Diabetes & its Complications

The Role of Paricalcitol In Urinary Albumin-To-Creatinine Ratio in Patients with Type 2 Diabetes and Chronic Kidney Disease

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ABSTRACT

Background: Albuminuria is an important marker of chronic kidney disease (CKD) progression. Many drugs have been tested to reduce residual albuminuria, among them, vitamin D analogues.

Objectives: The aim of this study was to assess whether treatment with paricalcitol could be beneficial in the reduction of the urinary albumin-to-creatinine ratio (UACR) in patients with type 2 diabetes and CKD and to verify the impact of baseline vitamin D in the ratio.

Methods: Observational retrospective study, 42 patients enrolled with CKD secondary to type 2 diabetes, treated with paricalcitol, 1 µg daily during 3 months, followed in a Diabetic Nephrology consultation. Patients data were analysed at baseline (T0) and 12 weeks after treatment with paricalcitol (T1). Two groups were divided based on vitamin D baseline values to evaluate its impact on baseline UACR. We used descriptive analysis, Wilcoxon test, Sign test and the Student's t test and percentage change in geometric mean was applied.

Results: Between T0 and T1 there was a reduction in UACR (p=0.001) and parathyroid hormone (PTH) (p=0.001), whereas vitamin D increased (p=0.0001). Estimated glomerular filtration rate (eGFR) also increased (p=0.035). Change in UACR was -1.78% and in PTH was -3.85% after 12 weeks of treatment with the study drug. Conversely, vitamin D and eGFR change increased +3.38% and +0.10% respectively, compared to baseline. Regarding vitamin D effect on baseline UACR between groups [G1 (n=40) vitamin D <10 ng/mL vs G2 (n=40) vitamin D ≥ 10 ng/mL] G1 showed higher levels of UACR (p=0.002).

Conclusion: In our study paricalcitol showed renoprotective effects in renal disease associated with type 2 diabetes, by promoting a reduction in the urinary albumin-to-creatinine ratio in this population. Moreover, there is an inverse correlation between vitamin D and UACR at baseline.

Keywords

Albuminuria, Chronic kidney disease, Paricalcitol, Type 2 diabetes, Vitamin D.

Introduction

Chronic kidney disease (CKD) is a worldwide public health problem [1], with cardiovascular diseases among the main causes of morbidity and mortality in these patients [2]. Albuminuria is an important sign of CKD progression, cardiovascular disease and death [3].

Clinically, renal disease (RD) secondary to diabetes is characterized by the onset of microalbuminuria, progressing to proteinuria, azotemia and ultimately renal failure, and is the leading cause of end-stage renal failure in developed countries [4].

Diabetes mellitus represents a worldwide epidemic [5] and causes metabolic changes which result in the glomerular lesions observed in diabetic nephropathy. Hyperglycaemia is the major determinant in the pathogenesis and progression of RD secondary to diabetes, however this process can be modified by genetic

susceptibility or increased by other factors such as hypertension, proteinuria, hypercholesterolemia, smoking tobacco, among others [6,7]. Several kidney functional changes like hyperfiltration, hyperperfusion and increased capillary permeability to macromolecules occur before the establishment of proteinuria. Basal membrane growth and mesangial expansion are well known pathologic markers of diabetes [4].

Therefore, treatment of albuminuria is one of the main goals in the management of CKD patients. Drugs that block the renin-angiotensin-aldosterone system (RAAS) reduce albuminuria, however their effect is suboptimal and residual albuminuria persists [8].

Calcitriol is a natural activator of the vitamin D receptor, produced by the kidney, but its plasma concentration declines as estimated glomerular filtration rate (eGFR) reduces [9]. In a multivariable analyses of patients with CKD, lower calcitriol concentrations strongly correlated with higher urinary albumin-to-creatinine ratio (UACR) and lower eGFR [9].

