Alterations in Circulating Levels of Vitamin D Binding Protein, Total and Bioavailability of Vitamin D in Diabetes Retinopathy Patients

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Abstract

Aims

The aims of this study were to investigate the association between circulating levels of vitamin D binding protein (VDBP) and its genotypes and diabetic retinopathy risk.

Methods

In this study, 154 patients with type 2 diabetes mellitus; 62 with diabetic retinopathy (DR) and 92 without DR/and or diabetic nephropathy (DN), were recruited. Circulating levels of 25-hydroxyvitamin D3 and VDBP levels were measured in the patients. The genotype and phenotype of VDBP were evaluated based on two common VDBP variations; rs7041 and rs4588.

Results

Serum levels of VDBP were significantly lower in patients with DR than in patients without DR/and or DN (Ln-VDBP ($\mu\text{g/ml}$): 6.14 ± 0.92 vs. 6.73 ± 1.45 , $p = 0.001$) even after adjustment for age, sex, body mass index, disease duration, eGFR, HbA1C, and serum levels of 25(OH)D. The distribution of VDBP phenotypes and genotypes in the two studied groups were nearly the same, and the distribution was similar to that of the general population.

Conclusions

In this study, we found out the association between lower levels of VDBP and risk of DR. However, the precise mechanism linking these two remains unknown. Further and more in-depth research to find out the underlying causes of the relationship is needed.

Introduction

Diabetes retinopathy (DR) is one of the most common etiologies for visual loss in people between the ages of 20-64 [1]. It is the first etiology of preventable blindness in the working-age (20-45) population in developed countries, and the estimated prevalence of diabetic retinopathy is 18.45% [2–5]. Poor glycemic control and disease duration are the main important risk factors of DR. However, abnormal metabolic environmental factors like vitamin D deficiency increase the risk of developing diabetic retinopathy [6–11]. Vitamin D may have a role in the pathogenic mechanisms predisposing to diabetes complications through dysregulating in glucose homeostasis, modulating insulin resistance, and/or pancreatic β -cell function [12, 13].

A meta-analysis concluded that there is not enough evidence supporting the efficacy of vitamin D supplementation in complications of type 2 diabetes[14]. The study reported that vitamin D supplementation has no proved effect on fasting glucose levels, impaired glucose tolerance, improving insulin resistance and glycemic control in diabetic patients [14]. On the other hand it has been reported

that moderate to high doses of vitamin D may reduce the risk of cardiovascular disease [15]. Indeed using vitamin D supplementation may reduce the risk of progression from prediabetes to diabetes [16]. These discrepancies may be caused by vitamin D binding protein (VDBP) dysregulation in diabetes patients.

VDBP, the key determinant of vitamin D, can efficiently affect the sustainability, biocompatibility, and biological performance of vitamin D. Vitamin D and its metabolites should pass the cell membrane in VDBP complex form to have their function and attach to the nuclear vitamin D receptor [17]. VDBP (P02774), is a polymorphic protein from the albumin family, which is known as the main carrier of active metabolites of vitamin D (85–90%) [18, 19]. It is synthesized in the liver and its concentration is stable during life comparatively. However, liver failure, proteinuria, and malnutrition lead to lower circulating levels of VDBP [20, 21]. VDBP has a high affinity to the principal vitamin D metabolites; around 85% of circulating 25(OH) D and 1, 25(OH) 2D carries via binding to VDBP with high affinity as approximately 15% are connected to albumin and less than 0.4% of them are free [22, 23]. VDBP variations could regulate the bioavailability of 25(OH)D by presenting different phenotypes and circulating levels of total VDBP [24]. These findings provide novel insight into metabolic differences that may underlie racial variation of VDBP in the association between 25(OH)D and diabetes and microvascular complications. As a single marker, the total 25(OH)D level is not sufficient to accurately evaluate vitamin D status [25]. In cases where circulation of VDBP may be altered, VDBP measurements and bioavailable 25(OH)D calculations may help to determine vitamin D status accurately[25].

