Original Article

Approach to correction of apoptotic disorders in children with early diabetic nephropathy

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ABSTRACT

Diabetic nephropathy is a disease characterized by pathological changes in glomeruli that lead to the development of albuminuria, hypertension, and a decline in renal function. Prominent changes detected in apoptosis controlling factors, i.e. BcL-xL and caspase-3 and require a search for additional non-toxic therapeutic interventions, i.e. antioxidants, anti-apoptotic effectors in addition to glycemic control, hypertension management, as well as albuminuria. The present study included forty-four children (24 boys, 20 girls) diagnosed with T1D and DN around the ages of five-seventeen. Clinical data (age, gender, disease duration, blood pressure), conventional laboratory markers (complete blood count, serum cholesterol, Hb1Ac, GFR, MAU), and apoptosis markers (BcL-xL, caspase-3) were analyzed. We show that apoptosis-induced disorders in children with DN evaluated by dysbalance occurring in the cell death controlling system may be attenuated by the advanced scheme which includes conventional treatment and Vitamin D; the applied scheme has a long-term therapeutic effect on macroalbuminuria development.

Keywords: Treatment, Vitamin D, Diabetic nephropathy, BcL-xL, Bax, Apoptosis

Introduction

It is expected that more than 1 million patients below twenty years can be affected by type 1 diabetes (T1D) [1, 2]. T1D is an epidemic disease that resulted in an increase in diabetic nephropathy (DN) incidence worldwide [1-5]. It was seen that Hyperglycemia is the major biochemical disorder in diabetes and DN. This in turn leads to a toxic reaction via activation of inflammation, oxidative stress, and apoptosis [1]. The diagnosis of DN is based on the measurement of abnormal urinary albumin levels in T1D patients after excluding other causes of albuminuria. The gold standard in diagnostics of DN includes two out of three urine samples within the range of microalbuminuria (30 to 300 mg of albumin/24 h) or macroalbuminuria (more than 300 mg of albumin/24 h) [6-9].

Vitamin D is a lipid-soluble, secosteroid hormone substance. Vitamin D is a prehormone. In humans, it is obtained in 2 ways - the diet or via skin synthesis. It is subsequently activated in a 2-stage process of hydroxylation [10].

Vitamin D has a vital role in various physiological functions. Disorders in Vitamin D metabolism lead to many acute and chronic diseases – changes in calcium metabolism, autoimmune diseases, cancers, types 1 and 2 diabetes mellitus, cardiovascular disease, etc. Vitamin D deficiency is now concerned as a global pandemic. The major cause of vitamin D deficiency is the inadequacy of sun exposure [11-13]. We have previously shown that the majority of patients from the T1D group have a normal value of Vitamin D, only 27,7% of children have Vitamin D insufficiency. In contrast, in children from the DN group, only 16,7% of children have Vitamin D insufficiency and 83,3% have Vitamin D deficiency [14].

BCl-2 family is a well-known group of proteins known for the position it plays in cell death regulation. An in vivo model [15] showed their protective effect. It is known that Puromycin-induced podocyte apoptosis is p53-dependent and is associated with changes in Bcl-2-related proteins and AIF translocations.

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The protective effect of dexamethasone on this model of apoptosis has a relation to a decrease in p53, an increase in Bcl-xL, and inhibition of AIF translocation [16].

Children with early DN have disturbances in the apoptosis controlling system, i.e. down-regulation of anti-apoptotic agent BcL-xL and up-regulation of apoptosis factor caspase-3 were previously shown [17].

This research aim at evaluating whether Vitamin D administration together with conventional treatment of DN may treat apoptosis disorders in children with DN.

