REVIEW ARTICLE

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Relationship of Vitamin D with Diabetes *Mellitus* and Diabetic Nephropathy

Relação entre a Vitamina D com a Diabetes *Mellitus* e a Nefropatia Diabética

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ABSTRACT

Diabetic nephropathy (DN) is a major public health problem whose prevalence has been increasing in recent years. It is characterized by a progressive pattern and is associated with high morbidity and mortality rates. Several factors are associated with the onset and progression of DN, such as glycaemic control, hypertension, obesity and inflammation status. In addition, vitamin D also seems to be involved through a pleiotropic regulatory activity/protection with promising applicability, either in prevention or progression of renal disease. The purpose of this review was to summarize the scientific evidence that supports the role of vitamin D in diabetes *mellitus* and DN.

Key-Words: Diabetes *mellitus*; diabetic nephropathy; vitamin D.

RESUMO

A nefropatia diabética (ND) é um problema de saúde pública grave, cuja prevalência tem vindo a aumentar nos últimos anos. A ND é caracterizada por um padrão de progressão e está associada a taxas de morbilidade e mortalidade elevadas. Diversos fatores são associados com o surgimento e progressão da ND, tais como o controle glicémico, a hipertensão, a obesidade e o estado inflamatório. Além disso, a vitamina D parece também estar envolvida, através de uma actividade reguladora pleiotrópica/protectora com uma aplicabilidade promissora, tanto na prevenção como na progressão da doença renal. O objectivo deste artigo de revisão é sumarizar a evidência científica que suporta o papel da vitamina D na diabetes *mellitus* e na ND.

Palavras-Chave: Diabetes *mellitus*; nefropatia diabética; vitamina D.

INTRODUCTION

Proteinuria is a classic marker of glomerular impairment and it has been implicated as a key factor for the progression of kidney disease[1].

Diabetic nephropathy (DN) is clinically characterized by the onset of proteinuria and its progression to chronic renal disease is currently the leading cause of end-stage renal failure requiring renal replacement techniques in industrialized countries[2,3]. In addition to its increasing incidence, DN is associated with a high rate of cardiovascular mortality, whose risk is two to three times higher when associated with proteinuria[4].

The metabolic changes induced by diabetes *mellitus* (DM) are *per se* sufficient for the development of the glomerular lesions observed in DN. There are several retrospective and prospective studies that confirm it, particularly the Diabetes Control and Complications Trial (DCCT), and the United Kingdom Prospective Study (UKDPS), with type 1 and type 2 diabetic patients, respectively. These studies have demonstrated that the development of DN is influenced by metabolic control. However, some diabetic patients with good metabolic control developed DN, while others preserved a normal renal function and did not present proteinuria, despite a poorer glycaemic control. These observations suggest that in diabetes, hyperglycaemia is an important but not sufficient factor to cause renal lesions[5]. Although hyperglycaemia is the major determinant in the pathogenesis and progression of DN, this pathologic process can be modified by genetic susceptibility and accelerated by several other factors[6]. (Fig. 1)

Regarding vitamin D (1,25 dihydroxivitamin D3, the active form of Vitamin D3), and supported by the presence of its receptors in several organs and tissues, it is known that it has a role in other processes and pathways besides the activity on mineral metabolism[7-9]. Adequate levels of 1,25 dihydroxivitamin D3 are important for maintaining the good functioning of bone metabolism, and there is also evidence of its anti-proliferative effect in cellular differentiation, immunomodulation and inhibition of the renin-angiotensin system (RAS)[10].

There is evidence that in kidney disease, podocytes are affected in glomerular disease and that they play an important role in the progression to more advanced stages, including nephropathy in type 1 and type 2 diabetes[11].

Recently, some experimental studies have documented the existence of 1,25 dihydroxivitamin D3 receptors (VDR) in podocytes and have demonstrated an association between this vitamin and the number of podocytes observed. This association may contribute to the prevention and reduction of mesangial cells proliferation and to promote a decrease in proteinuria[12]. Supporting this evidence, Schwarz *et al.* demonstrated that treatment with 1,25 dihydroxivitamin D3 attenuates the development of glomerulosclerosis and the progression of albuminuria in rats submitted to subtotal nephrectomy[13]. Other studies also demonstrated that the administration of 1,25 dihydroxivitamin D3 reduces mesangial cells proliferation, glomerular hypertrophy and progression to glomerulosclerosis[14-16]. The 1,25 dihydroxivitamin D3 has a pleiotropic effect, which indicates a regulatory/protective role with promising applicability, either for preventing or treating chronic kidney disease[17-19].

