Vitamin D as a Multiple Player Under the Physiological and Pathological Conditions and in Diabetic Nephropathy in Children

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Abstract

Vitamin D deficiency is known to be a great problem worldwide. Vitamin D plays essential role in calcium and bone metabolism. Recently data about the role of vitamin D deficiency in pathogenesis of diabetes, diabetes complications, and cardiovascular disease published. The vitamin D receptors presented in all cell types. In vitro and in vivo studies show their role in transcription of multiple genes. This function is not dependent on calcium homeostasis. These effects provide anti-proliferative, immunomodulatory, angiogenic changes; inhibition of the renin-angiotensin-aldosterone system, and neurotrophic factor expression.

Diabetic nephropathy (DN) is a dangerous kidney-related complication of type 1 diabetes. DN is an important cause of end-stage kidney disease in the world. DN characterizes by presence of albuminuria, which in turn causes renal disease progression and cardiovascular complications. Main pathological processes associated with DN are the following: kidney hemodynamics changes, oxidative stress, inflammation, hypoxia, renin-angiotensin-aldosterone system (RAAS) activation. All mentioned above changes accompanied by fibrosis.

The aim of the study was to do the overview of the literature dealing with physiological and pathological effects of Vitamin D. Own data dealing with the level of hypoxic disorders, condition of apoptosis controlling system and Vitamin D3 levels in children with diabetic nephropathy discussed.

Thus, our results show that children with signs of DN have high level of cellular hypoxia and apoptosis, as one of the key mechanisms of kidney damage in DN. All these events are parallel in development with Vitamin D3 deficiency. Number of papers hypothesize about the role of vitamin D in the long-term complications of diabetes. It is also suggested that Vitamin D deficiency may enhance the symptoms of DN. It’s not studied if administration of vitamin D may prevent pathogenic processes related to DN progression. Finally, large well-designed randomized controlled trials of vitamin D supplementation in pediatric cohort with DN need to be done.

Keywords: Vitamin D; Diabetes Mellitus; Diabetic Nephropathy; Cardiovascular System; Kidney Diseases; Apoptosis; Albuminuria

Introduction

Vitamin D is defined as a lipid-soluble, secosteroid hormone. It plays primary essential role in calcium homeostasis regulation. Synthesis of vitamin D in in skin is fully dependent on sun exposure. The source is dehydrocholesterol. Ultraviolet B light of wavelengths between 280 and 315 nm is required for 7-dehydrocholesterol to previtamin D3 transformation, which in turn converts to vitamin D3. Second source of vitamin D is Vitamin D-containing food. Vitamin D obtained from mentioned above sources undergo transformation to 25(OH)D in liver with subsequent hydroxylation to 1,25-dihydroxyvitamin D [1,25(OH)2D3 (activated vitamin D)] in kidneys [1].
To evaluate the vitamin D status in humans the 25(OH)D measurement in blood is commonly used method [1,2]. Following scale using to evaluate the results: > 30 ng/mL (> 75 nmol/L) - sufficient, 20 - 30 ng/mL (50 - 75 nmol/L) - insufficient, 10 - 20 ng/mL (25 - 50 nmol/L) - deficient, and < 10 ng/mL (< 25 nmol/l) as severe deficient (Table 1).

<table>
<thead>
<tr>
<th>Classification of 25-OH-vitamin D levels (according to 1, 5, 10-13, e3, e4)</th>
<th>25-OH-vitamin D (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxic</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Optimum</td>
<td>31 to 60</td>
</tr>
<tr>
<td>Suboptimum</td>
<td>21 to 30</td>
</tr>
<tr>
<td>Moderate deficiency</td>
<td>11 to 20</td>
</tr>
<tr>
<td>Severe deficiency</td>
<td>≤ 510</td>
</tr>
</tbody>
</table>

Table 1: Blood levels of Vitamin D.

It is known that Vitamin D insufficiency doesn’t cause the bone disease. However, by these levels of Vitamin D cardiovascular and kidney disease may be induced. Vitamin D receptors (VDR) expressed in all human cell types. VDR and Vitamin D regulate multiple biological processes through the gene regulation. Vitamin D and it’s role in understanding the basic processes of kidney and cardiovascular diseases pathogenesis are crucial for further treatment strategies [3].

