Original Article

A Pilot Study of 25-Hydroxy Vitamin D in Egyptian Diabetic Patients with Diabetic Retinopathy.

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Abstract:

Background: Many cellular, preclinical and observational studies support a role of vitamin D in the pathogenesis of type 2 diabetes. Vitamin D is suggested to be an inhibitor of angiogenesis. A growing body of evidence suggests an association between vitamin D inadequacy and diabetic microvascular complications e.g. diabetic nephropathy but little data are available about the association with diabetic retinopathy (DR). **Objective:** To explore a hypothesized association between 25 (OH) vitamin D level and DR in patients with type 2 diabetes mellitus and to evaluate for the presence of any relation between 25 (OH) vitamin D level and different stages of DR. **Research Design and Methods:** This pilot study was conducted on 50 type 2 diabetic patients divided into two groups: group (I) including 25 patients with diabetic retinopathy and group (II) including 25 patients without diabetic retinopathy. 50 healthy volunteers matched by age and sex were selected as a control group. The patients were selected from Ain Shams University Hospital from April to June 2010. Fasting blood sugar, HbA1c, renal functions, liver functions, lipid profile, serum calcium, serum phosphorous, intact parathyroid hormone (iPTH) and serum 25 hydroxyvitamin D_3(25(OH)D_3) levels were done to all participants in the study. A complete ophthalmic and fundus examination was done for all patients. **Results:** Mean 25(OH) vitamin D level was lower in type 2 diabetic cases than in control group (21.48±5.24 ng/ml vs. 45.79±11.08ng/ml) (P<0.01). Mean 25(OH) vitamin D level was lower in type 2 diabetic cases with DR than type 2 diabetic cases without DR (18.78±4.62 ng/ml vs. 24.17±5.80 ng/ml) (P<0.01). Patients with PDR have the lowest mean 25(OH) vitamin D level compared to patients with moderate NPDR and severe NPDR (13.53±3.36 ng/ml vs. 21.57±5.38 ng/ml and 16.20±4.00 ng/ml) (P<0.01). 25(OH) vitamin D level was inversely correlated with age, duration of type 2 diabetes mellitus, degree of microalbuminuria, fundus findings, BMI, SBP, DBP, glycemic parameters (including FBS and HbA_1c%), urinary ACR, total cholesterol, triglycerides, LDL-C (P<0.01) and iPTH level (P<0.05). 25(OH) vitamin D level was positively correlated with GFR, HDL-C and total calcium level (P<0.01) among all studied groups. **Conclusions:** There was a significant reduction of 25 (OH) vitamin D level in patients with type 2 diabetes and more so in patients with DR. Moreover, this study demonstrated a negative correlation between 25 (OH) vitamin D level and severity of diabetic retinopathy among patients with type 2 diabetes mellitus. These findings reflect a possible causality relationship between vitamin D deficiency and the development and progression of diabetic retinopathy. We recommend to validate our results in larger trials and to study the effect of vitamin D supplements on the prevention of DR.

Keywords: 25-Hydroxy Vit D - Diabetes - Retinopathy.

Introduction:

Diabetic retinopathy is a highly specific vascular complication and a sight-threatening problem related to diabetes. It is characterized by gradually progressive alterations in the retinal microvasculature, leading to retinal nonperfusion, increased vascular permeability and pathologically intraocular proliferation of retinal vessels[(1)].

The cause of complications in the diabetic state has been a subject of intense research for over half of a century. However, two major clinical trials, DCCT and UKPDS, established the relationship of poor glycemic control to diabetic retinopathy[(2,3)]. The precise relationship of other factors to diabetic complications is still not clear.

Abbreviations: 25(OH) D_3, 25(OH) vitamin D_3; DR, diabetic retinopathy; FBS, fasting blood sugar; GFR, glomerular filtration rate; HbA_1c%, glycated hemoglobin; HDL-C, high density lipoprotein cholesterol; iPTH, intact parathyroid hormone; LDL-C, low density lipoprotein cholesterol; NPDR, non proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; urinary ACR, urinary albumin creatinine ratio.