Materials and Methods

Patients

54 children (34 males, 20 females) diagnosed with an early stage of DN were included in the analysis. Subsequently, a follow-up session was performed in the Endocrinology ward at Clinical Pediatric Hospital №6 (Kyiv, Ukraine). The approval of this research was attained from the ethics committee of the Bogomolets National Medical University (approval № 142). The informed consents were signed by the children between twelve or above years and/or also their parents and then documented medical records. Records data were analyzed in all patients. All diabetic patients with an early DN were followed-up every three months. The patients were put under multiple flexible intervals of dosage of management with insulin. Abiding by the parameters which are; weight, chronological age, diabetes duration, height, body mass index (, BP, Hb1Ac, serum cholesterol, CBC, urinalysis, and urine albumin excretion measured and fixed at each visit.

All patients with early DN were treated accordingly to local protocols. TREATMENT INCLUDED ACE inhibitors, antioxidants (Vitamin E) (main group, named “Before treatment I”, after treatment I”). Vitamin D (Aquadetrim, POLPHARMA) in a dose of 500 IU/day was administered to patients from the experimental group for 3 months (experimental groups named below “Before treatment II”, after treatment II”).

Patients with an early DN were those with T1D studied for one year following the initially registered episode of albuminuria. Other reasons for albuminuria were excluded in all patients. Two out of three urine samples of microalbuminuria (30-300 mg albumin / 24 hours) or macroalbuminuria (300 mg albumin / 24 hours or more) confirm DN. Severe chronic and acute diseases, i.e. chronic inflammatory diseases, autoimmune diseases, transplantation, Viral Hepatitis B or C, liver cirrhosis, or other severe liver diseases, acute and chronic gastrointestinal diseases, previous Acute Kidney Injury, Chronic Kidney Disease, major surgery 12 months before the study, heart disease, cancer AIDS selected as patient exclusion criteria from the included study.

Measurements

Complex examination of all patients included conventional methods, i.e. blood pressure measurement, blood tests, urinalysis, renal ultrasound, ECG, etc. Urinary microalbumin/albumin excretion was measured with 24-hour urine collection samples. Bcl-xL levels were measured using ELISA assay and kit Human BCL2L1 / BCL-XL (LSBio, USA). Caspase-3 levels were measured using an ELISA assay and a commercially available Caspase 3(Cleaved) Human ELISA Kit (Invitrogen, USA).

Statistics

The data is represented as means ± SEM. ANOVA followed by the *post hoc* Kruskal-Wallis test for multiple comparisons employed in testing the significance of differences. Main demographic and clinical data were compared using Pearson chi-squared test. Data were analyzed using GraphPad Prism 9.0 Software for Windows (USA, San Diego, CA). P values <0,05 are considered as those statistically significant.

Results and Discussion

Clinical characteristics of patients

Table 1 shows the demographic and clinical features of the individuals who participated in this research. The DN group (n = 44) consisted of children who received DN follow-up care for 1 year after the first recorded albuminuria. The duration of TID in this group was shown to be 6.0±0.51 years on averagely. The distribution of the individuals’ gender is as follows: 24 males and 20 females. The average age of children is 12.95±0.56 years. The average age of the males was 12.12±0.76 years, and the average age for was girls – 13.2±0.56 years. Body mass index (BMI) was analyzed in all children. Average BMI in DN group – 19.72±0.55. BP was measured at a visit before inclusion in the study. Systolic PB in DN group - 127,4±1,34 mmHg (p<0,001). Diastolic BP value 71,8±1,11 mmHg. GFR is a direct marker of kidney function measured in all children. GFR level was evaluated at level 85.89±2.9 mL/min/1.73 m^2. The mean Hb1Ac value was 10.22±9.55 %. The Control group included 44 healthy children matched by age and sex **(Table 1)**.

