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Clinical Study

Associations between Pulse Wave Velocity, Aortic Vascular Calcification, and Bone Mineral Density in Chronic Hemodialysis Patients and General Population

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The aims of this study were to compare PWV, vascular calcification (VC), and bone mineral density (BMD) in chronic hemodialysis patients (CHP) with those of the general population patients (GPP). We evaluated 60 GPP (aged 57.5±10.9) compared with 80 CHP (aged 59.3 ± 11.8). PWV was determined from time diversity propagation of the carotid and femoral artery by Doppler ultrasound. Lateral lumbar radiography (LLR) of the abdominal aorta was used to determine the overall abdominal aortic calcification (AAC) score. BMD of the hip and the spine was assessed by dual energy X-ray absorptiometry (DEXA). Biochemical parameters (PTH, Ca^{2+}, P, albumin, CRP, etc.) were determined in all participants according to standard laboratory procedures. The mean PWV was 9.2 ± 2.0 m/s in the GPP and 12.5 ± 2.0 m/s in the CHP. The mean AAC score was as follows: GPP: 2.2 ± 2.69, CHP: 7.98 ± 5.65. The mean BMD at hip was as follow: GPP: 0.875 ± 0.159 (g/cm^2), CHP: 0.729 ± 0.147 (g/cm^2). In the GPP, PWV correlate with VC ($P < 0.001$), BMD ($P < 0.001$), albumin ($P < 0.001$), and CRP ($P = 0.004$). In the CHP, PWV correlates with VC ($P < 0.001$), BMD ($P < 0.098$), albumin ($P < 0.094$), and CRP ($P = 0.004$). There is a strong inverse correlation between PWV and BMD and between VC and BMD. There is a positive correlation between PWV and VC.

1. Introduction

Chronic hemodialysis patients (CHP) belong to the group of patients with a high prevalence of cardiovascular disease. Atherosclerosis is the most frequent cause of cardiovascular morbidity in patients with end-stage renal disease [1].

A direct consequence of atherosclerosis is an increase in vascular stiffness. It has been known for a long time that a high percentage of all cardiovascular diseases are associated with stiffening of the arteries [2]. A Pulse wave velocity (PWV) has been used to assess the stiffness of large vessels and it was found that the stiffness is increased in patients with atherosclerosis. PWV measures the speed of the pressure wave propagation, not the displacement (flow) of blood.

Vascular calcification(VC) and arterial stiffening are independent predictors of all causes of cardiovascular mortality in chronic kidney disease (CKD) [3]. It has already been shown that vascular calcifications produce an increase in the rigidity of the vascular wall with deceleration of the progression of the PWV [3, 4]. Studies have reported increased VC in hemodialysis patients, compared to the general population patients (GPP), with the predominant differences being earlier age of onset and greater distribution. Chronic hemodialysis patients also have stiffer vessels compared to the general population, contributed to reduced arterial compliance and increased PWV [5].

Abnormalities of bone mineral metabolism in hemodialysis patients with decreased bone mineral density (*BMD*) may

contribute to the high incidence of cardiovascular diseases [6]. A large proportion of patients undergoing hemodialysis suffer from bone remodeling abnormalities caused by altered hormonal and metabolic pathway involving calcium, phosphate, and parathyroid hormone [7]. Hence, in the context of CKD, vascular, and bone diseases may often coexist suggesting that they share common pathogenetic mechanisms. Both, increased PWV and bone demineralization have been associated with an increased mortality risk in hemodialysis patients [8].

The aims of this study were to compare PWV, VC, and BMD in the CHP and compare them with those of the GPP. We also investigated serum markers of renal function and mineral metabolism in association with PWV, aortic VC, and BMD.

2. Methods

2.1. Patients. The *control group* (CG), named as *general population patients* (GPP) consisted of 60 patients selected from database of 150 patients from general populations (GP) by proportionate sampling strategies which began by stratifying the GP into relevant subgroups. The number of participants that we recruited from each subgroup was equal to their proportion in the population according to predefined criteria: age, smoking, diabetes, and hypertension. An exclusion criterion was reduced GFR (glomerular filtration rate) $\leq$60 mL/min/1.73 m^2 as determined by the Modification of Diet in Renal Disease (MDRD) formula. Thirty-six patients were males and 24 were females, aged 57.5 $\pm$ 10.9 years, their mean body mass index (BMI) was 27.8 $\pm$ 4.41 kg/m^2. Eighteen patients were smokers, 24 were hypertonic, and 12 were diabetics.

The study group, named as *Chronic Hemodialysis Patients* (CHP), included 80 patients undergoing hemodialysis (53 men and 17 women, aged 59.3 $\pm$ 11.8 years, their mean BMI was 23.5 $\pm$ 3.6. Twenty patients were smokers, 37 were hypertonic, and 16 were diabetics. The mean duration of the dialysis was 5.47 $\pm$ 5.16 years. All of them were on dialysis therapy for more than three months.

Demographic and clinical data were collected from the patient's chart and included age, sex, history of diabetes mellitus, smoking habit, hypertension, and dyslipidemia, cardiovascular history including myocardial infarction, vascular surgery, revascularization, and heart failure. All relevant data (gender, age, hypertension, diabetes, and smokers) in both groups were well matched. There was no statistical significance between the data collected from both groups.

