-38
- [24] Durik M, Kavousi M, van der Pluijm I, Isaacs A, Cheng C, Verdonk K, Loot AE, Oeseburg H, Bhaggoe UM, Leijten F, van Veghel R, de Vries R, Rudez G, Brandt R, Ridwan YR, van Deel ED, de Boer M, Tempel D, Fleming I, Mitchell GF, Verwoert GC, Tarasov KV, Uitterlinden AG, Hofman A, Duckers HJ, van Duijn CM, Oostra BA, Witteman JC,

- Duncker DJ, Danser AH, Hoeijmakers JH, Roks AJ. Nucleotide excision DNA repair is associated with age-related vascular dysfunction. *Circulation* 2012;**126**:468-478
- [25] Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. 4th ed. Oxford: Oxford University Press; 2007
- [26] Vostálová J, Galandáková A, Strebl P, Zadražil J, Kajabová M, Schneiderka P. Oxidative stress in kidney disease patients. *Vnitřní Lékařství*. 2012;**58**(3):202-207
- [27] Górriz JL, Molina P, Cerverón MJ, Vila R, Bover J, Nieto J, Barril G, Martínez-Castelao A, Fernández E, Escudero V, Piñera C, Adragao T, Navarro-Gonzalez JF, Molinero LM, Castro-Alonso C, Pallardó LM, Jamal SA. Vascular calcification in patients with nondialysis CKD over 3 years. *Clinical Journal of the American Society of Nephrology*. 2015;**10**(4):654-666
- [28] Shanahan CM, Cary NR, Salisbury JR, Proudfoot D, Weissberg PL, Edmonds ME. Medial localization of mineralization-regulating proteins in association with Monckeberg's sclerosis: Evidence for smooth muscle cell-mediated vascular calcification. *Circulation*. 1999;**100**:2168-2217
- [29] Osako MK, Nakagami H, Koibuchi N, Shimizu H, Nakagami F, Koriyama H, Shimamura M, Miyake T, Rakugi H, Morishita R. Estrogen inhibits vascular calcification via vascular RANKL system. Common mechanism of osteoporosis and vascular calcification. *Circulation Research*. 2010;**107**(4):466-475
- [30] Giguère V. To ERR in the estrogen pathway. *Trends in Endocrinology and Metabolism*. 2002;**13**:220-225
- [31] Razzaque MA, Masuda N, Maeda Y, Endo Y, Tsukamoto T, Osumi T. Estrogen receptor-related receptor gamma has an exceptionally broad specificity of DNA sequence recognition. *Gene*. 2004;**340**:275-282
- [32] Zirngibl RA, Chan JS, Aubin JE. Estrogen receptor-related receptor alpha (ERRalpha) regulates osteopontin expression through a non-canonical ERRalpha response element in a cell context-dependent manner. *Journal of Molecular Endocrinology*. 2008;**40**:61-73
- [33] Jeong BC, Lee YS, Park YY, Bae IH, Kim DK, Koo SH, Choi HR, Kim SH, Franceschi RT, Koh JT, Choi HS. The orphan nuclear receptor estrogen receptor-related receptor gamma negatively regulates BMP2-induced osteoblast differentiation and bone formation. *The Journal of Biological Chemistry*. 2009;**284**:14211-14218
- [34] Kim JH, Choi YK, Do JY, Choi YK, Ha CM, Lee SJ, Jeon JH, Lee WK, Choi HS, Park KG, Lee IK. Estrogen-related receptor γ plays a key role in vascular calcification through the Upregulation of BMP2 expression. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2015;**35**(11):2384-2390
- [35] Trujillo-Cuellar Hernando, Sierra-Lara Martínez J Daniel y Osorno-Solís Lucía. Alteraciones del metabolismo mineral y óseo asociadas a la enfermedad renal crónica. *Revista Médica MD*. 2015;**5**(3):151-160

- [36] Chen NX, Moe SM. Vascular calcification: Pathophysiology and risk factors. *Current Hypertension Reports*. 2012;**14**(3):228-237
- [37] Nikodimopoulou M, Liakos S. Secondary hyperparathyroidism and target organs in chronic kidney disease. *Hippokratia*. 2011;**15**(1):33-38
- [38] Leopold JA. Vascular calcification: Mechanisms of vascular smooth muscle cell calcification. *Trends in Cardiovascular Medicine*. 2015;**25**(4):267-274
- [39] Schiffrin EL, Lipman ML, Mann JF. Chronic kidney disease: Effects on the cardiovascular system. *Circulation*. 2007;**116**(1):85-97
- [40] Rojas-Campos E, Herrera-Llamas R, Montañez Fernandez JL, Martinez-Martinez P, Andrade-Sierra J, Avila-Baray AA, Cueto-Manzano AM. Vascular calcification in mexican hemodialysis patients. Elsevier. *Archives of Medical Research*. 2013;**44**:628-637
- [41] Puchades Montesa MJ, González Rico MA, Solís Salguero MA, Torregrosa Maicas I, Tormos Muñoz MC, Saez Tormo G, Juan Garcia I, Miguel Carrasco A. Estudio del estrés oxidativo en enfermedad renal avanzada. *Nefrología*. 2009;**29**(5):464-473
- [42] Al-Aly Z. Phosphate, oxidative stress, and nuclear factor-kB activation in vascular calcification. *Kidney International*. 2011;**79**:1044-1047
- [43] Norbury CJ, Hickson ID. Cellular responses to DNA damage. *Pharmacology and Toxicology*. 2001;**41**:367-401
- [44] Hegde ML, Hazra TK, Mitra S. Early steps in the DNA base excision / single strand interruption repair pathway in mammalian cells. *Cell Research*. 2008;**18**:27-47
- [45] Loeb LA, Preston BD. Mutagenesis by apurinic/apyrimidinic sites. *Annual Review of Genetics*. 1986;**20**:201-230
- [46] Cerrillos-Gutiérrez JI, Miranda-Díaz AG, Preciado-Rojas P, Gómez-Navarro B, Sifuentes-Franco S, Carrillo-Ibarra S, Andrade-Sierra J, Rojas-Campos E, Cueto-Manzano AM. The beneficial effects of renal transplantation on altered oxidative status of ESRD patients. *Oxidative Medicine and Cellular Longevity*. 2016;**2016**