**Vitamin D and molecular background of its role in cardiovascular physiology**

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide. In 2015, the World Health Organization (WHO) suspect that CVD may lead to more than 17.7 million deaths which is 31% of global deaths [4]. The main injury typical for CVD is atherosclerosis and atherosclerotic-type vascular damage. Vitamin D is known as a hormone beside being the vitamin. The proof of later is direct interaction of Vitamin D and VDR.

The VDR is essential for Vitamin D3 and calcitriol binding to nuclei with subsequent interaction with specific response elements. These events induce response of various genes inducing physiological reactions. After the diffusion through the cell and nuclear membranes calcitriol binds to the VDR, causing a conformational change in the receptor. Then its heterodimerization with retinoic acid X-receptor (RXR) occurs.

Many cells and tissues of the cardiovascular system abundantly express either calcitriol or VDR including cardiomyocytes, and vascular and endothelial cells [5]. VDR activation in endothelial cells can regulate their cell cycle. This requires vascular endothelial growth factor (VEGF) promoter activation. It is shown that higher VDR expression in stressed endothelial cells and increased concentrations of calcitriol decrease cytokine and adhesion molecule expression [5,6].

In heart cells cardiomyocytes calcitriol regulate cell cycle (i.e. maturity and differentiation) as well as in endothelial cells. In experiments done on animal models where lack of VDR expression induced increased ventricular mass and high levels of matrix metalloproteases (MMPs) and atrial natriuretic peptide (ANP) documented. This is a direct proof that vitamin D can regulate intracellular matrix status [7].

Currently many studies show that vitamin D deficiency has place in number of cardiovascular disorders. However, a basic disorders leading to these pathologies are not fully understand. Number of studies demonstrated that vitamin D deficiency is a direct cause of atherosclerosis progression and vascular calcification [7,8].

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Vitamin D regulates processes of macrophage maturation and infiltration into the vasculature. This in turn regulates the pro-inflammatory cytokines and adhesion molecules expression providing regulation of the atherosclerosis progression. In clinical study shown that vitamin D supplementation leads to decreased levels of inflammatory markers in patients with heart failure [9].

Number of studies show the association of vitamin D deficiency with prevalent and incident CVD as well. Vitamin D deficiency associated with conditions accompanied CVD such as dyslipidaemia, hypertension and diabetes mellitus [7].

It shown that increased up to normal level vitamin D concentrations provide improved lipid profile in patients with CVD. However, recent meta-analyses did not proof this hypothesis [10].

**Vitamin D in kidney and endocrine disorders**

The wide tissue distribution of VDR suggests that the vitamin D has additional physiological functions beyond calcium homeostasis. There are many experimental and clinical data showing the pleiotropic actions of vitamin D. Vitamin D and VDR shown to play important roles in immune, cardiovascular, reproductive system etc. Many of these effects of vitamin D are independent from calcium and phosphorus homeostasis.

Epidemiological studies show that diabetes mellitus type 1 has prevalence in countries with lower winter ultraviolet exposure. These data support hypothesis that vitamin D supplementation in early childhood may prevent the onset of diabetes mellitus type1 [11].

On experiments in vivo model shown that 1,25(OH)2D or its analogues have capacity to reduce the levels of proteinuria levels due to preserved glomerular podocyte structure, decreased levels of TGF-β1. This is due to renal fibrosis and inhibited mesangial cell proliferation reduction [12]. It is known about the inverse association of circulating vitamin D levels with blood pressure. Moreover, vitamin D administration reduces blood pressure [7].

Vitamin D has direct effect on RAAS through the VDR resulted in plasma renin activity, angiotensin II (Ang II) and myocardial hypertrophy attenuation. These effects observe as a result of profibrotic and proinflammatory processes and endothelial dysfunction inhibition [7].