Hence, new therapeutic approaches which prevent progressive renal failure are mandatory [10]. Several drugs have been tested to reduce residual albuminura, among them, vitamin D analogues. They have shown an antiproteinuric effect in different animal models through renin suppression, regulation of inflammation and fibrosis, direct effects on podocytes, antiapoptotic action and preservation of the slit diaphragm [8]. Paricalcitol is a selective activator of the vitamin D receptor that lowers parathyroid hormone (PTH) secretion and has little effect on mineral metabolites [11].

Despite the fact that its mechanisms of action are not fully understood, 1.25 dihydroxyvitamin D3 and its analogues, play a renoprotective role by reducing proteinuria, a well-known biomarker of renal involvement [12]. Therefore, the aim of the present study was to evaluate the effectiveness of paricalcitol in the reduction of the urinary albumin-to-creatinine ratio in patients with type 2 diabetes with CKD as well as to confirm if basal values of vitamin D influence the aforementioned ratio.

Material and Methods
Population
In this study, 80 patients with CKD secondary to type 2 diabetes who had been taking paricalcitol 1 µg daily, were enrolled. The classification of diabetes followed the guidelines established by the American Diabetes Association [13].

Patients were considered ineligible to participate in the study if they presented with at least one of the following exclusion criteria: previous cardiovascular disease six months prior starting paricalcitol, uncontrolled hypertension (BP ≥ 140/90mmHg), UACR > 1000, eGFR ≤ 15 mL/min or ≥90 mL/min, PTH <50 pg/mL, calcemia >11 mg/dl, vitamin D ≥ 25 ng/mL, type 1 diabetes, renal diseases other than diabetic nephropathy, and neoplastic or infectious diseases. Patients were not allowed to undergo therapy with analogues of native vitamin D and/or

therapy with paricalcitol for at least 3 months before screening. Those with inability to continue treatment and patients who started renoprotective antihypertensive therapy 3 months prior the study were also excluded. Patients with any gastrointestinal pathology that could possibly interfere with vitamin D absorption were also not included in this study.

Materials and methods
This observational study included diabetic patients with renal impairment. Patients were screened and recruited at an outpatient diabetic nephropathy consultation of Centro Hospitalar do Algarve, Faro. Clinical and laboratory data of our research was gathered between November 2015 and February 2016, through search of medical electronic records, previously authorized by local Ethics Committee. All principles of the Declaration of Helsinki were followed, and study procedures were only conducted after obtaining patients' written informed consent.

Patients data were analysed before starting paricalcitol at baseline (T0) and 12 weeks after treatment with the study drug (T1). Two groups were established according to baseline vitamin D (T0). This baseline value represents the average of three measurements of vitamin D analysed in winter, spring and summer. Patients whose values were <10 ng/mL were defined as Group 1 (G1) and those with vitamin D ≥10 ng/mL as group 2 (G2) in order to assess its impact on baseline UACR.

Procedures
Serum samples were collected at T0 and T1 in fasting patients. Several laboratory parameters were analyzed: serum creatinine, haemoglobin (Hb), glycated haemoglobin (HbA1c), serum glucose, lipid profile [total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol], mineral metabolism [calcium, phosphorus, PTH], 1.25 dihydroxyvitamin D3. Serum levels of 1.25 dihydroxyvitamin D3 were quantified with a radioimmunoassay (IDS, Boldon, UK). Total cholesterol, HDL and phosphorus were measured using the ARCHITECT c Systems and the AEROSET System (Abbott Diagnostics Division, Abbott Laboratories Abbott Park, IL, USA) and LDL cholesterol in human plasma was assessed using a MULTIGENT Direct LDL assay (Abbott Diagnostics Division, Abbott Laboratories Abbott Park, IL, USA). HbA1c and PTH levels were measured using a spectrophotometry technique and electrochemiluminescent immunoassays (ECLIA), respectively.