Table 1. Basic clinical characteristics of patients

Data Mean±SEM	DN group (T1D with diabetic nephropathy) (n=44)	Control group (n=44)
Age, years	12.95±0.5	12.75±0.7
Boys/girls	34/20	34/20
Boys, age, years	12.12±0.76	12.5±0.7
Girls, age, years	13.2±0.56	12.95±0.5
Duration of T1D	6.0±0,51	6.15±0,8
BMI, kg/m^2	19.72±0.55	19.22±0.98
Systolic blood pressure, mmHg	127,4±1,34	122,5±1,4
Diastolic blood pressure, mmHg	71,8±1,11	70,4±1,16
GFR, mL/min/1.73 m^2	85.89±2.9	95.19±2.01
Hb1Ac, %	10.22±9.55	1.22±0.13

Therapeutic correction of apoptosis-induced disorders in patients with diabetic Nephropathy

Two principal markers of apoptosis – BcL-xL, and caspase-3 were measured and compared in children from the main and experimental groups.

After a course of therapy, all children were checked for serum levels of anti-apoptotic marker BcL-xL. In the group named Before treatment, the level of BcL-xL was down-regulated and lower in comparison to the control group by 63,25 ± 0,36% ($p<0.001$). After the course of treatment BcL-xL value in this group (After treatment I) appeared lower than the control rate down to 74,2 ± 0,55% ($p<0.05$ as compared to the level before treatment). In the group named Before treatment II BcL-xL level is lower in comparison to the control group by 63,88 ± 0,3% ($p<0.001$). After the treatment, the level of BcL-xL was measured at a level close to the level of the control group ($p <0.001$ in comparison to the level before treatment) **(Figure 1)**.

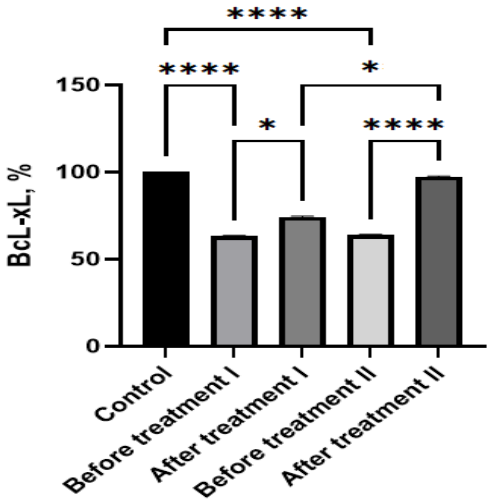

Figure 1. Levels of anti-apoptotic agent BcL-xL measured in children diagnosed with an early DN before and after the treatment. Histograms are presenting means ± SEM. Statistical analysis was performed using the *post hoc* Kruskal-Wallis test. * - P <0.05, **** - P <0.001.

All children were examined for serum levels of proapoptotic factor caspase-3 after the course of therapy. In children from the group named Before treatment II, the caspase-3 concentration was increased up to 139,4 ± 0,69% ($p<0.001$) in comparison to control. Value of control group assumed as 100%. After treatment, caspase-3 levels decreased to 115.3 ± 0.44% ($p <0.05$) compared to pretreatment levels. Children in the experimental group designated as Pretreatment II have 139.9 ± 0.83% ($p <0.001$) caspase-3 levels in comparison to the control group.. After the administered treatment caspase-3 was down-regulated to 103 ± 0,6% ($p<0.001$, in comparison to the level before treatment) **(Figure 2)**.

Both apoptotic markers – BcL-xL and caspase-3 changes under the treatment with Vitamin V in addition to conventional treatment show more prominent changes as compared to the effect of conventional treatment only.

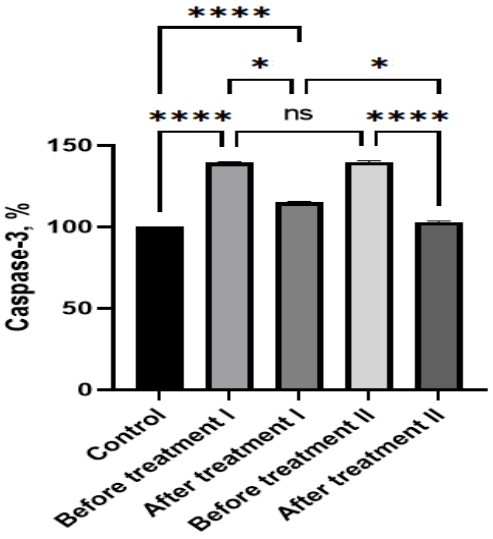

Figure 2. Levels of anti-apoptotic factor caspase-3 in children with an early DN before and after the treatment. Histograms represent means ± SEM. Statistical analysis was done using the *post hoc* Kruskal-Wallis test. - P <0.05, ** - P <0.01, **** - P <0.001.