In addition, it was demonstrated that VDR null mice show a sustained elevation of renin expression. At the same time they have normal level of blood electrolytes. Increased renin synthesis leads to increased plasma Ang II production from angiotensinogen. This causes increased water intake and intestinal salt absorption in VDR-null mice due to fact that Ang II is a potent thirst-inducing agent that acts on the CNS, and a stimulator of intestinal sodium absorption as well. To maintain the balance increased excretion of urine and salt documented in these mice. Besides being a potent vasoconstrictor, Ang also exerts proliferative effects. Since Ang is a potent vasoconstrictor, its augmentation also leads to the development of hypertension and cardiac hypertrophy in VDR null mice. Although the latter effect still needs more experimental proof [13].

The combined effect of ARB studied. It is documented that Losartan and non-calcemic vitamin D analogue paricalcitol have an effect on the development and progression of diabetic nephropathy. It was shown that combined administration of the AT1 blocker losartan and the vitamin D analogue paricalcitol reduce the levels of molecular and clinical markers of diabetic nephropathy [14].

Study done by Li and colleagues demonstrated that the combination of angiotensin receptor blockers plus paricalcitol slows down the progression of streptozotocin-induced diabetes. It was shown that combined therapy has better effect kidney disease progression. Positive effects found on kidney structure (kidney histology) and function (albuminuria, serum creatinine) in mice with type 2 diabetic nephropathy [15]. Renal protection under the administered treatment was due to podocytes preservation and TGF-β system blockage in glomeruli.

Renoprotection by VDRA in CKD patients who were not on dialysis shown at least in four studies. First of all, a reduction of proteinuria in stage 3 and 4 CKD patients included in 3 controlled trials of oral paricalcitol administration reported [16]. Moreover, reduced proteinuria levels in these patients occurred despite parallel RAS blockers administration.

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In study done by Alborzi, et al. [17] 24 patients with stage 3 CKD were randomly divided into three groups received 0, 1, or 2 μg paricalcitol for 1 month. After 1 month of therapy C reactive protein levels decreased in group of patients received 2 μg of paricalcitol. At the same time 24-h albumin excretion rate improved under the paricalcitol treatment. Thus, paricalcitol causes prominent reduction of albuminuria and inflammation levels. At the same time positive effects on hemodynamics (glomerular filtration rate and ambulatory blood pressure monitoring) documented. Parathyroid hormone level reduced under the administered treatment.

Another trial where ten patients with Ig-A nephropathy and persistent proteinuria under the angiotensin converting enzyme inhibition and angiotensin II receptor blockade included done. In this study patients treated with 0.5 μg Calcitriol twice a week during 12 weeks [18]. In the end of treatment period a significant decrease in urine protein-creatinine ratio found. However, any positive changes of blood pressure profile and renal function noted. Significant decrease in serum TGF-β level detected. This positive effect significantly correlated with percentage of proteinuria levels changes.

In order to understand the mechanism of the vitamin D and RAS interaction mentioned above study done. It was shown that VDR-null mice maintain a high level of renin expression. The underlying physiological cause of this event can be complicated. Obtained data show that the renin expression in VDR-null mice reacts properly to high salt load or dehydration. This is an evidence that the mechanism underlying the renin elevation is not dependent on classical pathways activated by tubular salt load or volume depletion.

Thus, taken together the RAS suppression under the Vitamin D treatment on animal models have beneficial effects on albuminuria, mesangial cell proliferation, inflammation, and extracellular matrix formation. Low vitamin D levels is a potent risk factor for the renal disease progression [1].

Vitamin D must be administered to patients with CKD in order to maintain bone health as well as a key agent in immunological, cardiovascular and renal diseases prevention. An appropriate dose of exogenous Vitamin D should maintain the serum level of vitamin D at level greater than 30 ng/ml [19]. Despite the positive effect of Vitamin D on proteinuria and hypertension values, no positive influence on end-stage renal disease course documented yet.

Vitamin D and diabetes mellitus
VDR have also been identified in pancreatic beta cells. Vitamin D deficiency has been shown to effect the insulin synthesis and secretion in animal models of type 2 diabetes. Several studies, including one published by Scragg, et al. [20] demonstrated that lower vitamin D levels were associated with an increased risk of diabetes. A double-blinded placebo trial [21] adults with high risk of type 2 diabetes development were administered Vitamin D. A short-term supplementation with Vitamin D significantly improved function of beta cells; HbA1c levels were decreased.