The objective of this article was to summarize the scientific evidence regarding the role of 1,25 dihydroxivitamin D3 in DM and DN.

VITAMIN D

The 1,25 dihydroxivitamin D3 and its receptors have a role in the transcription of several genes responsible for the activation or inhibition of many proteins, thereby playing an important role in the pathophysiology of certain diseases[6,20].

1,25 dihydroxivitamin D3 receptor

The 1,25 dihydroxivitamin D3 is an important modifier of gene transcription, even in tissues that are not involved in calcium homoeostasis, and acts by regulating the synthesis of messenger RNA (mRNA). This biological action is triggered after binding to VDR, with predominantly nuclear localization[6]. This receptor forms a complex with the Retinoid-X-receptor (RXR), resulting in a heterodimer complex with VDR that migrates into the cell nucleus and interacts with the 1,25 dihydroxivitamin D3 response element (VDRE)

in the DNA, leading to the transcription, or not, of genes implicated in several biological functions[21].

Plasma membrane-associated VDR (mVDR) is responsible for the rapid responses of 1,25 dihydroxivitamin D3, associated with its non-genomic action. The biochemical structure of these receptors is not yet known but their action is achieved through the opening of calcium or chlorine channels and mitosis activator proteins[22,23].

Genomic and non-genomic action of Vitamin D

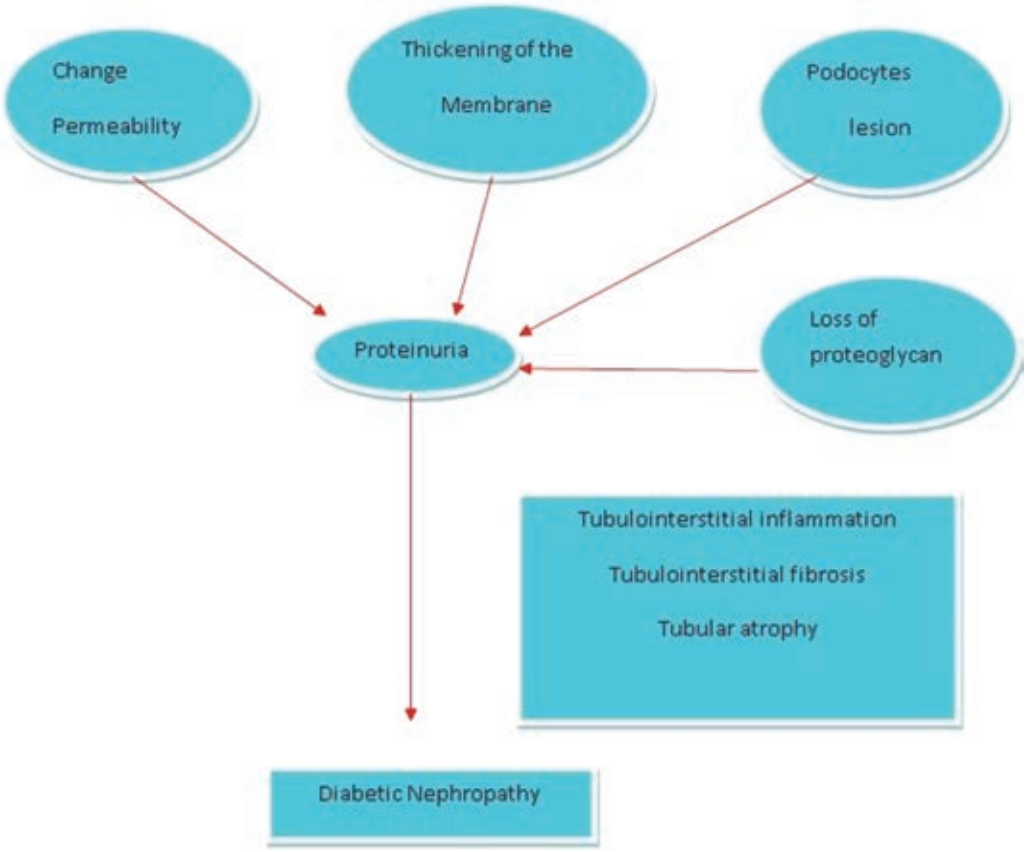
The 1,25 dihydroxivitamin D3 has a dynamic and configurationally flexible structure that allows multiple physiological responses on target tissues through the activation of genomic and non-genomic mechanisms[22,23]. Through the nuclear VDR, it causes activation or inhibition of the expression of genes responsible for the transcription of specific proteins. This process may occur for hours or days and it is recognized as its genomic response[24].