Pulse wave velocity (PWV) was determined in all participants from both groups. Lateral lumbar radiography (LLR) of the abdominal aorta was used to determine the overall abdominal aortic calcification (AAC) score. BMD of the femoral neck and the spine was assessed by dual energy X-ray absorptiometry (DEXA). Biochemical parameters (urea, creatinine, erythrocytes, hemoglobin, albumin, C-reactive protein, calcium, ionized calcium, alkaline phosphatase, parathyroid hormone, glucose, ferritin, cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides) were determined in all participants according to standard laboratory procedures.

All participants signed an informed consent and the study was approved by an ethics committee.

2.2. Diagnostics Methods

2.2.1. Pulse Wave Velocity. The standard method of measuring PWV is to record a proximal and distal flow waveforms at two different sites on the arterial tree [9].

The proposed method includes the following steps: first acquire flow waveforms by Doppler ultrasound at two locations within left common carotid artery (LCCA) and left femoral artery (LFA), next detect the delay or difference in arrival time of the flow wave at the two arterial locations. Distances between sampling sites S (m) are measured as straight lines between the points on the body surface. We used linear 7,5 MHz probe (Toshiba SSA-340A, Toshiba Medical System Corporation, 1385, Shimoishigami, Otara-Shi, Tochigi, Japan) at sequential recording, first was recorded the flow wave from the proximal and than from distal site. At both sites, the ECG was also recorded. The time differences between the R wave of the ECG signal and the onset of the flow waveforms at the two sites (carotid and femoral), T_1 and T_2, yield the time delay: $\Delta T = T_2 - T_1$. The speed of pulse wave (V) was calculated with the standard equation for the speed: S (m) $= V$ (m/s) $\cdot \Delta T$ [s]. Indication of increased arterial stiffness is PWV >9 m/s [8, 10]. The PWV in the HD patients was measured after a dialysis session.

2.2.2. Vascular Calcification. Lateral lumbar X-ray of the abdominal aorta was used to determine AAC (abdominal aortic calcification) scores, which is related to the severity of calcific deposits at lumbar vertebral segments L1 to L4. Lateral lumbar radiography was performed in the standing position using standard radiographic equipment (Shimadzu RADSpeed 324-DK, Nishinokyo-Kuwabarachou. Nakagyo-ku. Kyoto 604-8511. Japan. The film distance was 1 m and the estimated dose of radiation was approximately 15 mGy. Abdominal aortic calcification is typically seen as a linear stippling at the anterior or posterior wall of the aorta with linear edge corresponding to the aortic wall.

Calcifications of the aorta were estimated using a previously validated system [9, 11]. The scores were summarized using the composite score for anterior and posterior wall severity (range score 0–3), where the scores of individual aortic segment calcifications, both for the anterior (maximum score for 4 vertebral segments, $4 \cdot 3 = 12$) and posterior walls (max. 12) were summed (maximum score 24). The total number of aortic segments scores as the total number of aortic segment showing any level of calcification was presented as affected segment score (maximum score 4) [12, 13].

2.2.3. Bone Mineral Density. Bone density scanning, also called dual-energy X-ray absorptiometry (DXA or DEXA) or bone densitometry, is an enhanced form of X-ray technology that is used to measure bone loss. DEXA is today's established standard for measuring bone mineral density (BMD).

DEXA scans were performed on Hologic Delphi QDR4500A/SL (Fort Myers, 33912 FL, USA). BMD was

measured by DEXA in the lumbar spine and hip. Two X-ray beams with differing energy (about 140 kVp and 14,3 mAS) has been used for measuring the BMD. When soft tissue absorption was subtracted out, the BMD was determined from the absorption of each beam by bone [14, 15].

To assess the spine, the patient's legs were supported on a padded box to flatten the pelvis and lower (lumbar) spine. To assess the hip, the patient's feet were placed in a brace that rotates the hip inward. In both cases, the detector was slowly passed over the area, generating images on a computer monitor.

Absolute BMD values, *Z*-scores, and *T*-scores (number of standard deviations below the BMD of a younger reference group) for lumbar spine and right femoral neck were reported and mean scores for all patients were calculated.

A summary of the results provided by the DEXA scans was presented as printed report: BMD (g/cm^2), *T*-score, and *Z*-score (for femoral neck, total and L1–L4 region).

2.3. Laboratory Methods. The serum markers measured were those addressing mineral metabolism, including calcium, phosphate, calcium-phosphate product (Ca × P), parathyroid hormone (PTH) and alkaline phosphatase (ALP), as well haemoglobin, albumin, C-reactive protein, ferritin, glucose, and lipid profile. Blood samples were drawn in the fasting state, before hemodialysis (in the CHP) by COBAS Mira S analyzer, Roche Diagnostics, 333 Fiske Street, Holliston, MA 011746, USA.

2.4. Statistical Analysis. The results were expressed as means ± standard deviations. Univariate associations between PWV, VC, and BMD were explored using linear regression. Univariate regression analysis between PWV and other variables were performed. The following baseline independent categorical variables was entered into linear regression analysis as determinants of PWV, AAC score, and BMD of lumbar spine: age, gender, diabetes (yes/no), hypertension (yes/no), dialysis stage, PTH, ionized calcium, calcium, phosphorus, Ca × P product, alkaline phosphatase, CRP, cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, urea, creatinine, and so forth.