Vitamin D is now recognized as an important prohormone in health and disease[4]. Recently, vitamin D has sparked widespread interest in the pathogenesis and prevention of diabetes[5]. Diaz and his colleagues[6] demonstrated an association between vitamin D deficiency and vitamin D insufficiency with nephropathy in individuals with diabetes even after controlling for factors such as race/ethnicity, presence of hypertension and use of ACE inhibitors or ARBs. The high prevalence of vitamin D deficiency and vitamin D insufficiency in individuals with diabetes suggest that studies to further describe the role of vitamin D as a possible risk marker or risk factor in diabetic nephropathy are needed to evaluate the impact of maintaining an adequate level of vitamin D on the progression of diabetic nephropathy.

Taverna and his colleagues[7] demonstrated that the vitamin D receptor (VDR) is extensively expressed in retina. However, little data are available about the association with DR. The aim of the present study was to examine the potential relationship between serum 25 (OH) vitamin D level and DR and to evaluate for the presence of any relation with different stages of DR.

Material and Methods:

Study Design

This study comprised 100 subjects. 50 type 2 diabetic patients divided into two groups: group (I) including 25 patients with diabetic retinopathy and group (II) including 25 patients without diabetic retinopathy. 50 healthy volunteers matched by age and sex were selected as a control (group III). The patients were selected from Ain Shams University Hospital from April to June 2010. This study was approved by the Ethical Committee of Ain Shams University. An informed detailed consent was taken from all subjects before the start of the study.

Methodology:

Calculation of BMI and measurement of arterial blood pressure were performed for all subjects. The estimated glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease formula (MDRD). Complete ophthalmic examination and fundus examination were done for all patients. Based on their ophthalmic findings, the type 2 diabetic patients were divided into two groups: group (I) including 25 patients with DR and group (II) including 25 patients without DR. Fluorescein angiography was done for patients of group (I) and accordingly the patients were further classified into 3 subgroups: moderate nonproliferative diabetic retinopathy (moderate NPDR), severe nonproliferative diabetic retinopathy (severe NPDR) and proliferative diabetic retinopathy (PDR). Each subgroup is then divided according to presence or absence of clinically significant macular edema (CSME). When the diabetic retinopathy was asymmetric, the subject was assigned to the group corresponding to the eye with the worse retinopathy findings.

Biochemical Measurements:

Subjects were asked to faste for 8-12 hours over night. Venous blood sample was withdrawn for analyzing the fasting blood sugar, serum creatinine, serum calcium, serum phosphorus, AST, ALT, serum albumin, prothrombin time, HbA1c, lipid profile, 25 (OH) vitamin D and iPTH. Serum 25 (OH) vitamin D level was measured by using Immundiagnostik direct ELISA kit[8]. Levels of serum 25(OH) vitamin D_3 >30 ng/ml were sufficient, levels from 12 ng/ml to 30 ng/ml were insufficient, while levels <12 ng/ml were deficient[9,10]. iPTH was measured by using Calbiotech iPTH direct ELISA kit[11]. A morning urine sample was collected from each subject for complete urine analysis and measurement of urinary albumin/creatinine ratio. Values of urinary albumin/creatinine ratio between 30-300µg/mg and over 300µg/mg in spot urine were considered as microalbuminuria or macro albuminuria respectively.[12]

Exclusion Criteria:

Exclusion criteria included type 1 diabetic patients, type 2 diabetic patients who are receiving vitamin D or VDR agonist or calcium supplements or any medications that may affect vitamin D level (as antiseizures, glucocorticoids, orlistat, ketoconazole or cholestyramine), pregnant women, smokers, patients with acute hyperglycemic states, patients with chronic liver disease, patients with serum creatinine >1.5 mg/dl in male or >1.4 mg/dl in female, patients with GFR < 90 ml / min/1.73 m^2 and cancer patients. Information on age, duration of type 2 diabetes, medical history and drug history was obtained from patients.

Statistical Analysis:

All data processing and statistical analysis were done with SPSS version 15. Description of data was in the form of mean ± standard deviation for quantitative variables and percentage for qualitative variables. Student's t-test, ANOVA test and Chi-square (X^2) tests were used to compare variables between groups. Correlation-coefficient test (r-test) was used to rank different variables against each other either positively or negatively. The significance of the results was assessed in the form of P-value differentiated into: non-significant when P-value >0.05, significant when P-value ≤0.05 and highly significant when P-value ≤ 0.01.