Nuclear VDR is a ligand dependent transcription factor, modulating the expression of genes 1,25 dihydroxivitamin D3 dependent, via three mechanisms[25]:

- Upregulation of the expression of certain genes by allowing binding to VDRE present in the DNA promoter region (osteocalcin, osteopontin, Nuclear Factor-KappaB [NF-Kb] activator receptor);
- Downregulation of the expression of other genes, by not allowing binding to VDRE;
- Inhibition of the expression of some genes through the antagonistic action of specific transcription factors,

Figure 1

Pathophysiological mechanisms of diabetic nephropathy.



which are the nuclear factors, namely the nuclear factor of activated T-cells (NFAT) and NF-KB[26,27].

The genomic mode of action of this active form involves a direct interaction of VDR with the DNA sequence, with 200 genes being regulated by this hormone, however, little is known about the mechanisms involved[28]. On the other hand, it can also act through an alternative receptor, mVDR, which stimulates the rapid formation of second messengers, protein kinases and the opening of calcium and chlorine channels, triggering a variety of cellular responses within seconds or minutes. This process is recognized as a non-genomic response to 1,25 dihydroxivitamin D3[25-28]. Therefore, this hormone may have a key role in the prevention and treatment of some diseases, including type 1 and type 2 diabetes, as well as in microvascular (nephropathy, retinopathy and diabetic neuropathy) and macrovascular (coronary artery disease, cerebrovascular and peripheral arterial disease) complications[28].

VITAMIN D AND DIABETES *MELLITUS*

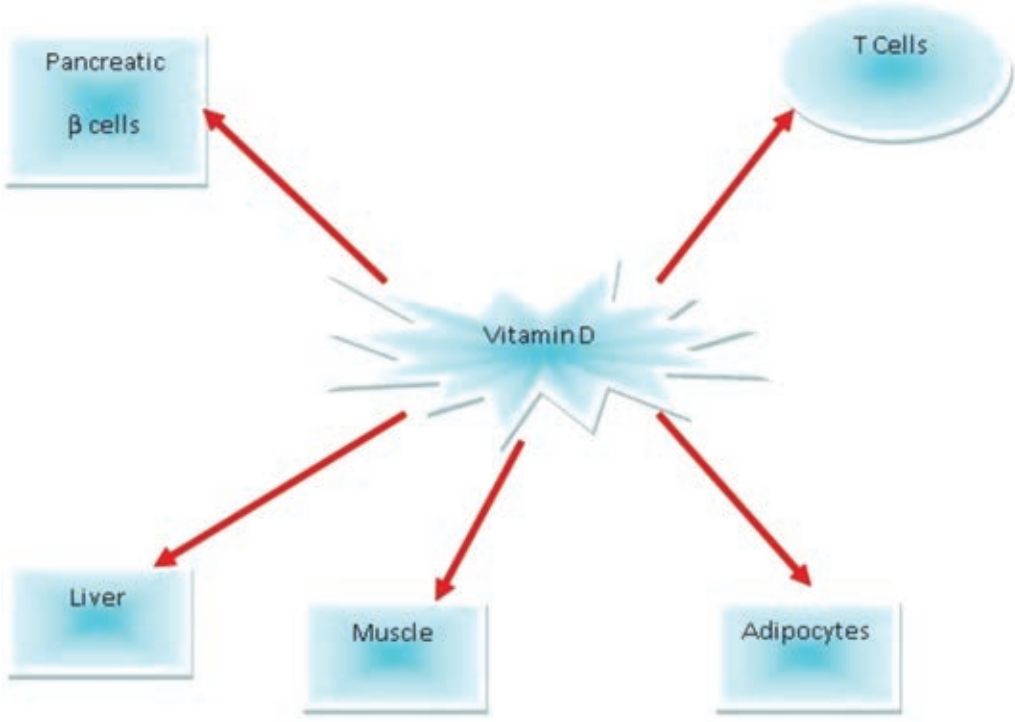
Diabetes *mellitus* is associated with microvascular and macrovascular complications. Some observational studies have reported the association between 1.25 dihydroxivitamin D3 receptors and type 1 and type 2 DM, glucose intolerance, sensitivity to insulin secretion and serum calcitriol levels[29-38].