Regarding renal function, values of serum creatinine were obtained through an enzymatic method, using the ARCHITECT® device (Abbott Diagnostics Division, Abbott Laboratories Abbott Park, IL, USA), while GFR was estimated using a formula derived by the Modification of Diet in Renal Disease (MDRD) study group [14]. The urine albumin-to-creatinine (µg/mg) ratio (UACR) was determined in early morning spot urine [15].

Paricalcitol prescription and weekly consumption was obtained through search of pharmaceutical electronic records Gestão Hospitalar de Armazém e Farmácia (GHAF).

Statistical analyses

Descriptive statistics was employed to compare both groups T0 and T1 and inferential statistics with non parametric tests, namely Wilcoxon and Sign Test for paired samples were applied. For independent samples the Student's t test was used in association with the homogeneity of variance test, Levene test. To calculate the variation between T0 and T1 the percentage change in geometric mean was applied. Statistical significance was considered with p<0.05. All analyses were performed with the SPSS program version 23 (SPSS Inc., Chicago, IL, USA) and Excel version 2007 (Microsoft Office system).

Results

The number of patients screened for eligibility were 260, of whom 80 (30.7%) were enrolled. Baseline demographic, clinical, and biochemical characteristics are summarized in (Table 1). Overall, the great majority of our patients 52 (65%) were male and the mean age was 71.07 ± 8.60 years. Duration of diabetes was approximately 18 years (17.92 ± 6.38). Mean HbA1c values were 7.84 ± 1.60 % and serum glucose 168.14 ± 74.61 g/dl. All patients were taking glucose-lowering treatments of which 69% were on oral anti-diabetics drugs and the remaining were insulin-treated.

Characteristics of patients at baseline (n=42)		
Demographic characteristics		
Age (years)		71.07 ± 8.60
Male sex		52 (65%)
Clinical characteristics		
Evolution of diabetes (years)		17.92 ± 6.38
Follow-up diabetic nephropathy (years)	Mean (SD)	3.58 ± 3.04
	Median	3.00
Blood pressure (mm Hg)	Systolic	136.89 ± 14.21
	Diastolic	74.56 ± 10.37
	Pulse pressure (mmHg)	62.33 ± 13.36
Urinary albumin-to-creatinine ratio (µg/mg crea)	Mean (SD)	503.07 ± 14.53
	Median	354.54
	Serum creatinine (mg/dL)	2.03 ± 1.06
GFRe (mL/min por 1,73 m²)	Mean (SD)	43.07 ± 26.66
	Median	33.34
	Haemoglobin (g/L)	130.79 ± 18.33
	Serum glucose (g/dL)	168.14 ± 74.61
	HbA1c (%)	7.84 ± 1.60
Cholesterol (mg/dL)	Total	202.26 ± 43.55
	LDL	115.58 ± 36.73
	HDL	51.13 ± 16.57
	Serum calcium (mg/dL)	9.34 ± 0.71
	Serum phosphorous (mg/dL)	3.79 ± 0.82
Serum intact parathyroid hormone (pg/mL)	Mean (SD)	234.02 ± 26.48
	Median	139.50
1.25(OH)$_2$D3 (ng/mL)	Mean (SD)	9.93 ± 5.41
	Median	10.00

	High blood pressure	92.9%
Comorbidities	Hyperlipidemia	81%
	Arterial coronary disease/AMI	11.9%
	Cerebrovascular accident	16.7%
	Diabetic neuropathy	7.1%
	Congestive heart failure	11.9%
Glucose-lowering treatments	Oral antidiabetic medication	69%
	Insulin or insulin analogues	31%
Antihypertensive drugs		92.9%

Table 1: Patients baseline characteristics.
Data are mean ± SD (standard-deviation), median or numbers (%).
eGFR: estimated glomerular filtration rate; HgA1c: glycosylated haemoglobin; LDL: Low Density Lipoprotein; HDL: (High Density Lipoprotein; AMI: Acute myocardial infarct.