Moreover, in two other independent studies shown that 25(OH)D administration decreased levels of HbA1c in patients with and without diabetes, respectively [21,22]. In study done by Devaraj, et al. [23] gradual improvement of diabetes mellitus markers (HOMA index, glucose consumption) under the treatment with Vitamin D found. A small placebo controlled study where Vitamin D3 supplementation provided for 6 months to patients with T1D reduced insulin resistance documented. All patients included into this study were Vitamin D deficient. Supplementation with Vitamin D is able to reduce mortality rate in diabetic patients and prevent nephropathy and retinopathy in type 1 diabetes as well [24].

Vitamin D in children with TD1 and diabetic nephropathy
According to the data from the International Diabetes Foundation in 2014, there were approximate 380 million people with diabetes who accounted for 8.3% of the world population. Among the causes of ESRD in the world, DM accounted for 30-47%. In the United States, about 54.4% of patients with type 1 diabetes mellitus will eventually receive renal replacement treatment (RRT) [25].
Albuminuria is one of the most characteristic clinical signs in diabetic nephropathy (DN). In the past, especially from the observations in patients with type 1 DM, the clinical stages of DN were considered to begin from early glomerular hyperfiltration, followed by the development of microalbuminuria, macroalbuminuria, and then declined GFR (Figure 1) [26].

Renal fibrosis, the final common pathway in the pathophysiology of DN, is caused by at least renal hemodynamic changes, ischemia and glucose metabolism abnormalities-associated oxidative stress increases, inflammatory processes and overactive renin-angiotensin-aldosterone system (RAAS) [27] (Figure 2).

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Glomerular hyperfiltration leads to the occurrence of DN. Hyperglycemia causes afferent arteriolar dilatation by release of vasoactive mediators, such as insulin-like growth factor 1 (IGF-1), glucagon, nitric oxide (NO), vascular endothelial growth factor (VEGF) and prostaglandin. On the other hand, alterations in renal tubular function also occur in the early stage of DM and related to the degree of glycemic control.

Due to high filtrated load of glucose, reabsorption of both glucose and sodium chloride is increased because of upregulation of sodium glucose cotransporter 2 (SGLT2) in the proximal tubules. Thus, the delivery of sodium chloride to the macula densa of distal tubules decreased, and then causes dilatation of afferent arteriole because of tubule glomerular feedback. At the same time, constriction of efferent arteriole occurs due to high local level of angiotensin II, and then causes changes of autoregulation and glomerular hypertension [28].

On the other hand, hyperglycemia, insulin resistance and compensatory hyperinsulinemia independently cause endothelial dysfunction by promoting some intracellular mechanisms, such as increased reactive oxygen species (ROS) production, activation of protein kinase C (PKCs) and advanced glycation end-products (AGE)-induced pro-inflammatory signaling [29].

The interactions of mediators produced by endothelial cells disrupted and tend to imbalance. Within these mediators, endothelin-1 (ET-1) is the most potent vasoactive peptide produced by endothelial cells in regulation of vascular homeostasis. In the endothelium, compensatory hyperinsulinemia increases ET-1 secretion, thus results in vasoconstriction and vascular dysfunction. In the kidney, activation of endothelin-receptor A is not only associated with vasoconstriction, but also podocyte injury, oxidative stress, inflammation, and fibrosis [30].

36 children with T1D (aged 6 to 17 years) treated in Endocrinology unit of Children’s Clinical Hospital №6 (Kyiv, Ukraine) included into the study. Examinations (general physical examination, blood pressure monitoring, blood tests, urinalysis, kidney ultrasound, etc.) done in all patients. Plasma levels of HIF-1α, caspase-3, Vitamin D3 measured in all patients. 16 healthy children included into the control group.

High blood level of HIF-1α in all patients with T1D and DN detected. The levels of HIF-1α in group with T1D exceeded control group by 18.5 ± 1.76% (p < 0.01 compared to control group) and by 30.03 ± 3.75% (p < 0.01 compared to control group) in patients with DN (Figure 3).