The purposes of our study were to explore the association of serum VDBP and bioavailable 25(OH)D with diabetic retinopathy and to determine the risk association of VDBP polymorphisms and diabetic retinopathy.

Subjects, Materials And Methods

Study population

The study population, the inclusion and exclusion criteria, and retinopathy and nephropathy diagnosis criteria have been described previously[26, 27] and it was summarized as follow:

Patients with the diagnosis of T2DM from a referral diabetes clinic affiliated with Tehran University of medical sciences (Tehran, Iran) were recruited in this case-control study; 62 of them had retinopathy and 92 of them didn't have retinopathy or nephropathy (control group).

Biochemical measurements

Patients had overnight fasting for 10-14 hours before taking blood samples. The separated sera of the patients were kept at -80°C before the analysis. The serum levels of fasting serum glucose, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol, triglycerides (TG), blood urea nitrogen (BUN), creatinine (Cr) uric-acid, and albumin were measured by an

enzymatic colorimetric assay [Pars-Asmun kits, Iran] using an auto-analyzer (Hitachi 902, Japan) with intra-assay precision of 0.63–1.23% and intraassay precision of 1.09–1.8%. Urine microalbumin and creatinine levels were measured by the same auto analyzer (Hitachi 902, Japan).

Estimating glomerular filtration rate (eGFR) was calculated based on CKD EPI Equation; for Cr > 61.9 $\mu\text{mol/l}$ [if female]: $\text{GFR} = 144 \times (\text{Scr}/61.9)^{-1.209} \times (0.993)^{\text{Age}}$, for Cr > 79.6 $\mu\text{mol/l}$ [if male] $\text{GFR} = 141 \times (\text{Scr}/79.6)^{-1.209} \times (0.993)^{\text{Age}}$. In our population study, serum Cr levels were over 61.9 $\mu\text{mol/l}$ in women and over 79.6 $\mu\text{mol/l}$ in men. [28].

Ion exchange chromatography with a DS5 set (DREW, UK) was used for measuring glycated hemoglobin (HbA1c) levels. To assess the glycemic control status in the patients, the mean HbA1c > 7% was considered as poorly controlled diabetes [29].

Serum 25(OH)D was measured by radioimmunoassay using a Biosource kit (Biosource Europe SA, Belgium); intra- and inter-assay coefficients of variation (CV) were 5.2% and 7.5%, respectively. P1NP was measured by quantitative sandwich enzyme immunoassay technique (intra- and inter-assay CV was <8% and <10%) (Cusabio). Parathyroid hormone (PTH) was measured by quantitative two-step sandwich immunoassay using Chemiluminescent Microparticle Immuno technique (Architect intact PTH, 8K25, Germany) with a sensitivity of $\leq 5\text{pg/ml}$ at a total CV of 20% and cross-reactivity of ≤ 0.01 .

Polyclonal ELIZA kit (Mybiosource, MBS564062) was used to measure VDBP serum levels according to the manufacturer's instructions; inter-assay precision: CV < 10% and intra-assay precision: CV < 8%. To estimate vitamin D bioavailability, we used the equation "[Bio D] = [DFree] + [DAlb] = (Kalb · [Alb] + 1) · [DFree]". The vitamin D bioavailability was calculated based on serum levels of vitamin D, VDBP and albumin [30].

Genotyping and phenotyping

The extraction of DNA, the genotyping and phenotyping of VDBP have been described in our previous study [31].

The polymorphisms of VDBP in exon 11 were determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis (RFLP); rs7041 at codon 416 (Asp→Glu) and rs4588 at codon 420 (Thr→Lys). The analysis of RFLP shows that rs4588 genotype marked as CC (Thr/ Thr) (809 bp), AA (Lys/Lys) (584 and 225 bp), and AC (Lys/Thr) (584, 225, and 809 bp); and rs7041 genotype marked as GG (Glu/Glu) (577 and 232 bp), TT (Asp/Asp) (nondigested bond at 809), and TG (Asp/Glu) (577, 232, and 809 bp). We sequenced 10% of the PCR samples directly to confirm the result of PCR-RFLP.