Multivariate linear regression was performed to determine significant associations between VC, PWV, and other variables, adjusted for potential confounders. All tests were two-tailed and a P value of <0.05 was considered statistically significant. Pearsons's correlation coefficient (r) was calculated and tested for significance of linear relationship among variables. A P value <0.05 was considered to be significant. All analyses were performed using the SPSS 20.0 for Windows.

3. Results

The patients from the GPP group were predominantly males (60%) with a median age 57.5 years (range 37–80), 30% (18/60) smokers, 40% (24/60) patients had a history of hypertension, and 20% (12/60) patients had diabetes.

Table 1: The demographics and clinical characteristics of the patients studied.

		GPP	CHP	*P*
Gender	M	36/60 (60%)	53/80 (66.2%)	*0.481*
	F	24/60 (40%)	27/80 (33.8%)	
Age	(years)	57.5 ± 10.9	59.3 ± 11.8	*0.358*
Height	(cm)	165.7 ± 9.9	162.3 ± 8.9	0.035
Weight	(kg)	78.1 ± 16.0	63.5 ± 11.9	**<0.0001**
BMI	(kg/m^2)	27.8 ± 4.4	23.4 ± 3.6	**<0.0001**
HT	(y/no)	24/60 (40%)	37/80 (46.2%)	*0.494*
Diabetes	(y/no)	12/60 (20%)	16/80 (20%)	*1.0*
Smokers	(y/no)	18/60 (30%)	20/80 (25%)	*0.546*

GPP: general population patients; CHP: chronic hemodialysis patients; HT: hypertension; M: male; F: female; BMI: body mass index; *P*: two-tailed probability *P* value; high statistical differences $P <$ **0.001** in bold, statistically significant values $P <$ 0.05 in underline, not significant $P >$ *0.1* in italic.

The patients from the CHP group were predominantly male too (66.2%) with a mean age 59.3 years (range 29–84), 25% (20/80) smokers, 46.25% (37/80) patients had a history of hypertension and 20% (16/80) patients had diabetes. The demographics and clinical characteristics of the patients studied are presented in Table 1.

3.1. Pulse Wave Velocity. The mean aortic PWV in the GPP group was 9.2 ± 2.0 m/s (range 5.2–14.2), male 9.2 ± 2.0 m/s (range 6.1–14.2), female 9.18 ± 1.9 m/s (range 5.2–13.1). The mean aortic PWV in the *CHP group* was 12.5 ± 2.0 m/s (range 8.2–18.2), male 12.5 ± 2.3 m/s (range 8.2–18.2), female 12.4 ± 1.4 m/s (range 8.9–14.3).

The mean aortic PWV in the GPP group with slightly or no presence of calcifications, (AAC score ≤ 4) was 8.6 ± 1.5 m/s (mean age 54.0 ± 8.9 years) versa PWV in the CHP group 9.5 ± 0.9 m/s (mean age 64 ± 10 years) with the same presence of calcifications. The mean aortic PWV in the GPP group with present of calcifications (AAC score ≥ 4) was 11.3 ± 1.4 m/s (mean age 69 ± 6.5 years) compared to the PWV in the CHP group whose was 12.7 ± 1.8 m/s with the same presence of calcifications (mean age 63.16 ± 9.7 years).

The mean aortic PWV in the CHP group with severe calcification (AAC score ≥ 10) was 14.0 ± 1.3 m/s. There was only one patient from the GPP group (male, 80 years of age) with severe calcification (AAC score ≥ 10) whose PWV was equal to14.2 m/s.

The aortic PWV greater than 12 m/s was present in 47.5% (38/80) patients of the CHP group (AAC score = 12.8 ± 3.0 and BMD = 0.765 ± 0.167) and in only 11.7% (7/60) patients from the GPP group (AAC score = 6.8 ± 1.95 and BMD = 0.73 ± 0.14). The aortic PWV greater than 9 m/s was present in 76.25% (61/80) patients from the CHP group (AAC score = 10.3 ± 4.1 and BMD = 0.803 ± 0.157) and in 43.0% (26/60) patients from the GPP group (AAC score = 4.7 ± 2.17 and BMD = 0.845 ± 0.142). The mean arterial PWV in elderly patients (>60 years of age) was 10.8 ± 1.6 m/s in the GPP group and 13.2 ± 1.4 m/s in the CHP group.

Table 2: Variation of PWV and AAC according to absolute hip BMD in the GPP and the CHP.

GPP, CHP	GPP		CHP	
BMD (g/cm^2)	PWV (m/s)	AAC	PWV (m/s)	AAC
<0.7	12.0 ± 1.0	6.4 ± 0.9	12.5 ± 2.5	9.5 ± 5.7
0.7–0.9	9.1 ± 2.2	1.9 ± 2.8	11.7 ± 2.4	8.0 ± 5.7
>0.9	8.9 ± 1.5	1.7 ± 2.1	11.2 ± 2.0	6.6 ± 4.7

GPP: general population patients; CHP: chronic hemodialysis patients; BMD: bone mineral density; PWV: pulse wave velocity; AAC: abdominal aortic calcification.

Variation of PWV and AAC according absolute hip BMD in the GPP group and in the CHP group is presented in Table 2.

3.2. Calcifications. The mean (±SE) AAC score of the GPP group was 2.2 ± 2.69 (range 0–10), male 2.19 ± 2.76 (range 0–10), female 2.21 ± 2.64 (range 0–8). The mean (±SE) AAC score of the CHP group was 7.98 ± 5.65 (range 0–22), male 7.69 ± 5.79 (range 0–22), female 8.44 ± 5.42 (range 0–17).