The pathophysiology of type 2 diabetes is complex, but we know that there is an involvement of changes in pancreatic β-cell function and tissue resistance to insulin. It is generally accepted that as the major hormone regulating calcium metabolism, 1,25 dihydroxivitamin D3 produces effects on the immune system and on the pancreatic β cells by facilitating insulin production[38] (Fig. 2).

The presence of 1,25-dihydroxyvitamin D3 receptors in the pancreas raises the possibility of a direct action of this vitamin in the synthesis, regulation and secretion of pancreatic hormones. It was recently reported

Figure 2

Role of vitamin D in the regulation of glucose homoeostasis. Vitamin D is essential for the function of pancreatic β cells and sensitivity to insulin action in cells and target tissues. These mechanisms provide a protective effect of this vitamin on the development of type 2 diabetes.



the presence of 1,25 dihydroxivitamin D3-dependent--calcium-binding protein (DBP) in the pancreatic β cells and an additional factor in favour of the action of this vitamin in regulating the synthesis and secretion of insulin verified that 25-hydroxyvitamin D is converted to its active form, 1.25-dihydroxyvitamin D, in the pancreatic β cells through the action of the 25-hydroxyvitamin D-1α-hydroxylase[39-40].

Hypovitaminosis D is a risk factor for the development of type 2 diabetes and metabolic syndrome since it causes pancreatic β cell dysfunction and peripheral resistance to insulin action[41]. Supplementation in patients with type 2 diabetes and non-diabetic patients with hypovitaminosis enhances the secretion of insulin, suggesting that this vitamin improves the action of the islets of Langerhans β cells[42].

The mechanism by which 1,25 dihydroxivitamin D3 deficiency contributes to the onset of type 2 diabetes is not fully understood, and more studies are necessary to further examine this association; however, it is clear that pancreatic β cell dysfunction, resistance to insulin action and low-grade inflammation are important factors for the development of glucose intolerance and type 2 diabetes[42-44].

In both animal and human studies, it was described that pancreatic β cell dysfunction is associated with a state of 1,25 dihydroxivitamin D3 deficiency and that its supplementation contributes to the conversion of pro-insulin into insulin[45]. Despite the evidence of a protective role of this vitamin in diabetes, it is unclear whether its deficiency is related to the several risk factors presented in this disease[46-47].

VITAMIN D AND DIABETIC NEPHROPATHY

The mechanisms associated with DN are multiple and complex, being all interconnected, and this process is not yet fully understood.

Role of cellular and molecular mediators in diabetic glomerulopathy

The key determinant factor for the pathogenesis and progression of DN is hyperglycaemia, and this process can be modified by genetic susceptibility or accelerated by other factors, such as hypertension, proteinuria, hypercholesterolaemia, and smoking habits, among others[48].

The glomerulus is the location of histological lesions associated with DM, where mesangial cell proliferation, the excessive production of extracellular matrix (fibronectin, laminin, collagen type IV), occurs 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)[49-51]. This factor has an important role on the onset and progression of DN, by inducing the production and accumulation of fibronectin and collagen IV in the extracellular matrix resulting in structural changes in the glomerulus (interstitial fibrosis and sclerosis)[52-54].