Three common variants of VDBP have been recognized regarding the VDBP genotypes, considering the fact that humans do not have the combination of rs4588 (T) and rs7041(C): Gc1F (rs7041 (A) and rs4588 (G)), Gc1S (Gc1S = rs7041(C) and rs4588 (G)), and Gc2 (Gc2 = rs7041 (A) and rs4588 (T)). Six phenotypes can be determined considering the genotypes: 1 s/1 s, 1 s/1f, 1 s/2, 1f/1f, 1f/2, and 2/2.

Statistical analysis

The data were analyzed by SPSS statistical software (version 20). Data were expressed as number (%) for categorical values and mean $\pm$ standard deviation for continuous variables. Data normality was analyzed by the Shapiro–Wilk test. Circulating levels of VDBP, PTH, and BUN had no normal distribution, so a natural Log transformation was applied to correct their normality distribution. Thereafter, the comparisons between the study groups were performed using the Student's t-test and Chi-square test for continuous variables and categorical variables (control as a reference group), respectively. Vitamin D bioavailability did not have a normal distribution. The data were showed median and interquartile range and Mann-Whiney U test was used to analyze the differences in the vitamin D bioavailability in the case and control groups.

The Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) tests of SNP were also assessed. Notably, the participants' demographics and clinical measures were reported using descriptive statistics. The genotype and allele frequencies as well as phenotype analysis were performed by Chi-square and Fisher exact tests, respectively.

To examine the association between VDBP and DR risk, multivariable logistic regression models were used. Adjusting covariates were selected based on the parameters associated with DR, including age, sex, body mass index (BMI), poor control of glycemia, kidney dysfunction, and the disease duration. If P-value was less than 0.2 (P-value <0.2), it was adjusted in multivariable logistic regression analyses. All tests were two-sided, and a P-value less than 0.05 was defined as statistically significant.

Results

Baseline characteristics

A total number of 154 diabetic patients participated in this case-control study; 62 patients were diagnosed with diabetic retinopathy and 92 of them had no distinguished DR and/or DN (control group). The age and sex ratio were nearly the same in the two groups, but DR group patients were older and disease duration was longer in them. More details on the characteristics of the subjects are available in Table 1. There were no significant differences in circulating levels of FBS, TG, LDL, and TC. Patients with DR had significantly lower levels of HDL even after adjusting for age, sex, and disease duration (p=0.001). In the course of diabetes poor control (HbA1c>7%), both groups were nearly the same; %60.9 in the DR group vs %69.4 in the control group (p-value=0.3).

Table 1

Demographic characteristic and biochemical analysis in diabetic mellitus without retinopathy diabetic mellitus with retinopathy

Demographic characteristic	Diabetic mellitus with retinopathy (N=62)	Diabetic mellitus without retinopathy and/or nephropathy (N=92)	p-value
Age (years)†	59.6±6.0	55.0±6.0	0.001
Sex (Women)‡	27 (43.5%)	53 (57.6%)	0.16
BMI (kg/m ²)†	28.9±4.6	29.0±5.6	0.86
Disease duration (year)	16.5±6.7	10.1±6.2	0.00
Laboratory outcomes			
FBS (mg/dL)	150.1±64.4	143.6±46.2	0.45
Ln BUN (mg/dL)	3.1±0.4	2.7±0.3	0.001
Uric acid (mg/dL)	5.62±1.57	5.10±1.33	0.02
TG (mg/dL)	133.42±68.05	157.82±94.17	0.05
TC (mg/dL)	155.67±40.71	152.82±36.76	0.64
HDL (mg/dL)	42.19±10.13	49.00±10.33	0.001
LDL (mg/dL)	86.25±25.91	81.56±23.82	0.23
eGFR (mL/min)	69.80±30.10	92.69±27.11	0.001
Cr (mg/dL)	1.5±0.9	1.1±0.5	0.001
Serum albumin (g/dL)	5.07±0.55	5.26±0.96	0.12
Urine micro albumin (mg/L)	184.11±45.87*	8.36±0.97*	0.001
25(OH)D (ng/mL)	18.5±11.6	21.4±10.2	0.08
Ln PTH (pg/mL)	3.8±0.6	3.5±0.4	0.01
Abbreviations: BMI; body mass index, BUN; blood urea nitrogen, Cr; creatinine, eGFR; Estimating glomerular filtration rate, FBS; fasting blood serum, HDL; high-density lipoprotein, LDL; low-density lipoprotein, Ln; natural logarithm, PTH; parathyroid hormone			
†mean±SD			