Interestingly, more children from the group of DN treated with the concentional scheme (67%) show episodes of macroalbuminuria within 3 years as compared to the group treated with Vitamin D in addition to the conventional scheme (33%, $p<0.05$) **(Figure 3)**.

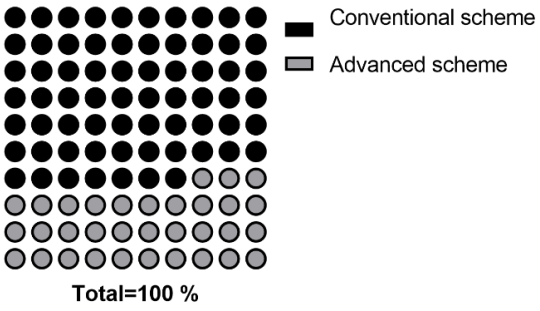

Figure 3. Distribution of children treated with concentional scheme and with Vitamin D in addition to the conventional scheme in terms of macroalbuminuria progression.

Diabetic nephropathy is one of the crucial causes of mortality in type 1 diabetes patients [12, 13]. It is known that above ¼ of T1D suffering from DN develop ESRD. Likewise, DN is an important risk factor for heart disease, i.e. coronary artery disease (CAD). The development of DN in most of the scenarios is categorized through a long silent period that lacks any clinical signs of nephropathy. This leads to under-treatment in adolescents with T1D and early complications. Interestingly, only a 1/3 of patients aged <20 years with a diagnosis of microalbuminuria

received necessary renoprotective treatment - ACE inhibitors (ACEi)/angiotensin-receptor blockers (ARB) [6-8].

By the time when GFR reduced down to <60mL/min/1.73m^2, renal function dramatically declined due to irreversible renal structural changes. This is a direct cause of subsequent ineffective therapeutic interventions including blood pressure and glycemic control [18].

Vitamin D is an important regulator of calcium and bone metabolism. The classical functions of vitamin D are dealing with mineral metabolism and skeletal balance. Recently, the non-classical functions of vitamin D were discovered. They are dealing with the regulation of the cell cycle, differentiation, apoptosis, and inflammation.

The extremely wide tissue distribution of VDR is the main background for Vitamin D's additional, non-classical functions. Mitri *et al*. show that T1D has prevalence higher incidence rate in countries with low ultraviolet exposure [19].

It was shown in vivo that 1,25(OH)$_2$D or its analogues can reduce the levels of proteinuria via preserved glomerular podocyte structure, and decreased levels of TGF-β1. The inverse association of circulating vitamin D levels with BP is a well-known fact [20].

In an animal model study, the potential renoprotective effects of active vitamin D and its counterparts were shown. Vitamin D decreases kidney injury by suppressing fibrosis, inflammation, and apoptosis. Also, Vitamin D can provide protective effects by inhibiting multiple pathways, including the renin-angiotensin-aldosterone system (RAAS) [21, 22], the nuclear factor-κB (NF-κB) [23, 24], the transforming growth factor-β (TGF-β)/Smad, and the Wnt/β-catenin pathways [25].

Moreover, paricalcitol weakens the increasing expression of phospho-p53 and p21, which are known to be crucial agents in apoptosis activation in a cisplatin-induced rat model. Increased Bax/Bcl-2 ratio and the cleaved form of caspase-3 can be reversed by paricalcitol in kidney damage induced by gentamicin. 1,25(OH)$_2$D$_3$ prevents apoptosis of podocytes induced by aminonucleoside [26, 27].