The mechanisms associated with DN are not fully understood, and many of the molecular factors associated with the renal lesion are under investigation. However, it is known that there are four mechanisms responsible for the onset of DM complications that are triggered by the increase of intracellular glucose. These mechanisms are:

- Increased flow through the polios pathway, with consequent increase of sorbitol in the tissues, including the renal tubules and glomeruli. This increase in sorbitol causes tissue lesion by changing the cellular osmoregulation. This is the primary mediator of protein kinase C (PKC) cellular activation[55];

- The increase in the non-enzymatic advanced glycosylation end products (AGEs) causes an increase in plasma proteins and extracellular matrix. When AGEs bind to specific receptors, identified in the macrophages of endothelial and mesangial cells, they induce the synthesis and secretion of cytokines (IL TNF-α, IL-1, IL-6) and insulin growth factor (IGF1), stimulating mesangial cells proliferation and collagen IV production. Moreover, by crosslinking with collagen, AGEs can induce an increase in extracellular matrix synthesis by stimulating growth factors (TGF-β1, CTGF, VEGF, PDGF)[56];

– Increase of the flow through the hexosamine pathway, causing changes in gene expression of glomerular cells and endothelial cells[55].

These mechanisms are, among others, responsible for the changes in the glomerular filtration barrier whose manifestation is the emergence of proteinuria.

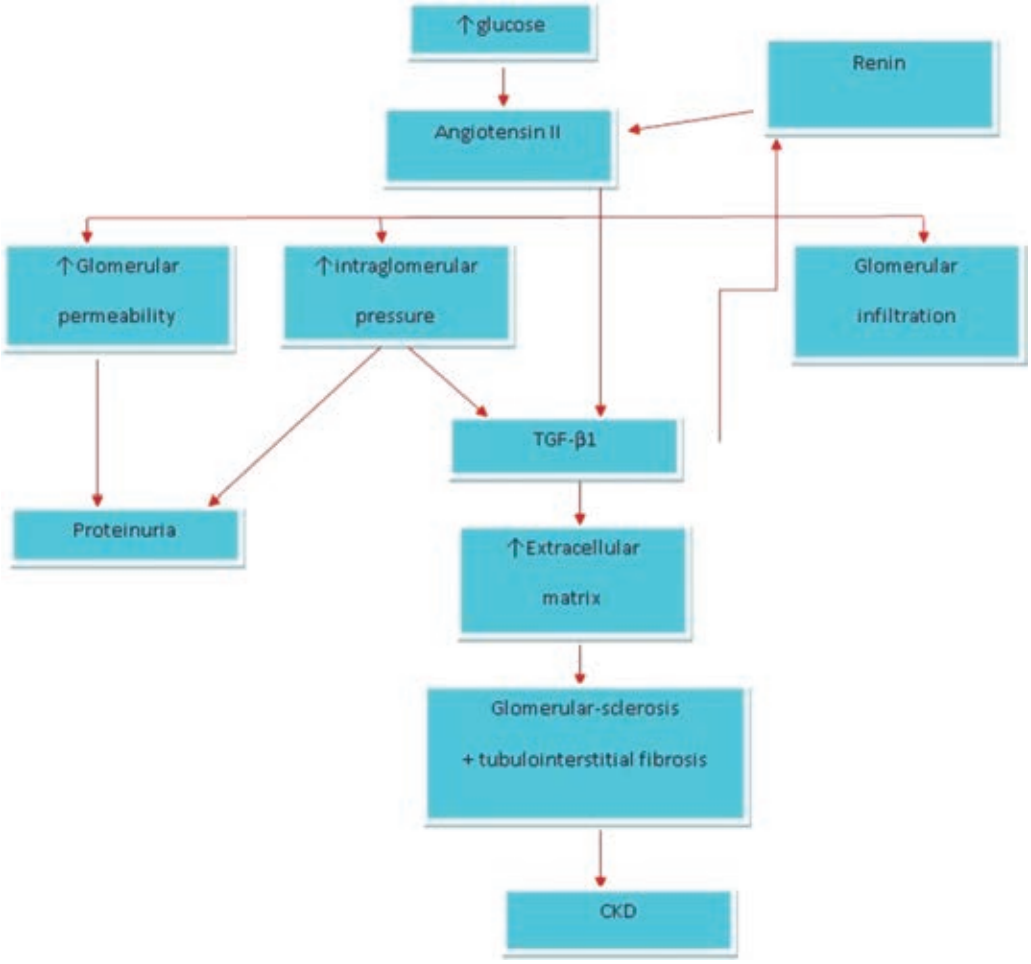
Role of the renin-angiotensin-aldosterone system (RAAS):

Hyperglycaemia stimulates the production of cytokines, including the angiotensin II (Ang II). This is a vasoactive peptide with glomerular hemodynamic actions that contributes to the onset of proteinuria (Fig. 3).