Results:

Comparison of different variables between the studied groups (Table 1) revealed that there is no statistical difference between studied groups as regard age, body mass index, serum creatinine, glomerular filtration rate, total calcium or serum phosphorous levels. Type 2 diabetic patients with diabetic retinopathy had longer duration of the disease than those without retinopathy with a statistically significant difference (P<0.05). there was a statistically highly significant difference between the studied groups as regard systolic and diastolic blood pressures, parameters of glycemic control including fasting blood sugar and HbA1C%, urinary ACR, total cholesterol, triglycerides, LDL-C and iPTH being higher in type 2 diabetic patients without DR than control group and the highest in type 2 diabetic patients with DR. Additionally, HDL-C was lower in type 2 diabetic patients without DR than control group and the lowest in type 2 diabetic patients with DR with a highly significant statistical difference. 25(OH) vitamin D level was lower in type 2 diabetic cases with DR (n = 25) than type 2 diabetic cases without DR (n = 25) (18.78±4.62 ng/ml versus 24.17±5.80 ng/ml) (P<0.01).

Correlation study of 25(OH) vitamin D with different variables (Table 2) revealed that 25(OH) vitamin D level was inversely correlated with age, duration of type 2 diabetes mellitus, stages of diabetic nephropathy, fundus findings, BMI, SBP, DBP, glycemic parameters, urinary ACR, total cholesterol, triglycerides, LDL-C (P<0.01) and iPTH level (P<0.05) among type 2 diabetics. 25(OH) vitamin D level was positively correlated with GFR, HDL-C and total calcium level (P<0.01) among all studied groups.

Comparison of different variables between different grades of DR (Table 3) revealed that patients with PDR have the highest mean serum creatinine (0.86±0.11mg/dl), urinary ACR (136.27±25.73 µg/mg) and iPTH (60.43 ± 15.08 pg/ml) and the lowest mean glomerular filtration rate (93.67±7.55 ml/min/ 1.73m^2) (P<0.05). Patients with PDR have the lowest mean 25(OH) vitamin D level compared to patients with moderate NPDR and severe NPDR (13.53±3.36 ng/ml versus 21.57±5.38 ng/ml and 16.20±4.00 ng/ml respectively) (P<0.01) (Table 3). Type 2 diabetic patients with DR associated with CSME had a lower mean serum 25(OH) vitamin D_3 level compared to those without CSME (16.60±3.86 ng/ml versus 22.67±5.06 ng/ml) (P<0.01).

Table (1): Descriptive data and comparison of different variables between the studied groups

	Group I (type 2 diabetics with diabetic retinopathy) (N=25) Mean±SD	Group II (type 2 diabetics without diabetic retinopathy) (N=25) Mean±SD	Group III (control) (N=50) Mean ±SD	P
Age (years)	53.80 ± 8.67	53.08 ± 7.77	51.60 ± 6.85	>0.05
Duration of type 2 diabetes mellitus (years)	17.96 ± 4.08	15.44 ± 3.54	-	<0.05*
Body mass index (Kg/m²)	28.85 ± 3.90	27.93 ± 3.89	27.02 ± 3.06	>0.05
Systolic blood pressure (mmHg)	140.60 ± 13.10	131.40 ± 15.11	121.30 ± 5.23	<0.01**
Diastolic blood pressure (mmHg)	89.60±7.90	81.98 ± 4.68	79.70 ± 4.09	<0.01**
Fasting blood sugar (mg/dl)	252.60 ± 50.50	210.08 ± 51.40	83.18 ± 10.59	<0.01**
Glycated hemoglobin (HbA1c %)	10.01 ± 1.82	8.24 ± 1.76	4.82±0.47	<0.01**
Serum creatinine (mg/dl)	0.80 ± 0.08	0.85 ± 0.14	0.80 ± 0.06	>0.05
Glomerular filtration rate (ml/min/1.73 m^2)	109.32 ±18.77	110.44 ± 6.67	115.38 ± 18.47	>0.05
Urinary albumin/creatinine ratio (µg/mg)	124.44±30.32	86.07±20.80	12.84 ± 2.44	<0.01**
Total cholesterol (mg/dl)	250.64 ± 61.51	234.76±51.40	155.30 ± 19.18	<0.01**
Triglycerides (mg/dl)	178.48 ± 44.26	173.24±42.69	88.36 ± 21.19	<0.01**
Low density lipoprotein cholesterol (mg/dl)	175.52 ± 43.65	163.17 ± 40.09	86.89 ± 19.33	<0.01**
High density lipoprotein cholesterol (mg/dl)	39.44 ± 7.74	36.68 ± 6.61	50.86 ± 4.55	<0.01**
Total calcium (mg/dl)	9.28 ± 0.56	9.16 ± 0.56	9.26 ± 0.47	>0.05
Serum phosphorus (mg/dl)	3.59 ± 0.48	3.68 ± 0.46	3.69 ± 0.57	>0.05
Intact Parathyroid Hormone (pg/ml)	46.68 ± 11.26	41.21 ± 7.62	34.01 ± 8.03	<0.01**
25 (OH) vitamin D_3 (ng/ml)	18.78 ± 4.62	24.17 ± 5.80	45.79 ±11.08	<0.01**