The current study aimed to evaluate whether Vitamin supplementation besides conventional management can reverse the dysbalance in factors controlling apoptosis in children with DN.

Tocopherol is a fat-soluble antioxidant that can scavenge free radicals in the plasma membrane. This in turn can assist in stopping the oxidative damages. Individuals with DN possess a decreased amount of tocopherol in plasma. This is a direct indication of its use in therapies [28, 29]. It was shown that prescribing tocopherol in those suffering from DN assisted in reducing cardiovascular complications, and improved the activity of internal antioxidant systems - GPX, and catalase [30]. Management with an antioxidant substantially reduced the hypoxia-induced cellular apoptosis and elevation of HIF-1alpha in vitro [31]. Regarding local protocols established in our hospital antioxidants are included in conventional schemes of DN treatment in children. In order to eliminate negative effects induced by HIF-1alfa which level is increased in children with DN.

In our previous study, we show the presence of Vitamin D3 deficiency, activation of the apoptotic effector caspase-3, and a high level of cellular hypoxia in children with DN [13, 16]. Moreover, we have previously demonstrated that tocopherol prescription has a positive protective effect on hypoxia-induced apoptosis in children diagnosed with nephrotic syndrome. This in turn provided subsequent restoration of the proapoptotic factor Bax activation. We speculate that this effect has a place in DN as well [32].

Previously, it showed a gradual increase in the marker of apoptosis (caspase-3) in children with T1D and DN. In addition, all diabetic patients had decreased vitamin D levels. Insufficient anger was exhibited in the group of T1D vitamin D levels found in patients with DN vitamin D deficiency [14]. Thus, we can't exclude the fact that the leak of Vitamin D protective functions on blood vessels, endothelium, and kidney cells [27, 29] may have a case in diabetic children and children with DN.

There are in vivo and in vitro data showing that Vitamin D3 deficiency and apoptosis activation may have a role in microvascular, vascular, and direct nephron damages in T1D. In our previous study [33, 34] we show that children with T1D and DN have decreased levels of Vitamin D and apoptosis activation. Here in this study, we evaluated that administration of Vitamin D in children with an early DN in addition to conventional treatment has a potent effect on changes in BcL-xL level which is a key molecule in the Bcl family. Moreover, we have found that under this treatment the level of caspase-3 has been substantially reduced. These antiapoptotic effects were more prominent in juveniles with an early DN treatment with Vitamin D in addition to the conventional scheme including (ACEi, antioxidants, i.e. Vitamin E). Interestingly, more children from the group of DN treated with concentional scheme show episodes of macroalbuminuria within 3 years.

Conclusion

- Administration of Vitamin D in children with an early DN in addition to conventional treatment has a potent effect on changes in BcL-xL level which is a key molecule in the Bcl family.
- Under applied treatment, the level of caspase-3 has been substantially reduced. These antiapoptotic effects were more prominent in children with an early DN treatment with Vitamin D in addition to the conventional scheme including (ACEi, antioxidants, i.e. Vitamin E).
- More children from the group of DN treated with concentional scheme show episodes of macroalbuminuria within 3 years.

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Conflict of interest: None

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Ethics statement: The study was approved by the ethics committee of the Bogomolets National Medical University.