Regarding kidney function, circulating levels of BUN, uric acid, and Cr and urine microalbumin levels were significantly higher in the DR patients. Consequently, the eGFR levels were significantly lower in DR patients compared to diabetic patients without microvascular complications.

Serum levels of vitamin D, vitamin D binding protein, and bioavailability of vitamin D

In the total study population, the mean serum levels of vitamin D were 20.9 ± 9.6 ng/ml and only 19% of patients were vitamin D sufficiency (>30 ng/ml).

In the course of DR, the prevalence of vitamin D deficiency was as similar as in the control group; 82.9% vs. 77.8%, respectively, while PTH was significantly higher in the DR group.

The serum levels of VDBP were significantly lower in the DR group (Ln- VDBP (μ g/ml): 6.14 ± 0.92 in DR group vs 6.76 ± 1.51 in the control group, $p = 0.002$). As DR patients were older and had longer duration of diabetes, and lower eGFR and serum levels of vitamin D, the logistic regression model was used to minimize their effects. After adjusting age, sex, eGFR, vitamin D levels, and disease duration, there was an independent association between lower levels of VDBP and DR risk (Beta = 0.7, 95% CI lower-upper: 0.2–0.9, $p = 0.03$).

In the subgroup analysis of the DR group, the VDBP levels were similar in patients with proliferative and non-proliferative diabetic retinopathy ($p=0.9$) (Fig. 1).

There was a decreasing trend in circulating levels of VDBP in relation to retinopathy progression; (LnVDBP (ug/ml): with PDR, 6.1 ± 0.8 ; NPDR, 6.2 ± 0.8 ; and without PDR or NPDR, 6.7 ± 1.5 ($p=0.01$)). In the post hoc test, there was no significant difference in the circulating levels of VDBP between the PDR and NPDR subgroups ($p=0.7$) (Fig. 1).

The estimation of vitamin D bioavailability showed higher rates in the DR group than in the control group (median (IQR): 1.02 (1.75) ng/ml vs. 0.44 (1.29), $p=0.03$). There was no significant constant association between vitamin D bioavailability and DR after adjusting for confounder factors including, age, sex, eGFR, and disease duration ($p=0.11$).

Polymorphisms in the vitamin D-binding protein gene

Genotyping was performed in 81 diabetic patients without retinopathy and 59 diabetic patients with retinopathy for rs4588 and rs7041 of the VDBP gene. Table 2 presents the distribution of genotype, allele, and phenotype frequency in the DR and control groups. The genotypes based on both SNPs were consistent with Hardy-Weinberg equilibrium in the DR group ($p = 0.96$ for rs4588, $p = 0.16$ for rs7041) and control ($p = 0.33$ for rs4588, $p = 0.14$ for rs7041) group.

Table 2

Genotype, allele frequency and phenotype of Vitamin D-Binding Protein Gene in diabetes retinopathy patients and control groups