The mean values of pulse pressure and systolic blood pressure were 62.33 ± 13.36 mmHg and 136.89 ± 14.21 mmHg respectively. Of note, most patients were hypertensive 92.9% all of which were taking antihypertensive drugs with renoprotective action namely RAAS inhibitors 52.4% angiotensin receptor blocker (ARB); 40.5% angiotensin-converting enzyme inhibitors (ACEI) and 7.1% taking lercanidipine.

Baseline UACR was 503.07 ± 14.53 µg/mg and the mean eGFR was 43.07 ± 26.66 mL/min. The PTH median was 139.50 pg/mL and the mean value was 234.02 ± 26.48 pg/mL which is too close to the standard deviation. 1.25(OH)$_2$D3 at baseline was on average around 10 ng/mL (9.93 ± 5.41 ng/mL). Finally, mineral metabolites serum phosphorous and serum calcium were within reference values.

Variation analysis of parameters between T0 and T1

UACR mean values decreased approximately 122 µg/mg (p=0.001) between T0 and T1. Specifically, mean baseline UACR decreased from 503.07 to 381.40 µg/mg after a period of 12 weeks. This reduction was observed in 65 (83%) patients, which means a decline in the UACR among 4/5 of patients (Table 2 and Figure. 1).

Variable[1]	T0 Mean ± SD	T1 Mean ± SD	Confidence Interval (95% CI)		p value
eGFR	43.07 ± 26.66	44.77 ± 28.68	-5.39	2.00	0.359
PTH	234.02 ± 16.48	168.31 ± 19.68	29.70	301.73	0.001
1.25(OH)$_2$D3	9.93 ± 5.41	13.98 ± 6.39	-5.58	-2.52	0.0001
UACR	503.07 ± 44.53	381.40 ± 53.75	51.20	692.15	0.001
Serum calcium	9.34 ± 0.71	9.60 ± 0.81	-0.49	-0.03	0.029
Serum phosphorous	3.79 ± 0.82	3.99 ± 0.95	-0.37	-0.03	0.026
Total cholesterol	210.65 ± 35.45	196.00 ± 30.86	5.18	224.13	0.004

Table 2: Comparison of difference between paired samples of the variables in study.
[1]Paired samples (between T0 e T1). eGFR: estimated glomerular filtration rate; PTH: serum intact parathyroid hormone (pg/mL); 1.25(OH)$_2$D3: vitamin D; UACR: urinary albumin-to-creatinine ratio.
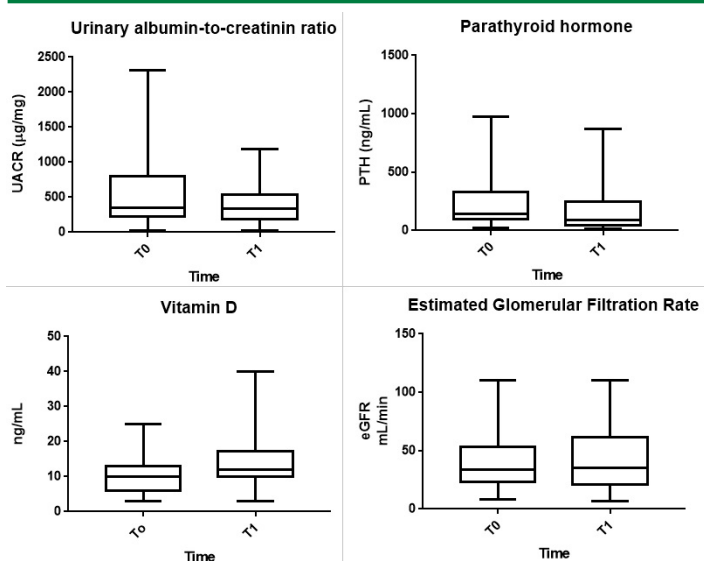
Figure 1: Variation of the parameters UACR, PTH, VITD and eTFG between T0 and T1.