* Significant difference. ** Highly significant difference.

Table (2): Correlation coefficient study of 25 (OH) vitamin D level with different variables

	Variables	r	P
25 (OH) vitamin D_3 (ng/ml)	Age (years)	- 0.52	<0.01**
	Duration of type 2 diabetes mellitus (years)	- 0.59	<0.01**
	Stages of diabetic nephropathy	-0.62	<0.01**
	Fundus findings	-0.40	<0.01**
	Body mass index (Kg/m²)	- 0.48	<0.01**
	Systolic blood pressure (mmHg)	- 0.73	<0.01**
	Diastolic blood pressure (mmHg)	- 0.69	<0.01**
	Fasting blood sugar (mg/dl)	- 0.84	<0.01**
	Glycated hemoglobin (HbA1c %)	- 0.75	<0.01**
	Serum creatinine (mg/dl)	- 0.16	>0.05
	Glomerular filtration rate (ml/min/1.73 m^2)	0.46	<0.01**
	Urinary albumin creatinine ratio (µg/mg)	- 0.82	<0.01**
	Total cholesterol (mg/dl)	- 0.71	<0.01**
	Triglycerides (mg/dl)	- 0.58	<0.01**
	Low density lipoprotein cholesterol (mg/dl)	- 0.71	<0.01**
	High density lipoprotein cholesterol (mg/dl)	0.47	<0.01**
	Total calcium (mg/dl)	0.61	<0.01**
	Serum phosphorus (mg/dl)	0.20	>0.05
	Serum albumin (gm/dl)	0.27	>0.05
	Intact Parathyroid Hormone (pg/ml)	- 0.33	<0.05*

Table (3): Comparison of different variables between different grades of diabetic retinopathy