	Diabetic mellitus with retinopathy (N=59)	Diabetic mellitus without retinopathy and/or nephropathy (N=81)	P-value
Genotype-rs4588			
CC (Thr/Thr)	54.2%(32)	64.2%(52)	0.48
AA (Lys/Lys)	6.8%(4)	6.2%(5)	
AC (Lys/Thr)	39.0%(23)	29.6%(24)	
Genotype-rs7041			
TT (Asp/Asp)	8.5%(5)	8.6%(7)	0.99
GG (Glu/Glu)	37.3%(22)	38.3%(31)	
TG (Asp/Glu)	54.2%(32)	53.1%(43)	
allele Freq. rs4588			
C	0.74	0.75	0.3
A	0.26	0.25	
allele Freq.rs7041			
T	0.29	0.29	0.3
G	0.71	0.71	
GC phenotype			
Gc1f, Gc1f	1.2% (1)	1.7%(1)	0.7*
Gc1s, Gc1s	38.3%(31)	37.3%(22)	
Gc2, Gc2	6.2%(5)	6.8%(4)	
GC1f,GC1s	24.7%(20)	15.3%(9)	

*fisher's exact test

	Diabetic mellitus with retinopathy (N=59)	Diabetic mellitus without retinopathy and/or nephropathy (N=81)	P-value
GC1f,GC2	2.5%(2)	1.7(1)	
GC1s,GC2	27.2%(22)	37.3%(22)	
*fisher's exact test			

In both groups, the minor-allele frequency for SNP rs4588 was “A” and the dominant genotype was recognized “CC”. In both groups, the minor-allele frequency for SNP rs7041 was T and the dominant genotype was recognized as the heterozygous TG. The frequencies of the genotypes according to both SNPs rs4588 and rs7041 were no different significantly between the two groups; DR and control ($P > 0.05$). There was no significant difference in the frequency of minor-alleles of both SNPs rs4588 and rs7041 between the two studied groups ($P > 0.05$). Table 2 shows the distribution of the 6 possible haplotypes according to the 2 variants in both groups. The phenotype GC2-GC2 frequency was not significantly different between the DR and control groups (6.8% in the DR group and 6.2% in the control group).

Discussion

In this study, we considered serum levels of vitamin D, VDBP and vitamin D bioavailability in patients with DR. Our data showed that serum VDBP levels were lower in DR patients than those without DR and/or DN.

There are a few studies that considered circulating levels of VDBP in diabetes and its complications. In this line of research, Fawzy and Alsel assessed the serum and urine levels of VDBP in diabetes patients. They reported a significantly increased level of urine VDBP with the degree of albuminuria. Also, the authors showed that serum VDBP was also higher in patients with microalbuminuria or macroalbuminuria compared to patients with normal levels of urine albumin [32]. Another study conducted by Tian and et al. presented elevated urine VDBP levels as a biomarker for DN [33]. Elevated levels of urine VDBP are identified in diabetes patients and are likely associated with tubule-interstitial dysfunction in patients with nephropathy [34–36]. In the normal kidney, to prevent loss of vitamin D in the urine, VDBP bounds to 25OHD and other vitamin D metabolites to reabsorb them.

In the current study, we measured serum levels of VDBP that showed a negative correlation with microalbuminuria in both groups. In our study population, DR patients were older and had longer duration of diabetes and lower eGFR compared with diabetes patients without DR. Consequently, DR patients with tubular dysfunction lose more VDBP through urine and have lower circulating levels of VDBP. Grymonprez et al. showed that there is a negative correlation between serum VDBP levels and urine VDBP levels [37]. Obviously, tubular dysfunction should be the main reason for lower serum VDBP levels in diabetes patients.

One of the molecular mechanisms that explain the lower serum VDBP levels in DR patients with tubular dysfunction is megalin/Cubilin receptors. Megalin is a big protein receptor in the membrane that belongs to low-density lipoprotein receptors [38] and is expressed in the epithelial cells of the proximal tubule in the kidney [39]. Cubilin is known as a co-receptor of megalin [40] and it binds and internalizes many small proteins, including VDBP, retinol-binding protein, and transcobalamin [38]. The VDBP bounded to 25-OH vitamin D transports freely through the glomerulus to the proximal tubules and then the complex is reabsorbed by megalin and its co-receptor [41]. The reabsorption by megalin facilitates 1 α -hydroxylase activity in the epithelial cells of the proximal tubule to produce 1, 25-dihydroxyvitamin D [42, 43]. A previous study by Thrailkill et al. showed that urinary excretion of megalin is enhanced in patients with microalbuminuria due to diabetes type 1 [44]. *In vivo* studies reported vitamin D deficiency, enhanced urinary excretion of VDBP in mouse models [43, 45]. Enhanced urinary excretion of VDBP and 25(OH)D, and also decreased expression of megalin in the kidneys have been reported in animal models with diabetes [46, 47]. There is a hypothesis suggesting that lower levels of serum VDBP can be secondary to megalin deficiency in diabetic patients with tubular dysfunction [48].