Angiotensin II has a pro-fibrotic, pro-angiogenic and pro-inflammatory actions. It is the main mediator of TGF β1 and connective tissue growth factor (CTGF) production at the level of mesangial and tubular cells, leading to an increased production of extracellular matrix and contributing to the development and progression of glomerulosclerosis and tubulointerstitial fibrosis, typical features of DN[57]. It regulates mesangial cell growth, by promoting glomerular proliferation or hypertrophy, and also promotes an increased expression and synthesis of extracellular matrix proteins, such as fibronectin, laminin, and collagen IV[58].

Figure 3

Role of angiotensin II in the pathogenesis of diabetic nephropathy.



In its pro-angiogenic action, hypoxia triggers increased expression and synthesis of VEGF, contributing to the progression of renal lesion[59].

Currently, the role of Ang II in slit diaphragm of podocytes is under investigation, especially on the integrity of components (nephrin, podocin and α-actin – 43) and haemodynamic mechanisms responsible for proteinuria[60,61]. Angiotensin II and growth factors interfere with the haemodynamic mechanisms and structural/metabolic manifestations of the DN.

Cellular changes of podocytes and diabetic nephropathy

The onset of proteinuria in diabetic patients is a sign of changes in the glomerular filtration barrier, which consists of endothelium, basement membrane and podocytes. The function of this barrier is to limit the passage of macromolecules, primarily according to size, electrical charges (molecules with negative charges have more difficulty in crossing) and shape of the molecule itself[62]. Podocytes are responsible for maintaining the structure and functioning of the barrier. These are highly differentiated cells and their lesion causes dysfunction of the barrier with immediate consequences in the pathogenesis of proteinuria[11].

Podocytes also express a number of proteins, which contribute to this high degree of differentiation, some of which are specific of these cells. These proteins, localized in the podal processes, are responsible for maintaining cell shape and function. Some seem to be involved in the pathophysiology of proteinuria by modulating the function of the subpodocytic slit diaphragm. Some studies revealed that haemodynamic mechanisms modulate the changes of podocytes specific proteins, including nephrin, with a decrease of its expression and consequently an increased urinary excretion of proteins[63]. Histomorphometric studies in animal models of type 1 diabetes with proteinuria, showed an apparent fusion of podocytes, which assume a flattened shape with disappearance of the space between the podal processes[64]. This morphological change triggers a breakdown of the subpodocytic slit diaphragm, with the podocytes losing much of their ability to contain the passage of macromolecules, causing urinary excretion of increased amounts of proteins, as long as the lesion persists. However, an analysis of renal biopsies during the European Study for the Prevention of Renal Disease Type 1 Diabetes (ESPRIT), in normotensive patients with albuminuria, showed no significant reduction in the number of podocytes as compared to the non-diabetic control group[65]. However, longitudinal studies have demonstrated a clear correlation between the reduction in the number of podocytes during follow-up and proteinuria[66,67].

In renal biopsies performed in Pima Indians with type 2 diabetes, it was found not only a deletion but also a reduction in the number of podocytes[68]. Schiffer *et al,* demonstrated that hyperglycemia alters the expression of regulatory proteins and induces apoptosis in renal tubular and endothelial cells. There is also evidence that TGF-β1 plays an important role in the pathogenesis of DN and causes apoptosis in podocytes in culture[69].

Vitamin D mechanisms of action in DN

Hyperglycaemia causes the 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 dihydroxivitamin D3 reabsorption with increased levels of protein urinary excretion[70].

The combination of hyperglicaemia and the absence of VDR results in an intrarenal increase of RAAS activation, as demonstrated by Zhang, and simultaneously there is evidence that deficits in the active metabolite of 125 dihydroxivitamin D3 indirectly stimulate the activation of TGF-1β[71] (Fig. 4).