There was a significant statistical difference in the fall of PTH mean values in 76% patients quantified as 66 pg/mL (p=0.001). The same trend was observed with vitamin D which had a substantial (p=0.0001) increase between baseline evaluation and 12 weeks after treatment with the vitamin D analogue, mean value of 13.98 ng/mL at T1. This rise was observed in 81% patients. There was a discrete rise in eGFR, however less than 2 mL/min, and with no statistical significance (p=0.359). With regard to mineral metabolites, serum calcium increased (p=0.029), however values stayed within the normal range for this population. After paricalcitol treatment, serum phosphorous also increased as mean values recorded were of around 4 mg/dL (p=0.026). Total cholesterol decreased to values under 200 mg/dL (p=0.004) (Table 2 and Figure 1).

Figure 2 displays the results of percentage change in geometric mean between T0 and T1 regarding the parameters studied. After 12 weeks of treatment with paricalcitol, UACR declined 1.78% as well as PTH with a reduction of 3.85%. Conversely, vitamin D and eGFR increased 3.38% and 0.10% respectively compared to baseline.
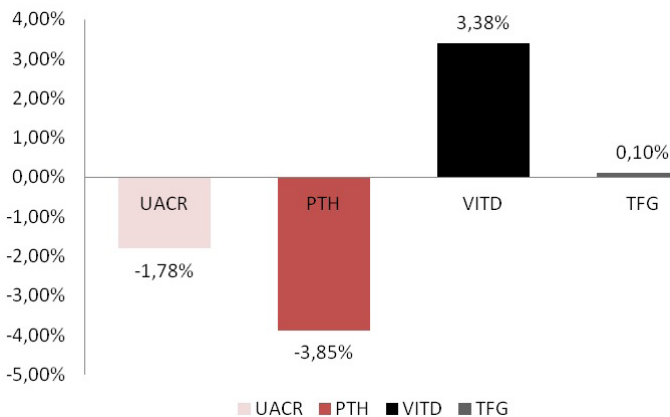

Figure 2: Percentage change in geometric mean between T0 and T1.

Baseline Vitamin D vs baseline UACR

$1.25(OH)_2D3$ median values of the population studied were 10 ng/mL G1 is the group which presented lower vitamin D values (<10 ng/dL), and in this same group the UACR was 730.45 ± 87.06 µg/mg, while in G2 UACR was inferior 315.24 ± 21.20 µg/mg. When analysing the effect of vitaminD on the baseline UACR, we found a significant statistical difference between groups (p=0.002) (Figure 3).

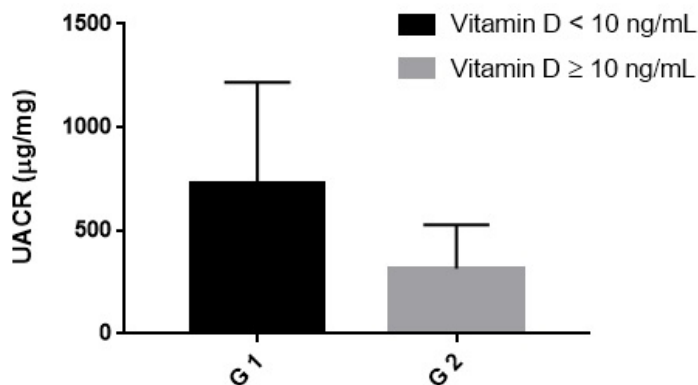**Figure 3:** Comparison of baseline UACR, according to vitamin D values.

Discussion

Our study has shown that after 12 weeks of treatment with 1µg paricalcitol daily, there was a reduction in the residual urinary albumin-to-creatinine ratio in patients with renal disease associated with type 2 diabetes. This reduction was not only significant, moreover it was observed in more than half of the patients in this study.

Based on current knowledge, paricalcitol seems to have several mechanisms of action which lower albuminuria [10]. This can be explained because activation of the vitamin D receptor interferes in pathways well known to be involved in progressive renal and vascular disease [10].