References

1. Patterson CC, Karuranga S, Salpea P, Saeedi P, Dahlquist G, Soltesz G, et al. Worldwide Estimates of Incidence, Prevalence and Mortality of Type 1 Diabetes in Children and Adolescents: Results From the International Diabetes Federation Diabetes Atlas, 9th Edition. Diabetes Res Clin Pract. 2019;157:107842. doi:10.1016/j.diabres.2019.107842
2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and Regional Diabetes Prevalence Estimates for 2019 and Projections for 2030 and 2045: Results From the International Diabetes Federation Diabetes Atlas, 9(Th) Edition. Diabetes Res Clin Pract. 2019;157:107843. doi:10.1016/j.diabres.2019.107843
3. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 Diabetes. Lancet. 2014;383(9911):69-82. doi:10.1016/S0140-6736(13)60591-7
4. Thomas NJ, Jones SE, Weedon MN, Shields BM, Oram RA, Hattersley AT. Frequency and Phenotype of Type 1 Diabetes in the First Six Decades of Life: A Cross-Sectional, Genetically Stratified Survival Analysis From UK Biobank. Lancet Diabetes Endocrinol. 2018;6(2):122-9. doi:10.1016/S2213-8587(17)30362-5
5. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of Type 1 Diabetes. Endocrinol Metab Clin North Am. 2010;39(3):481-97. doi:10.1016/j.ecl.2010.05.011
6. Gheith O, Farouk N, Nampoory N, Halim MA, Al-Otaibi T. Diabetic kidney disease: world wide difference of prevalence and risk factors. J Nephropharmacol. 2015;5(1):49-56.
7. Piccoli GB, Grassi G, Cabiddu G, Nazha M, Roggero S, Capizzi I. et al. Diabetic Kidney Disease: A Syndrome Rather Than a Single Disease. Rev Diabet Stud. 2015;12:87-109. doi:10.1900/RDS.2015.12.87
8. Lim AKh. Diabetic nephropathy - complications and treatment. Int J Nephrol Renovasc Dis. 2014;7:361-81. doi:10.2147/IJNRD.S40172
9. Wang J, Xiang H, Lu Y, Wu T, Ji G. New progress in drugs treatment of diabetic kidney disease. Biomed Pharmacother. 2021;141:111918. doi:10.1016/j.biopha.2021.111918
10. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol. 2014;21(3):319-29. doi:10.1016/j.chembiol.2013.12.016
11. Basit S. Vitamin D in health and disease: a literature review. Br J Biomed Sci. 2013;70(4):161-72. doi:10.1080/09674845.2013.11669951
12. Lindblad AJ, Garrison S, McCormack J. Testing vitamin D levels. Can Fam Physician. 2013;60(4):351.
13. Zmijewski MA. Vitamin D and Human Health. Int J Mol Sci. 2019;20(1):145. doi:10.3390/ijms20010145
14. Burlaka IA. Beyond Proteinuria: Apoptosis and Vitamin D3 State in Children with Diabetic Nephropathy. J Clin Exp Pathol. 2016;6(2):266. doi:10.4172/2161-0681.1000266
15. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. Science. 1985;228(4706):1440-3. doi:10.1126/science.3874430
16. Wada T, Pippin JW, Marshall CB, Griffin SV, Shankland SJ. Dexamethasone prevents podocyte apoptosis induced by puromycin aminonucleoside: role of p53 and Bcl-2-related family proteins. J Am Soc Nephrol. 2005;16(9):2615-25. doi:10.1681/ASN.2005020142
17. Burlaka LA. Analysis of Apoptotic, Clinical, Laboratory Parameters in Type 1 Diabetes and Early Diabetic Nephropathy in Frame of Clustering and Potential Groups Evaluation for Additional Therapeutic Interventions. J Clin Res Pediatr Endocrinol. 2022. doi:10.4274/jcrpe.galenos
18. Alicic RZ, Rooney MT, Tuttle KR. Diabetic Kidney Disease: Challenges, Progress, and Possibilities. Clin J Am Soc Nephrol. 2017;12(12):2032-45. doi:10.2215/CJN.11491116
19. Mitri J, Dawson-Hughes B, Hu FB, Pittas AG. Effects of vitamin D and calcium supplementation on pancreatic β cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial. Am J Clin Nutr. 2011;94(2):486-94. doi:10.3945/ajcn.111.011684
20. Mittal S, Sandhu HS, Singh B. Study of Vitamin D levels in patients with Chronic Kidney Disease. Bangladesh J Med Sci. 2018;17(4):652-60. doi:10.3329/bjms.v17i4.38332
21. Zhang Y, Deb DK, Kong J, Ning G, Wang Y, Li G, et al. Long-term therapeutic effect of vitamin D analog doxercalciferol on diabetic nephropathy: strong synergism with AT1 receptor antagonist. Am J Physiol Renal Physiol. 2009;297(3):791-801. doi:10.1152/ajprenal.00247.2009
22. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest. 2002;110(2):229-38. doi:10.1172/JCI15219
23. Sanchez-Niño MD, Bozic M, Córdoba-Lanús E, Valcheva P, Gracia O, Ibarz M, et al. Beyond proteinuria: VDR activation reduces renal inflammation in experimental diabetic nephropathy. Am J Physiol Renal Physiol. 2012;302(6):F647-57. doi:10.1152/ajprenal.00090.2011
24. Dugbartey GJ, Alornyo KK, N'guessan BB, Atule S, Mensah SD, Adjei S. Supplementation of conventional anti-diabetic therapy with alpha-lipoic acid prevents early development and progression of diabetic nephropathy. Biomed Pharmacother. 2022;149:112818. doi:10.1016/j.biopha.2022.112818