Megalin is also expressed in other tissues including the lung, uterus, and eye [49]. It is detected in the outermost layer of the retina and ciliary body of the eye [50]. The role of megalin in the development of the normal eye seems to be essential, but the details of the process have remained unknown to date. Our data also showed that the association between serum VDBP and DR was independent of vitamin D levels and eGFR. It is likely; the lower levels of serum VDBP in DR patients may have a pathological role in developing DR by megalin function in retinal cells.

Another mechanism that could explain the possible role of serum VDBP in developing DR is PTH imbalance due to vitamin D deficiency. Growing evidence shows that low concentrations of 1, 25-dihydroxyvitamin D are correlated with the risk of DR and other diabetes complications [51, 52]. However, the marked deficiency in our population may have confounded the study. In our study, the prevalence of vitamin deficiency in DR patients was as similar as in diabetic patients without DR and/or DN and in both groups over 70% patients were deficient/insufficient (25(OH)D <30 ng/mL). Although the serum levels of vitamin D were similar in the two groups, serum PTH levels were higher in patients with DR than those without retinopathy, which is in agreement with the results of other studies [53, 54]. The higher levels of PTH as a compensatory mechanism of vitamin D deficiency in diabetes patients can stimulate inflammatory cytokines that have a role in the pathogenesis of DR [53, 55].

Regarding vitamin D bioavailability, our data showed higher rates in the DR group than in the control group, but the association disappeared after adjusting for confounder factors including, age, sex, eGFR, and disease duration. As expected, lower VDBP concentrations led to lower VDBP-bound 25(OH) D levels and higher vitamin D bioavailability among the DR patients. The longer disease duration and tubular dysfunction in DR patients are likely to impair 1,25-dihydroxyvitamin D reabsorption in the kidney.

Although VDBP is known as a vitamin D carrier, only 5% of its function is allocated to transporting vitamin D metabolites to its target cells [56]. VDBP binds to actin and it can depolymerize polymeric actin

[57]. Monomeric actin easily turns into polymeric form in the plasma and results in clogging of the microvascular system, such as retinal microvessels. VDBP prevents polymerization and the subsequent clogging [58]. It is worth mentioning that in our data, the DR patients were older and had higher disease duration. By modeling, we tried to minimize the effect of the main confounding factors. However, after adjusting for eGFR, age, sex, and the disease duration, there was an independent association between lower circulating VDBP levels and DR. Indeed, there was a decreasing trend in circulating levels of VDBP in terms of progressing retinopathy; PDR and NPDR, but not statistically significant.

It points out a new concern that circulating levels of VDBP have a causality role in the development of retinopathy. One of the important functional roles of VDBP is activating macrophages [59]. Macrophage activation may be associated with DR by several mechanisms. Macrophage adhesion to the capillary endothelium can result in capillary occlusion and finally retinal ischemia [60, 61] by stimulation of cytokines [62–67]. TNF- α is a cytokine produced by macrophages; it has an important role in DR pathogenesis [62]. It increases the permeability of retinal endothelium through downregulation of tight junction proteins' expression. Increased endothelial permeability may result in rupture of the blood-retinal barrier [63]. TNF- α is also a chemoattractant of leukocytes, which can also stimulate leukocyte adhesion and cause oxidation and produce reactive oxygen species as a result of optic nerve degeneration and retinal ganglion cells death [64, 65]. TNF- α levels have been increased in animal models of proliferative retinopathy due to retinal neovascularization [65]. IL-1 β is another cytokine mainly synthesized by macrophages. It can induce angiogenesis and ocular neovascularization [66]. The role of IL-1 β in DR has been demonstrated in diabetic mouse models [67]. Taken together, macrophage activation may mediate the association of VDBP with DR.