	Moderate Non Proliferative Diabetic Retinopathy (N=15) Mean±SD	Severe Non Proliferative Diabetic Retinopathy (N=4) Mean±SD	Proliferative Diabetic Retinopathy (N=6) Mean ±SD	P
Age (years)	46.87 ± 2.88	55.25 ± 2.50	66.00 ± 3.74	<0.01**
Duration of type 2 diabetes mellitus (years)	14.80 ± 3.34	19.25 ± 1.50	25.00 ± 3.16	<0.01**
Body mass index (Kg/m²)	28.73 ± 3.76	27.08 ± 4.22	30.33 ± 4.19	>0.05
Systolic blood pressure (mmHg)	136.30 ± 13.69	142.50 ± 9.57	151.67 ± 7.53	<0.05*
Diastolic blood pressure (mmHg)	87.67 ± 7.99	86.25 ± 4.79	96.67 ± 5.16	<0.05*
Fasting blood sugar(mg/dl)	223.20 ± 33.57	273.00 ± 40.61	301.50 ± 38.71	<0.01**
Glycated hemoglobin (HbA1c %)	8.91 ± 0.94	10.63 ± 1.52	12.35 ± 1.26	<0.01**
Serum creatinine (mg/dl)	0.77 ± 0.05	0.78 ± 0.02	0.86 ± 0.11	<0.05*
Glomerular filtration rate (ml/min/1.73 m^2)	115.47 ±18.23	109.75 ± 22.23	93.67 ± 7.55	<0.05*
Urinary albumin/ creatinine ratio (µg/mg)	101.52 ± 25.06	107.35 ± 25.86	136.27 ± 25.73	<0.05*
Total cholesterol(mg/dl)	231.47 ± 50.84	258.50 ± 39.53	293.00 ± 36.27	<0.05*
Triglycerides (mg/dl)	159.27 ± 33.56	188.75 ±14.27	207.83 ± 41.62	<0.05*
Low density lipoprotein cholesterol (mg/dl)	158.49 ± 35.70	182.05 ± 38.59	213.27 ± 34.54	<0.05*
High density lipoprotein cholesterol (mg/dl)	41.13 ± 7.84	38.75 ± 3.59	31.90 ± 6.46	<0.05*
Total calcium (mg/dl)	9.39 ± 0.45	9.20 ± 0.87	9.03 ± 0.62	>0.05
Serum phosphorus(mg/dl)	3.59 ± 0.50	3.70 ± 0.34	3.50 ± 0.54	>0.05
Serum albumin (gm/dl)	4.17 ± 0.28	4.35 ± 0.25	4.43 ± 0.30	>0.05
Intact Parathyroid Hormone (pg/ml)	42.25 ± 10.56	47.65 ± 11.83	60.43 ± 15.08	<0.05*
25 (OH) vitamin D_3 (ng/ml)	21.57 ± 5.38	16.20 ± 4.00	13.53 ± 3.36	<0.01**

* Significant difference. ** Highly significant difference.

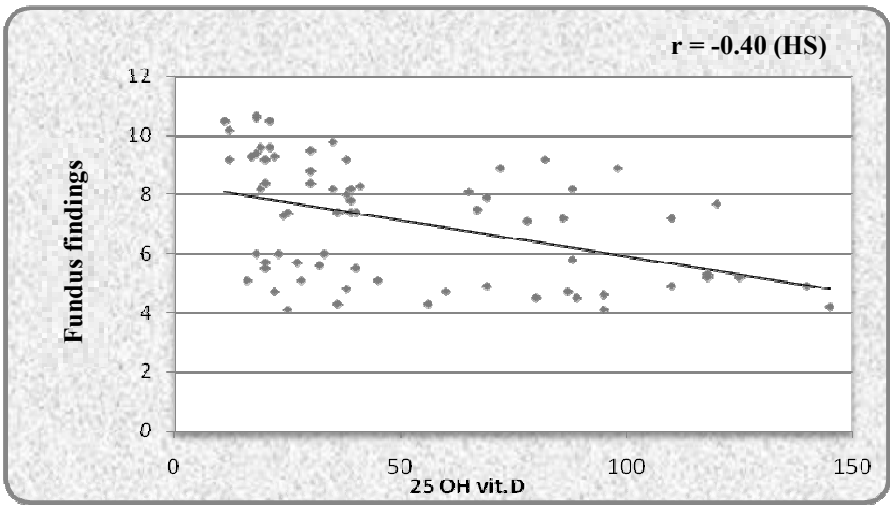

Figure (1): Correlation study of 25 (OH) vitamin D level and severity of diabetic retinopathy

Discussion:

Our results showed that mean serum 25(OH) vitamin D_3 level was lower in the 50 type 2 diabetic patients compared with the healthy control group with a highly significant statistical difference. This was in agreement with results of the previously conducted studies[13-16]. Some intrinsic factors unique to type 2 diabetes could be used as predictors for developing this state of hypovitaminosis. The significant higher age, BMI, glycemic indices and frequency of diabetic complications in the group with vitamin D insufficiency could represent the answer.

In our study we observed that type 2 diabetic patients with DR had the highest body mass index with no statistically significant difference if compared to type 2 diabetic patients without DR and statistically significant difference if compared with the healthy control counter partners. Dirani and colleagues[17] had presented several biological theories to explain the pathophysiological mechanisms of the association between higher BMI and DR. These include the potential involvement of platelet function, blood viscosity, aldose reductase activity and vasoproliferative parameters such as vascular endothelial growth factor. Apart from these parameters, lifestyle factors such as physical activity and weight loss provide some evidence to support this relationship[17].