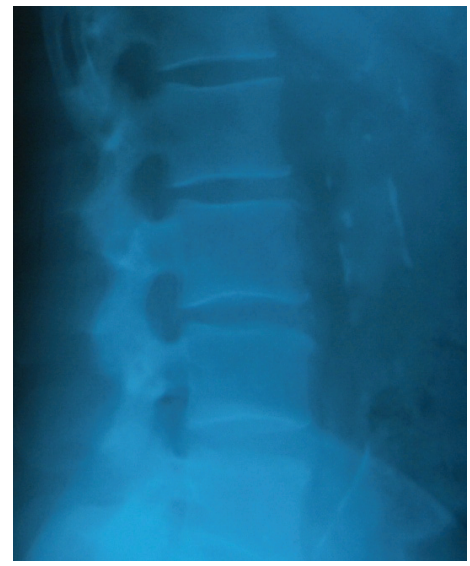
The percent age of patients from the GPP group with slightly or no presence of calcifications (AAC ≤ 4) was 81.66% (49/60), compared to the patients from the CHP group, with slightly or no presence of calcifications (AAC ≤ 4) with smaller percent age of 33.75% (27/80). The percent of patients from the CHP group with no presence of calcifications (AAC = 0) was 20% (16/80). The percent of patients from the CHP group with severe calcifications (AAC ≥ 10) was 41.25% (33/80, AAC = 13.57 ± 2.55). Only one patient (1.67%) from the GPP group had severe calcification (1/60, AAC = 10). The AAC severity increased significantly from L1 to L4 ($P < 0.0001$) with independent predictors for the presence of AC: age, and duration of dialysis.

An example AAC-9 score of 24, five on the posterior wall and 4 on the anterior aortic wall, estimated by Lateral Lumbar X-ray, is presented in Figure 1.

3.3. Bone Mineral Density. The mean (±SD) BMD of the femoral neck, spine BMD (L1–L4 region), average BMD of neck and spine, and DEXA scoring results (T-score, Z-score) for femoral neck and spine, among the GPP group and the CHP group, respectively, were as follows.

(i) For the GPP group: BMD: 0.875 ± 0.159 (g/cm^2), range (0.443–1.298); 0.924 ± 0.167 (g/cm^2), range (0.581–1.334); 0.899 ± 0.145 (g/cm^2), range (0.553–1.308). *DEXA scoring*: −0.77 ± 1.26, range [(−4.6)–(+2.3)]; 0.25 ± 1.18, range [(−3.8)–(+2.7)]; −1.25 ± 1.51, range [(−4.2)–(+2.6)]; −0.3 ± 1.50, range [(−2.8)–(+4.1)].

(ii) For the CHP group: BMD: 0.729 ± 0.147 (g/cm^2), range (0.388–1.224); 0.865 ± 0.172 (g/cm^2), range (0.368–1.484); 0.800 ± 0.14 (g/cm^2), range (0.378–1.354). *DEXA scoring*: −1.86 ± 0.9, range [(−2.8)–(+0.5)]; −0.75 ± 0.98, range [(−2.8)–(+2.0)]; −1.73 ± 1.56, range [(−6.6)–(+3.6)]; −0.67 ± 1.74, range [(−6.1)–(+5.4)].



An example AAC-9 score
(40 year male, HD duration 9 year)

Figure 1: Lateral lumbar X-ray of the abdominal aorta.

The results from unpaired t-test for a difference in mean of PWV, AAC, and BMD when we compared the GPP group and the CHP group are presented in Figure 2.

The changes of mean PWV, mean absolute BMD of the femoral neck and mean AAC according to dialysis duration are shown in 5-year intervals in Figure 3.

The results from biochemical markers of renal function, mineral and bone markers, lipids, glucose, and routine analysis in the GPP and the CHP groups are presented in Table 3.

The results from univariate regression analysis between PWV, aortic VC, and BMD with biochemical parameters of blood are presented in Table 4 for the GPP group and in Table 5 for the CHP group.

When we analyzed the independent determinants of PWV and aortic VC with multivariate regression analysis, we identified that *age* and *triglyceride* levels in the *GPP group* were significant: for PWV (triglycerides, P value = 0.011; age, P value = 0.035) and for VC (triglycerides, P value = 0.005; age, P value = 0.003). The results for the same independent determinants in the *CHP group* were: for PWV (triglycerides, P value = 0.003; age, P value = 0.027) and for VC (triglycerides, P value = 0.002; age, P value = 0.001).

Using the same regression model to analyze BMD, we found the following results significant: parathormone, P value = 0.046; age, P value = 0.0001; PWV, P value = 0.025 and parathormone, P value = 0.036; age, P value = 0.0003; PWV, P value = 0.018, for the GPP and the CHP groups, respectively, (BMD in correlation with parathormone and age).