The 1.25 dihydroxyvitamin D3 and its receptors play an important role in the transcription of several genes responsible for the activation or inhibition of several proteins [6]. It is an important modifier of gene transcription and acts by regulating the synthesis of messenger RNA (mRNA). This biological action is triggered after binding to vitamin D receptor (VDR), with predominantly nuclear localization in cells [6]. The 1.25 dihydroxyvitamin D3 allows multiple physiological responses on target tissues through the activation of genomic and non -genomic mechanisms [16]. Due to its pleiotropic effects, this hormone plays a key regulator and protective role both in the prevention and treatment of CKD [17].

In the context of type 2 diabetes complex patophysiology, it is known that 1.25 dihydroxyvitamin D3 produces effects on the immune system and on the pancreatic β cells by facilitating insulin production [18].

In renal disease associated with diabetes, mesangial cell proliferation and excessive production of extracellular matrix (fibronectin, laminin, type IV collagen), occur due to increased levels of intracellular glucose. This high glucose concentration induces the overexpression of glucose transporter (GLUT-1) mRNA, triggering production of the GLUT1 protein in mesangial cells, which leads to an increased urinary excretion of transforming growth factor β1 (TGF-β1) [19,20]. This factor has a major role on the onset and progression of renal disease [21].

The onset of proteinuria in diabetic patients is a marker of changes in the glomerular filtration barrier, which consists of endothelium, basement membrane and podocytes [22]. The latter are highly differentiated cells and their lesion causes dysfunction of the barrier which ultimately correlates with the pathogenesis of proteinuria. Some studies suggest that hemodynamic mechanisms modulate the changes of podocytes specific proteins, namely nephrin, with a decrease of its expression and consequently an increased urinary excretion of proteins [23].

Hyperglycaemia induces intrarenal production of factors by downregulating VDR and 1α-hydroxylase in proximal tubule cells, resulting in a decreased tubular megalin expression and consequently a decrease in 1.25 dihydroxyvitamin D3 reabsorption with increased levels of protein urinary excretion [24]. There is evidence that a deficit in the active metabolite of 1.25 dihydroxyvitamin D3 indirectly stimulates the activation of TGF -1β [25].

Among the various mechanisms of action of 1.25 dihydroxyvitamin D3 in the pathogenesis of proteinuria, it seems to inhibit myofibroblasts proliferation in the renal interstitium by stimulating hepatocyte growth factors, thus performing a renoprotective effect by suppressing the activation of myofibroblasts production in the matrix [12,26,27]. In some experimental models, 1.25 -dihydroxyvitamin D3 administration decreased the loss of podocytes and inhibited their hypertrophy [28]. This beneficial effect is due to a direct action on signal modulation, by inhibiting TGF -b1 and bone morphogenetic protein (BMP -7) expression [29].

In some studies with diabetic and non-diabetic models, activation of the vitamin D receptor supressed TGF-β1 and macrophage infiltration which substantially reduced glomerulosclerosis [30-32]. Concurrently, this receptor activation results in nephrin upregulation and nuclear factor-κB (NFkB) [33]. Some experimental study results suggest that renoprotection is due to supression of renin translation, antiproliferative or antifibrotic effects, or both [34,35].

Our results corroborate the findings of Alberto de Lorenzo et al. [34], whose work has shown an important reduction in proteinuria with the administration of a low dose of paricalcitol in CKD patients, for 6 months. Although substantial, the difference we obtained was less expressive.

In the VITAL study, a prospective, randomized, double-blinded placebo-controlled, multicentre study, Dick de Zeeuw et al. [10] demonstrated that the selective activation of vitamin D receptor with paricalcitol has unique hemodinamic effects, by lowering urinary albumin with slight secondary hypercalcemia. In this trial, patients were equally allocated into three groups to receive, 0, 1 or 2 μg of oral paricalcitol for a 24 -week period. Reduction in UACR was observed in the 1 μg paricalcitol-group and in the 2 μg paricalcitol-group, however with a statistical difference observed only in the latter. In our research a statistically significant result was found in patients taking 1 μg of paricalcitol. These authors suggest that the major effect of paricalcitol occurs during the first 12-16 hours after the drug is taken, and has real and reversible effects in albuminuria reduction [10]. Change in UACR seemed to have a dose-response relation with paricalcitol and eGFR had also reduced substantially in the 2 μg paricalcitol group [10]. Regarding mineral metabolites our results were consistent with those found in the literature, with a slight rise in serum phosphorus and calcium [35,36].