The mechanisms of action of 1,25 dihydroxivitamin D3 in the pathogenesis of proteinuria include haemodynamic and non-haemodynamic direct actions that regulate cell proliferation, apoptosis, angiogenesis and anti-inflammatory action. The 1,25-dihydroxyvitamin D3 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[72-76].

In some experimental models, administration of 1,25-dihydroxyvitamin D3 decreased the loss of podocytes and inhibited their hypertrophy[77-79]. This beneficial effect is due to a direct action in signal modulation,

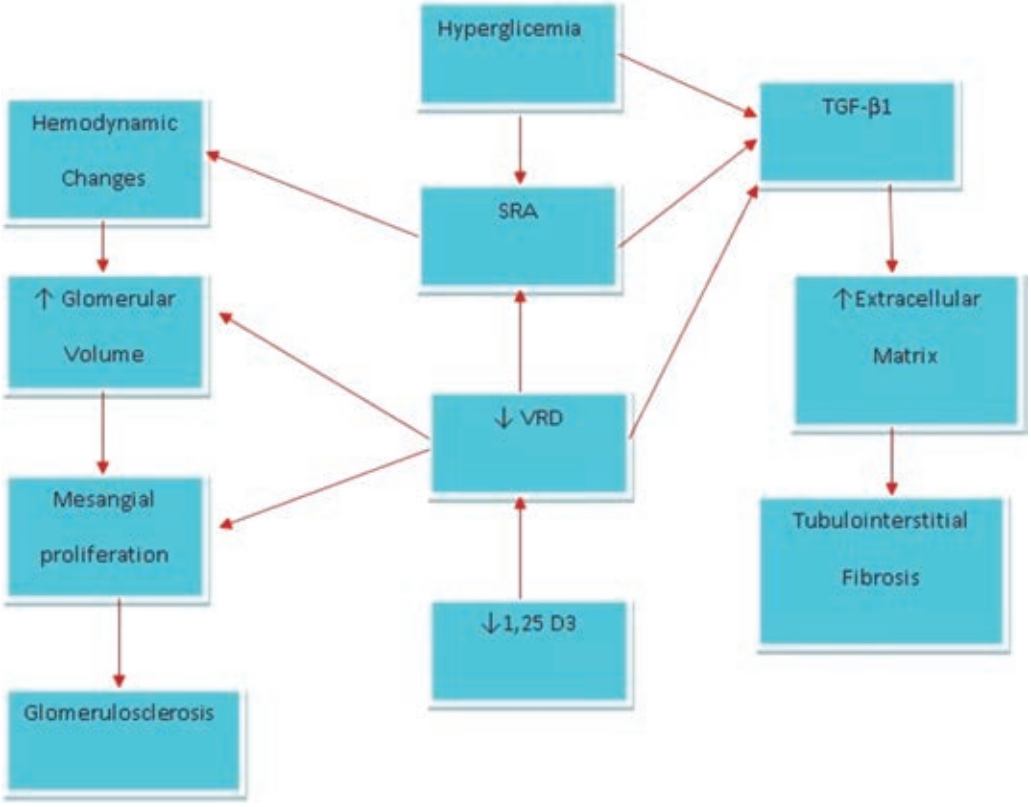
by inhibiting TGF-b1 and bone morphogenetic protein (BMP-7) expression[80]. In DN, the protective action of 1,25 dihydroxivitamin D3 is also due to its negative regulatory effect on the RAAS, by suppressing the production of renin which is one of the mechanisms responsible for renal injury, either by haemodynamic mechanisms, or through pro-inflammatory and pro--fibrotic mechanisms[81-83]. Although the mechanisms are not completely understood, 1,25 dihydroxivitamin D3 and its analogues, have protective functions by promoting the reduction of proteinuria, a biomarker of kidney involvement[84,85].