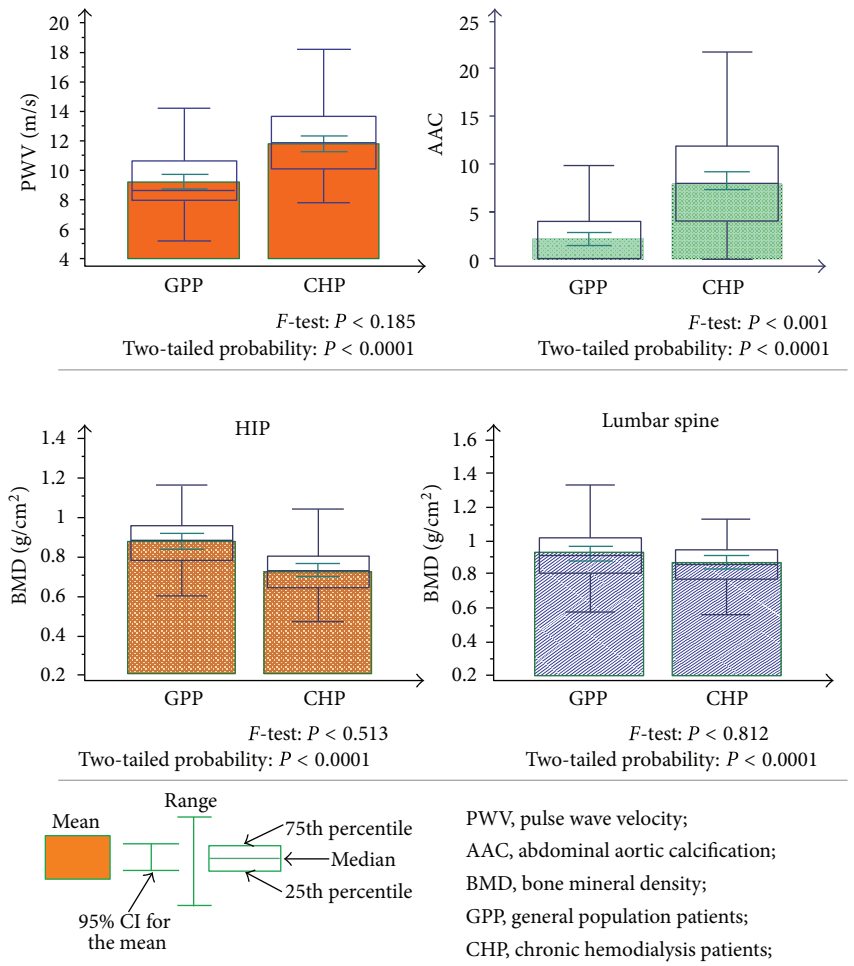


Figure 2: Box plots of PWV, AAC, and BMD of subjects in the two groups.

4. Discussion

Eighty patients from two hemodialysis centers and sixty GP patients from ambulatory internal medicine were recruited and examined during December 2009 and January 2010. All of them were subject to noninvasive diagnostic procedures: native lumbar radiography, DEXA, Doppler examination, and blood biochemistry tests.

The purpose of the study is to verify the correlation between vascular calcifications, bone mineral density and rigidity of the arterial wall in the patients undergoing hemodialysis with those of the general population patients. We also investigated serum markers of renal function and mineral metabolism in association with PWV, aortic VC, and BMD.

There is an association between decreasing vertebral bone density measured by DEXA and increasing vascular stiffness measured by Doppler. The abnormalities of bone metabolism and remodeling appear in two forms: *hyperdynamic* (excessive bone resorption with rapid accrual and removal of calcium and phosphorus from the bone) and *adynamic* (inactive phase with very little ongoing remodeling and accrual of calcium) bone disease. In both cases, poor bone mineralization and excessive calcium and phosphorus in the circulation likely favor deposition of hydroxyapatite crystals in soft tissues. Both minerals can induce a transformation of smooth muscle cells into osteoblast-like cells capable of initiating and sustaining calcification of the interstitium [9, 13, 16].

Alkaline phosphatase can promote vascular calcification by hydrolyzing pyrophosphate in the arterial wall. This increase was reduced by levamisole, a nonspecific inhibitor of alkaline phosphatase [17]. Higher levels of serum alkaline phosphatase in hemodialysis group than levels in control group were associated with progressive arterial calcification. This association was independent of serum calcium, ionized calcium, phosphorus, parathyroid hormone (PTH), and *C-reactive protein*. Clinical studies found that the presence and extent of vascular calcification were associated with higher CRP levels as mortality [18, 19]. We found strong positive correlation of CRP levels with abdominal aortic calcifications and aortic pulse wave velocity such an inverse correlation with bone mineral density in both groups. Our results showed that dialysis patient with high CRP levels had higher levels of

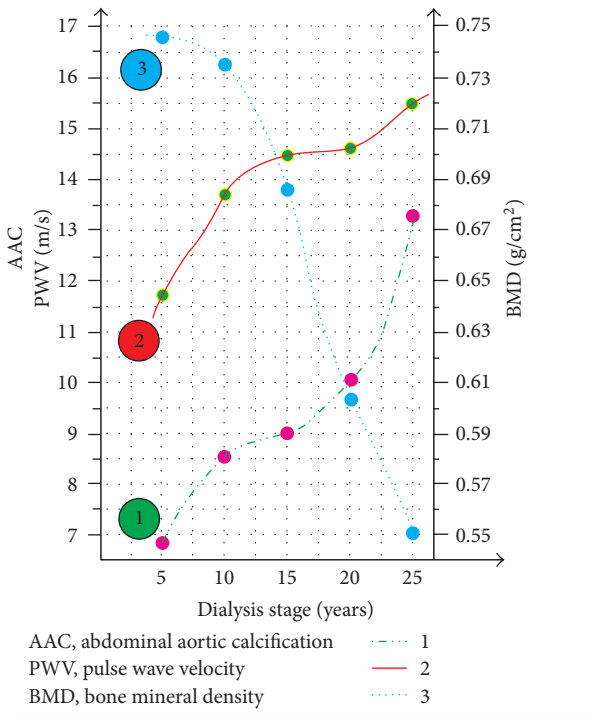


Figure 3: Changes in AAC, PWV, and BMD values in chronic hemodialysis patients.