Results from several studies enhance the role of vitamin D receptor activators in the reduction of albuminuria in patients with diabetic nephropathy [12,37,38] notably when combined with RAAS inhibitors [31,39,40].

Furthermore, Agarwal et al. [41], evaluated the efficacy of paricalcitol in patients with CKD, stages 3 and 4 throughout 24 months. A total of 107 patients were randomized in a double-blind essay, 57 of which were treated with paricalcitol. This group showed a greater reduction in proteinuria compared with the placebo group. The effect of paricalcitol in the reduction of proteinuria was independent with regard to demographic characteristics, comorbidities, and use of RAAS antagonists.

Regarding PTH, we obtained statistically significant results with the administration of paricalcitol which is consistent with the findings observed in the group treated with 1μ paricalcitol in the VITAL study [10]. Moreover, in a systematic review, Han et al. [42], confirmed the efficacy of paricalcitol in the decrease of PTH in CKD patients as well as in the reduction of proteinuria in diabetic patients with CKD.

In our survey, most patients had vitamin D deficiency, which according to the American Society of Endocrinology [43] is defined by $1.25(OH)_2D3$ concentration below 20 ng/ml. Several observational studies, established a pathophysiologic association between vitamin D deficiency and diabetic nephropathy [42,44]. Diaz et al. [30], showed an independent association between vitamin D deficiency and diabetic nephropathy.

It has also been demonstrated a higher prevalence of albuminuria in patients with lower $1.25(OH)_2D3$ [45]. The results of our study support this evidence. Results from a systematic review by Chokhandre et al. [46], emphasize the importance of vitamin D analogues in the improvement of renal function in patients with diabetic nephropathy.

In addition, Silva et al. [47], reviewed the relationship of vitamin D with diabetic nephropathy and concluded that through the activation of its nuclear receptors and consequent transcription of 200 genes, 1.25 dihydroxyvitamin D3, leads to the activation or inhibition of several proteins. Due to these mechanisms of action, this vitamin may have a key role in the prevention and treatment of some diseases, including type 1 and type 2 diabetes, as well as in its complications, namely in diabetic nephropathy.

Pulse pressure reflects increased large artery stiffness and is considered a major independent predictor of cardiovascular morbidity and mortality, especially in elderly patients [48]. Egido et al. [49], reviewed the pleiotropic effects of paricalcitol treatment and its positive effect on cardiovascular disease and proteinuria, in patients with CKD. The results we found in the population of our study could be explained by the observations found in several studies [50-52], where it was observed that both paricalcitol and an ACEI, as monotherapies, as well as the combination of the two, reduced the concentration of the enzyme aortic malondialdehyde (MDA) and increased levels of the enzyme glutathione peroxidase (GSH-Px). The protection against the inflammation and oxidative damage of atherosclerosis was greater with the combined therapy than with monotherapy. Plus, paricalcitol also appears to improve endothelial function in uraemic rats in a dose-dependent manner and independently of PTH levels and blood pressure according to this review.

In conclusion, the present study depicted an effective reduction in the urinary albumin-to-creatinine ratio and an inverse correlation between vitamin D values and UACR, therefore we conclude that the introduction of paricalcitol might have a beneficial role in the reduction of the UACR in patients with diabetic nephropathy.

However, there are several limitations in the current study such as the small sample size with subsequent limited statistical power of the tests applied and the restricted follow-up period. Nevertheless, the results we obtained are in agreement with currently evidence-based knowledge and future studies should be encouraged.

Acknowledgment

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