VDBP genetic variations were also considered in metabolic disorders like diabetes [68–71]. The VDBP gene is located on chromosome 4q12; which is a region that seems to be associated with plasma glucose and insulin concentrations in Pima Indians [12]. In this study, we have also investigated the two common missense VDBP polymorphisms, named rs7041 and rs8455. Based on the two SNPs, three major alleles have been recognized for the VDBP gene (GC1F, GC1S, GC2) [72]. The difference between GC1 and GC2 is due to alterations of four amino acids; 152 Gly / Glu, 311 Glu / Arg, 416 Asp / Glu, and 420 Arg / Thr [73]. The difference between GC1S and GC1F is for the substitution of aspartic acid by glutamic acid at position 416 [74]. There is controversy about the correlation between VDBP genotype polymorphisms and susceptibility to type 2 diabetes mellitus. Some studies have suggested a moderate correlation in Asians but no correlation in Caucasians [68, 69]. Szathmary et al. reported that homozygotes for Gc 1F-1F had lower fasting insulin among other phenotypes [70]. However, Wei-Zhen et al. have reported that the distribution of genotypes according to both codons and phenotypes was approximately similar in healthy controls and diabetic patients [71]. The results of genotyping and phenotyping of VDBP showed no difference between the two studied groups. The distribution of phenotypes and genotypes according to the variants of the two determined SNPs (rs4588, rs7041) in both groups was the same with the general population. Our findings are in line with the results of previous studies that have shown no correlation between the variants of these two codons with the risk of DR.

However, our study had some limitations. A case-control study could not show that variation in the circulating levels of VDBP is a consequence or its underlying cause of retinal or kidney damage in those patients. Therefore, further longitudinal studies will be conducted to investigate the VDBP roles in diabetic patients during the progression of DR.

Also, we did not measure urine VDBP levels and we could not conclude the exact association between microalbuminuria and urine VDBP concentration. It can be very useful for determining the role of VDBP and the more definite reason behind these findings.

Taken together, the results of this study could be beneficial in further identifying the role of VDBP in diabetic retinopathy. VDBP is a small protein from the albuminoid gene family, and its size is smaller than albumin and lost more by urinary excretion due to tubular dysfunction. Consequently, in future studies, it is possible to consider VDBP as a bio-marker for progressing microvascular complications.

Conclusion

We can conclude from the findings of this study that VDBP phenotypes and genotypes based on the rs4588 and rs7041 codons are not correlated with diabetic retinopathy. VDBP levels are lower in diabetic patients with retinopathy, which may be a result of urinary excretion of VDBP due to tubular dysfunction in diabetic patients. There is another possibility that lower levels of VDBP result in the progression of diabetic retinopathy by clogging retinal microvessels. Further investigations are needed to understand the mechanism relating VDBP to diabetic retinopathy.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. The experimental protocol was approved by Ethical Committee of the National Institute for Medical Research Development of Iran. The informed consent to participate in the study was obtained from all the study participants.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the European Variation Archive (<https://www.ebi.ac.uk/eva/?Submit-Data>) repository, with accession number # 10743821.

Competing interests

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Contributions

ZM was responsible for designing the review protocol and screening potentially eligible studies. She contributed to extracting and analyzing data, interpreting results and creating 'Summary of findings' tables.

SE conducted the writing the report, arbitrating potentially eligible studies, extracting and analyzing data and interpreting results.

AAM contributed to data extraction and provided feedback on the report.

AS was responsible for designing the review protocol, writing the protocol and report, conducting the search.

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Figures

Figure 1

Comparison of VDBP levels between proliferative retinopathy, non-proliferative diabetic retinopathy, and no retinopathy in diabetic patients