25. Kim CS, Joo SY, Lee KE, Choi JS, Bae EH, Ma SK, et al. Paricalcitol attenuates 4-hydroxy-2-hexenal-induced inflammation and epithelial-mesenchymal transition in human renal proximal tubular epithelial cells. PLoS One. 2013;8(5):e63186. doi:10.1371/journal.pone.0063186
26. Suh SH, Lee KE, Park JW, Kim IJ, Kim O, Kim CS, et al. Antiapoptotic Effect of Paricalcitol in Gentamicin-induced Kidney Injury. Korean J Physiol Pharmacol. 2013;17(5):435-40. doi:10.4196/kjpp.2013.17.5.435
27. Tourigny A, Charbonneau F, Xing P, Boukrab R, Rousseau G, St-Arnaud R, et al. CYP24A1 exacerbated activity during diabetes contributes to kidney tubular apoptosis via caspase-3 increased expression and activation. PLoS One. 2012;7(10):e48652. doi:10.1371/journal.pone.0048652
28. Oral E, Rowell SL, Muratoglu OK. The effect of alpha-tocopherol on the oxidation and free radical decay in irradiated UHMWPE. Biomaterials. 2006;27(32):5580-7. doi:10.1016/j.biomaterials.2006.07.017
29. Samsu N, Soeharto S, Rifai M, Rudijanto A. The Superiority of Rosmarinic Acid over Vitamin E in Preventing Development and Inhibiting Progression of Diabetic Nephropathy in Rats. Asian J Pharm Res. 2020;10(3):131-9. doi:10.5958/2231-5691.2020.00025.8
30. Wang W, Kang PM. Oxidative Stress and Antioxidant Treatments in Cardiovascular Diseases. Antioxidants (Basel). 2020; 9(12):1292. doi:10.3390/antiox9121292
31. Hielscher A, Gerecht S. Hypoxia and free radicals: role in tumor progression and the use of engineering-based platforms to address these relationships. Free Radic Biol Med. 2015;79:281-91. doi:10.1016/j.freeradbiomed.2014.09.015
32. Maidannyk VG, Burlaka IA. Management of Hypoxia-induced Disorders in Nephrotic Children. J Cytol Histol. 2015;6:6. doi:10.4172/2157-7099.1000362
33. Guo D, Zhang B, Liu S, Jin M. Xanthohumol induces apoptosis via caspase activation, regulation of Bcl-2, and inhibition of PI3K/Akt/mTOR-kinase in human gastric cancer cells. Biomed Pharmacother. 2018;106:1300-6. doi:10.1016/j.biopha.2018.06.166
34. Chang SK, Soo WK. Vitamin D and chronic kidney disease. Korean J Int Med. 2014;29(4):416-27. doi:10.3904/kjim.2014.29.4.416