Randomized Clinical trials of Active Vitamin D therapy in Proteinuria

Several small studies have demonstrated the benefit of using 1,25 dihydroxivitamin D3 in reducing the levels of proteinuria in renal disease. Agarwal and colleagues evaluated the efficacy of oral paricalcitol in 220 patients with stages 3 and 4 chronic kidney disease over a period of 24 months. In addition to other parameters, proteinuria was measured by dipstick at the start and at the end of the trial period[84]. The 107 patients were randomized in a double--blind pilot trial, with 57 patients receiving oral paricalcitol. At the end of the study, patients treated with paricalcitol showed a greater reduction of proteinuria, 51 vs. 25.5 % in the placebo group (OR = 3.2 for reduction of proteinuria, 95% CI:1.5-6.99). The effect of paricalcitol in reducing proteinuria was independent of demographic characteristics, comorbidities and use of antagonists of the renin-angiotensin system. The effect of paricalcitol in reduction proteinuria was independent of demographic characteristics,

Figure 4

Effects of "vitamin" D metabolites deficit in the development of diabetic nephropathy.



comorbidities and use of antagonists of the renin--angiotensin system[84].

Also in a study with non-diabetic chronic kidney patients, Alborzi *et al.* showed the advantage of paricalcitol over placebo. In a shorter trial, these authors found that paricalcitol (1 and 2µg / day) reduced albuminuria in about 50%, comparing with placebo (p › 0.001)[79]. Oral calcitriol also reduced the proteinuria in a small group of patients with biopsy proven IgA nephropathy[86]. More recently, Aperis *et al.* also found, in a non-randomized study, that paricalcitol decreased albuminuria, in a heterogeneous group including diabetic and non-diabetic patients[87].

Regarding diabetic patients, there are two clinical studies that only included type 2 diabetic patients. In both, all patients were already medicated with antagonists of the renin-angiotensin system. The VITAL study was a prospective, randomized, double--blinded placebo-controlled, multicentre study that evaluated the efficacy of oral paricalcitol on reducing albuminuria:creatinine ratio (UACR). In this trial, 281 patients were equally allocated into 3 groups to receive, 0, 1 or 2 µg of oral paricalcitol for a 24-week period. Patients on 2 µg showed a statistically significant reduction in UACR of 20% (p = 0.014 vs. placebo)[88].

In a prospective observational study, Kim *et al.* treated, with oral cholecalciferol, during four months, type 2 diabetic patients with low levels of 25(OH) D. They found that cholecalciferol reduced albuminuria and urinary TGF-β1 and concluded that dietary 1,25 dihydroxivitamin D3 repletion with cholecalciferol could have a beneficial effect on progression of DN[89].

Despite the existence of several studies reporting the ability of 1,25-dihydroxyvitamin D3 to lower proteinuria/albuminuria in diabetic and non-diabetic patients, quite a few questions remain unanswered. First of all, it is crucial to look at hard end-points, like the progression of kidney disease, conducting studies with longer follow-up time. It is known that in PRIMO study, the administration of paricalcitol was associated with a greater decrease of the renal function[90]. Although this study was not designed to address the progression of the renal disease and the paricalcitol group had worst renal function at baseline, this study reinforced the need of an answer to this issue. Secondly, cardiovascular is the main cause of morbidity and mortality in chronic kidney patients and there is a bulk of evidence showing that low levels of 1,25 dihydroxivitamin D3 are associated with greater risk of cardiovascular events in the general population[90-92]. It will be very important to ascertain if the treatment of chronic kidney disease patients with 1,25 dihydroxivitamin D3 will decrease cardiovascular morbidity and mortality in this particular population.

In an observational study including diabetic and non-diabetic patients, we could find that patients undergoing treatment with statins in association with 1,25 dihydroxivitamin D3 (alfacalcidol) had lower mortality[93]. It would be also important to see if the results are the same by administrating 1,25 dihydroxivitamin D3 or paricalcitol, an activator of the 1,25 dihydroxivitamin D3 receptor, since in terms of costs it is quite different.

The knowledge of the right moment of intervention, with former normalization of the 1,25 dihydroxivitamin D3 levels, also seems crucial. Will it be useful to administrate paricalcitol in 1,25 dihydroxivitamin D3 repleted patients?

The inclusion of type 1 diabetic patients and the evaluation of cardiovascular end-points are fundamental in future trials, in order to investigate the benefits of 1,25 dihydroxivitamin D3 in chronic kidney disease patients with DN.

CONCLUSION

Through the activation of its nuclear receptors and consequent transcription of 200 genes, 1,25 dihydroxivitamin 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 DN.

However, many questions remain and must be answered by conducting longer trials with stronger renal and cardiovascular end-points.

***Conflict of interest statement*: None declared.**

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