Table 3: Results of blood test levels from GPP and CHP.

	GPP Mean ± SD	CHP Mean ± SD	*P*
Urea (mmol/L)	6.6 ± 2.1	20.9 ± 5.5	**0.0001**
Creatinine (μmol/L)	84.6 ± 19.7	730.6 ± 204.1	**0.0001**
Haemoglobin (g/L)	125.5 ± 17.0	111.7 ± 18.2	**0.0001**
Albumin (g/L)	43.5 ± 4.9	38.4 ± 4.9	**0.0001**
C-react. protein (mg/L)	7.4 ± 5.7	9.4 ± 2.6	**0.0062**
Calcium (mmol/L)	2.30 ± 0.10	2.19 ± 0.15	**0.0001**
Ioniz. Ca (mmol/L)	1.21 ± 0.05	0.94 ± 0.01	**0.0001**
Phosphate (mmol/L)	1.30 ± 0.09	1.56 ± 0.36	**0.0001**
Ca × P ($mmol^2/L^2$)	2.97 ± 0.24	3.46 ± 0.83	**0.0001**
ALP (U/L)	123.7 ± 42.1	163.7 ± 152.7	**0.0506**
PTH (pmol/L)	5.04 ± 1.61	79.0 ± 77.3	**0.0001**
Glucose (mmol/L)	5.3 ± 1.6	5.6 ± 2.5	**0.4185**
Ferritin (μg/L)	116.1 ± 71.5	408.0 ± 277.2	**0.0001**
Cholesterol (mmol/L)	4.6 ± 1.08	4.5 ± 1.7	**0.6905**
LDL-Ch. (mmol/L)	2.3 ± 0.8	2.4 ± 0.9	**0.4964**
HDL-Ch. (mmol/L)	1.6 ± 0.8	1.2 ± 0.5	**0.0004**
Triglycerides (mmol/L)	1.7 ± 0.2	1.9 ± 0.9	**0.0935**

GPP: general population patients; CHP: chronic hemodialysis patients; C-react. protein: C-reactive protein; Ioniz. Ca: ionized calcium; Ca × P: calcium × phosphate product; ALP: alkaline phosphatase; PTH: parathyroid hormone; LDL-Ch.: low-density lipoprotein cholesterol; HDL-Ch.: high-density lipoprotein cholesterol.

calcium, phosphate, and Ca × P product than GP (general population), exactly; the dialysis patients have more vascular inflammation than GP patients. These findings indicate a close relationship between chronic inflammation and disturbance in calcium and phosphate. The Ca × P product has long been recognized as risk factors for extraosseous calcifications. In our study, this result is more expressed in the CHP group than in the GPP group. Because chronic inflammation is prevalent in the dialysis population, this generalized reaction may be associated with vasculopathy in uremia.

We found strong inverse correlation between CRP and *serum albumin*, more expressed in hemodialysis patients ($P < 0.001$) than the same correlation in general population ($P = 0.023$). However, some studies suggest that serum albumin is independently affected by both inflammation and nutritional intake [19]. Serum albumin also inversely correlated with increased PWV in both GPP and CHP groups, suggesting that increased arterial stiffness might be the link between hypoalbuminemia and accelerated atherosclerosis and increased cardiovascular mortality. The close association between lower serum albumin and increased PWV might be explained by the fact that hypoalbuminemia was associated with increased oxidative stress in dialysis patients. Using plasma protein thiol oxidation and protein carbonyl formation as indictors of oxidative, Danielski found that oxidative stress was significantly elevated in hypoalbuminemia group as compared with normoalbuminemia group. Increased oxidative stress, in turn, could accelerate atherosclerosis

Table 4: Correlation between vascular calcification, bone mineral density, and arterial stiffness: univariate regression analysis in the GPP group.

	GPP					
	PWV		Aortic VC		BMD (FN)	
	r	*P*	*r*	*P*	*r*	*P*
PWV	—	—	0.89	**<0.001**	−0.45	**<0.001**
Age	0.86	**<0.001**	0.85	**<0.001**	−0.51	**<0.001**
BMI	−0.11	ns	−0.11	ns	−0.36	**0.005**
Haemoglobin	−0.50	**<0.001**	−0.53	**<0.001**	0.26	**0.047**
Albumin	−0.51	**<0.001**	−0.51	**<0.001**	0.28	**0.027**
C-reactive protein	0.37	**0.004**	0.36	**0.005**	−0.38	**0.003**
ALP	0.30	**0.021**	−0.33	**0.009**	−0.04	ns
PTH	−0.03	ns	−0.03	ns	0.11	ns
Calcium	−0.20	ns	−0.19	ns	0.10	ns
Ca ionized	−0.22	0.092	−0.20	0.098	0.18	ns
Ca × P	−0.18	ns	−0.12	ns	0.11	ns
Urea	0.29	**0.024**	0.05	**0.013**	−0.18	ns
Creatinine	−0.11	ns	−0.11	ns	0.42	ns
Cholesterol	−0.14	ns	−0.19	ns	0.05	ns
HDL-Ch.	−0.04	ns	−0.12	ns	0.01	ns
LDL-Ch.	−0.11	ns	−0.08	ns	0.02	ns
Triglycerides	−0.08	ns	−0.18	ns	0.11	ns

Statistically significant values $P <$ **0.05** in bold; all $P <$ 0.1 in underline; ns: nonsignificant ($P \geq 0.10$).
PWV: pulse wave velocity; VC: vascular calcification; BMD: bone mineral density; FN: femur neck; BMI: body mass index; ALP: alkaline phosphatase; PTH: parathyroid hormone; Ca: calcium; Ca × P: calcium × phosphate product; HDL-Ch.: high-density lipoprotein cholesterol; LDL-Ch.: low-density lipoprotein cholesterol.