Figure 3: A) Western Blot showing expression of HIF-1α in children with T1D and DN. B) Densitometric quantification of HIF-1α. The density of the band from control group was set to 100%. Histograms show the mean ± SEM. *p < 0.05.
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The level of apoptosis assessed by pro-apoptotic effector caspase-3 measurement. High level of caspase-3 documented in all patients. In patients with DN expression of caspase-3 exceeded level of control group by 34,19 a.u. ± 4,36% (p < 0,001). This marker in T1D group was documented at level exceeded control group by 14,82 a.u. ± 2,35% (p < 0,01). Control group value set as 100%.

Blood levels of Vitamin D measured in all children with T1D and DN. Normal level of Vitamin D defined as ≥ 30 ng/mL; Vitamin D insufficiency and deficiency - 21 - 29 ng/mL and ≤ 20 ng/mL, respectively. Endogenous levels of Vitamin D3 synthesis are season-dependent. Due to this reason study done during the period from September to February. The lowest Vitamin D3 levels detected in patients with diabetic nephropathy. In control group Vitamin D3 was detected at level 35,68 ± 1,56 ng/mL, in patients with T1D - 32,37 ± 5,1 ng/mL, in patients with diabetic nephropathy - 19,39 ± 1,76 ng/mL (p < 0,01 as compared to control group). Negative correlation between the Vitamin D level and T1D course found (R = -0,79, p < 0,001) (Figure 4).

Moreover, Vitamin D3 levels negatively correlate with albuminuria levels in patients with diabetic nephropathy (R = -0,59, p < 0,001).

Our results have a proof of the deep and complex damages in children with DN, i.e. pro-apoptotic effector activation, increased hypoxic process, Vitamin D deficiency and its dependency on DN disease course and albuminuria levels.

Cellular and molecular experiments show that inflammation is an important key to the pathophysiology of DN. Key player in these events is nuclear factor kappa B - a transcription factor and major regulator of inflammatory factors. In DN increased tumor necrosis factor (TNF) and ROS activate NF-κB by phosphorylation. Moreover, NF-κB is associated with proteinuria and renal interstitial inflammatory cells infiltration [30].

The response of immune system is also related the development of DN. The predominant immune response in the development of DKD is innate immune response [30]. Hyperglycemia causes cellular stress and dysfunction due to disturbed function of mitochondria and endoplasmic reticulum, ROS production, and abnormal activation of intracellular signals pathways. As a result of these diabetic stress,
kidney cells produce pro-inflammatory responses and then facilitate innate immune response via release of chemokines, cell adhesion molecules (CAMs) and danger associated molecular patterns (DAMPs). The initiation of innate immune response is recruitment of macrophage [30,31].

All mentioned above processes accompanied by autophagy. Autophagy plays a key role in diabetes-related podocyte injury due to direct control of the cytoplasm by degrading proteins and peroxidases quality, maintaining the homeostasis of intracellular environment. Activated podocyte autophagy has a protective effect on DN through autophagy-related (Atg) protein conjugation system and mammalian target of rapamycin (mTOR) regulation [32]. In this paper we focus attention on autophagy as one of the key processes underlying the kidney damage in DN.

Conclusions

• Approximately 30%-50% of people documented to have low levels of vitamin D, and insufficiency and deficiency of vitamin D are recognized as global health problems worldwide.
• Low vitamin D levels are also associated with hypertension, cancer, and cardiovascular disease, diabetes mellitus (DM) and chronic kidney disease (CKD). Vitamin D deficiency has been linked to onset and progression of DN.
• Vitamin D insufficiency influences the renin-angiotensin system, inflammation, immune system, basic metabolic state and mineral bone disease, which may be associated with the cause and progression kidney disease in adults, including DN.
• Although at this time, supplementation with vitamin D has not been shown to improve glycemic control or prevent incident DM and DN progression in children. Clinical trials with sufficient sample size, study periods, and optimal doses of vitamin D supplementation are still needed.

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