Table 5: Correlation between vascular calcification, bone mineral density, and arterial stiffness: univariate regression analysis in the CHP group.

	CHP					
	PWV		Aortic VC		BMD (FN)	
	r	P	r	P	r	P
PWV	—	—	0.90	<**0.001**	−0.17	0.098
Age	0.59	<**0.001**	0.73	<**0.001**	0.07	ns
BMI	−0.08	ns	−0.07	ns	−0.36	**0.005**
Haemoglobin	−0.31	**0.01**	−0.48	**0.009**	−0.16	0.076
Albumin	−0.18	0.094	−0.17	0.097	0.30	**0.006**
C-reactive protein	0.37	<**0.001**	0.34	**0.002**	−0.14	0.062
ALP	0.22	**0.047**	−0.39	**0.015**	−0.14	ns
PTH	0.07	ns	0.07	ns	−0.10	ns
Calcium	0.05	ns	0.07	ns	−0.03	ns
Ca ionized	−0.03	ns	0.004	ns	−0.03	ns
Ca × P	0.06	ns	−0.18	0.095	0.13	ns
Urea	0.12	ns	0.05	ns	−0.15	ns
Creatinine	0.02	ns	−0.08	ns	0.04	ns
Cholesterol	−0.05	ns	−0.13	ns	−0.10	ns
HDL-Ch.	−0.13	ns	−0.13	ns	−0.09	ns
LDL-Ch.	−0.17	ns	0.24	**0.027**	−0.08	ns
Triglycerides	0.28	**0.035**	−0.18	ns	−0.01	ns
HD duration	0.52	<**0.001**	0.27	**0.015**	−0.27	**0.015**

Statistically significant values $P <$ **0.05** in bold; all $P <$ 0.1 in underline; ns: nonsignificant ($P \geq 0.10$).
PWV: pulse wave velocity; VC: vascular calcification; BMD: bone mineral density; FN: femur neck; BMI: body mass index; ALP: alkaline phosphatase; PTH: parathyroid hormone; Ca: calcium; Ca × P: calcium × phosphate product; HDL-Ch.: high-density lipoprotein cholesterol; LDL-Ch.: low-density lipoprotein cholesterol. HD: hemodialysis.

process. Our results showed inverse significant correlation between PWV and *triglycerides* levels only in hemodialysis group. Sometimes, this correlation is not expressed, because malnutrition hypoalbuminemia masks the triglyceride effect in atherosclerosis. There was no correlation between PWV, VC, and BMD with hypercholesterolaemia in our study (most study patients were on lipid-lowering therapy), but triglyceride levels were an independent determinate of both VC or PWV on multivariate analysis, more expressed in the CHP group than in the GPP group.

Our results showed that *CRP* positively correlated with increased PWV in the CHP group ($P < 0.001$) and in the GPP group ($P = 0.004$). This result suggests that PWV may be a surrogate marker for atherosclerotic vascular damages including an inflammatory component, both in GPP group and CHP group. The uremic state is associated with an altered immune response, which is associated with elevated proinflammatory cytokine levels. CRP concentrations are increased in a significant proportion of end-stage renal disease patients and have been associated with certain clinical outcome measures, including all-cause and cardiovascular mortality. This paper outlines the evidence linking CRP with atherosclerosis and proposes that elevated CRP concentrations may be involved in the initiation and progression of accelerated atherosclerosis in uremia. There was evidence link between CRP and glomerular filtration rate ($r = -0.32$; $P < 0.013$), and also between CRP and serum albumin ($r = 0.55$; $P < 0.001$) in the control group obtained in our study, but the above-mentioned correlations were more pronounced in the CHP group.

Calcification of the arterial system in undergoing hemodialysis patients occurs in the context of atherosclerosis and more representatives in the media layer of the vessel wall. This in particular may be responsible for increased vessel stiffness and increased PWV. An average PWV increases in a graded manner as the severity of AAC increases, more in the CHP group than in the GPP group. Abdominal aorta X-ray calcium remained always associated strongly with higher PWV, at slightly or severe present of calcification, both in the GPP group and in the CHP group. We found a strong correlation between VC and increasing age, C-reactive protein, alkaline phosphatase, LDL-cholesterol, triglycerides, and hemodialysis duration (for the CHP group) but more expressed in the CHP group than in the GPP group.

The reported factors associated with increasing severity and prevalence of VC in HD patients include age, duration of dialysis, and diabetes as well as abnormal mineral metabolism. The only consistent risk factors for stiffer arteries are increasing age and duration of the dialysis. Patients of undergoing hemodialysis have stiffer arteries compared with the nonrenal population, and abnormal PWV is apparent early in the development of CKD. The number of CHP, who have enormous PWV, is several times greater than GPP with enormous PWV. CHP at the same age as GPP are accompanied by pronounced double arterial calcification.

Increased PWV in CKD is consistently associated with elevated serum phosphate and elevated Ca × P product as well as with the total dose of calcium-based phosphate binders, all important risk factors for VC. We also report a positive correlation between PWV and elevated phosphate and Ca × P, as well as with age and triglycerides, in univariate regression analysis, and a significant association with age, triglyceride levels and Ca × P in multivariate analysis [18]. We have measured *ionized calcium* because the biological effect of calcium is determined by the amount of ionized calcium, rather than the total calcium. Ionized calcium does not vary with the albumin level, and therefore it is useful to measure the ionized calcium level when the serum albumin is not within normal ranges, or when a calcium disorder is suspected despite a normal total calcium level. We calculated the level of corrected calcium dependence of the total calcium and albumin levels in serum.

The *BMD* of the femoral neck, spine, and average BMD in HD patients is smaller than same location BMD in the GPP, presented like absolute value of density or reported as number of standard deviations below the average (relative results, compared the BMD to the optimal peak bone density to others in same age, weight, ethnicity, and gender). The absolute (g/cm^2) and relative (T-scores, Z-scores) BMD

results in the same regions are smaller in females in both GPP group and CHP group. Our finding that BMD at each time point was significantly lower in female than in male patients was in agreement with previous studies [10, 14, 17]. The reasons for these differences are explained by the significant abnormalities associated with hypophysis/gonad function in uremic women.

The higher BMD results for the spine than the femoral neck BMD may lie in the fact that DEXA uses dual-energy X-ray beams projected blindly through the body and measures the relative absorption of such beam, exactly, the X-ray beams could be absorbed by the densely calcified aorta rather than the spine, causing a falsely elevated BMD reading [8, 10]. The difference between bone density of the spine and femoral neck is less expressed in the GPP group than in the CHP group. The reason is a smaller degree of aortic calcification in the GPP with low absorption of X-ray beam with consequential elevated BMD value. However, the average of the absolute bone density obtained as the sum of bone density at the hip and spine bone density (divided by 2) is always in inverse correlation with PWV. The statement certainly applies to the GPP group, but is less evident, due to impaired bone mineral metabolism in patients on dialysis.

There is an evident correlation between absolute bone density and aortic deposits of calcification. With the increase of bone density, the degree of calcification is reduced, thereby reducing the rigidity of the vessel and decreased PWV. The eventual biologic link between vascular calcifications and bone changes is certainly part of the aging process, but in many studies, these bone-vascular associations remained significant after adjustment for age, which suggests an age-independent causal relationship [10]. The mechanisms responsible for bone-vascular interactions are not well understood.

Although, there is no sufficient number of studies that compare the above- mentioned parameters between the GPP and the CHP, however trove results for PWV, calcification and bone density (as well as dependence between them) are correlated with the results we obtained in our study.

The bone demineralization and calcium transfer from bone to blood vessels is consistent with deposits of calcium, which is evident earlier in the CHP. In humans with CKD, there appears to be a relationship between disorders of mineral metabolism (abnormal levels of serum calcium and phosphorus), abnormal bone (renal osteodystrophy), and vascular calcification [17, 20]. Most patients with progressive CKD develop elevated parathyroid hormone (PTH) and phosphorus.

The larger stiffness of the blood vessels in patients on hemodialysis, which occurs earlier, increases the speed of the pulse wave and the number of cardio vascular events.

Structural and functional vascular abnormalities, an almost invariable ESRD complication, have recently emerged as the most powerful predictors of a negative outcome in CKD populations. Increased aortic PWV and vascular calcification have demonstrated that total mortality by 39% with every 1 m/s increase in PWV is 1.9 times the increase in all-cause mortality hazard ratio for each unit increment in calcification [21].

The specificity of our study is that for assessment of calcification in the aorta and estimate of bone density, DEXA was used, instead of conventional computed tomography; for the PWV measurement, commercial appliances were not used and it was measured using a Doppler ultrasound machine. Parallel testing and comparing the two groups (the hemodialysis and the general population) provides important data for the influence of traditional factors for atherosclerosis in the general population and the combined impact of traditional and dialysis-specific factors in patients undergoing dialysis.

Knowing the correlation of the three mentioned diagnostic methods and the importance of *arterial stiffness*, *bone density*, and *vascular calcification* in the prognosis of CV morbidity and mortality in the CHP, we can bring important findings by measuring PWV: not only for arterial stiffness, but also for the status of arterial tree in relation to the vascular calcification, of bone status tissue density and of essential serum markers of renal function and mineral metabolism.

5. Conclusion

Our study reports that high incidence of VC, loss of bone mass and increased stiffness of the arteries becomes worse with increasing age and longer dialysis duration.

There is also an inverse relationship between VC and bone mineralization and strong inverse correlation between PWV and BMD. The femoral BMD is inversely associated with VC and may be more reliable measure of the BMD than measurement of the spine BMD in HD patients with aortic VC. There is a positive correlation between PWV and VC.

The PWV measuring by cheap and noninvasive diagnostic method gives as an adequate representation of vessels rigidity in both GPP and CHP. Detection of higher PWV as measure of high arterial stiffness, which becomes worse with increasing age, triglycerides, hypoalbuminemia, and elevated CRP, may allow accurate risk stratification and changes in treatment, as VC rarely regresses once developed.

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