In the present study, comparison between type 2 diabetic patients with diabetic retinopathy versus those without diabetic retinopathy revealed that patients with diabetic retinopathy had a significantly longer duration of type 2 diabetes mellitus, a significant higher systolic blood pressure and a highly significant higher diastolic blood pressure. Regarding parameters of glycemic control, there was a highly significant increase in FBS and HbA_{1C} in type 2 diabetic patients with diabetic retinopathy compared to type 2 diabetic patients without diabetic retinopathy. These results support findings of previous

studies[18,19]. It is presumed that duration of diabetes reflects total glycemic control and risk factor exposure over time. Additionally, DR can be affected by the hemodynamic changes induced by hypertension, such as impaired autoregulation and hyperperfusion. In addition, hypertension independent of hyperglycemia is known to upregulate the expression of vascular endothelial growth factor in retinal endothelial cells and ocular fluids, which can promote DR[19].

The results of the present study revealed that type 2 diabetic patients with diabetic retinopathy had a higher mean iPTH level compared to type 2 diabetic patients without diabetic retinopathy with a statistically significant difference. Patients with proliferative diabetic retinopathy had significantly the highest mean iPTH in comparison to other grades of diabetic retinopathy.

These results were in agreement with data from previous studies[20,21]. This finding may be explained by a compensatory mechanism to the low serum vitamin D3 concentrations. Parathyroid hormone excess can induce inflammatory cytokines which may play a role in the pathogenesis of proliferative DR[21].

The present study revealed that type 2 diabetic patients with severe NPDR have lower mean serum 25(OH) vitamin D level than type 2 diabetic patients with moderate NPDR and that type 2 diabetic patients with PDR have the lowest mean serum 25(OH) vitamin D level with statistically highly significant difference. This finding may suggest a permissive role of vitamin D deficiency in the pathogenesis of DR.

Additionally, vitamin D may play a role in the pathogenesis of diabetic retinopathy through its effects on the immune system. Inflammatory cytokines such as TNF-α, TNF-β, IL-6 and plasminogen activator inhibitor-1 are upregulated in patients with type 2 diabetes. It has been shown that vitamin D exerts an anti-inflammatory effect by decreasing the production of several proinflammatory cytokines such as IL-2, IL-6, IL-8, IL-12 and TNF-α as well as decreasing the proliferation of helper T-cells, cytotoxic T-cells and natural killer cells[22].

A recent study[23] found that vitamin D deficiency was associated with vascular endothelial dysfunction in middle aged and elderly adults. The authors concluded that this dysfunction was related to increased vascular endothelial cell expression of the proinflammatory transcription factor, nuclear factor κB.

Vitamin D may also contribute to diabetic retinopathy via angiogenesis mechanisms[14]. Albert and his colleagues[24] have shown that the active metabolite of vitamin D, calcitriol, was a potent inhibitor of both retinal neovascularization in vivo and retinal endothelial cell capillary morphogenesis in vitro.

In the current study we observed that patients with PDR have the highest mean serum creatinine, urinary ACR and iPTH levels as well as the lowest mean glomerular filtration rate and mean 25(OH) vitamin D level compared to patients with moderate and

severe NPDR. These findings may suggest that as renal function deteriorates, vitamin D synthesis decreases and stimulates iPTH which may play a direct role in the pathogenesis of DR and reveals the link between diabetic retinopathy and diabetic nephropathy.

Several complications in diabetic retinopathy such as macular edema and neovascularization are driven by VEGF production[25]. Therefore, vitamin D could exert its positive effect via calcitriol mediated VEGF reduction[24].

Vitamin D may also play a protective role through its effects on glycemic control and hypertension; both of them are significant risk factors for the development and progression of diabetic retinopathy[26].

There are several limitations to the current study. First, the cross sectional design of this study limits the ability to assess causality. It is not possible to determine whether the vitamin D insufficiency leads to diabetic retinopathy or if diabetic retinopathy leads to vitamin D insufficiency. Second, only one time point was recorded for the subjects in this study. It would be valuable to follow these patients with serial fundoscopic examinations and blood testing.

Conclusion:

There was a significant reduction of 25 (OH) vitamin D level in patients with type 2 diabetes and more so in patients with DR. Moreover, this study demonstrated a negative correlation between 25 (OH) vitamin D level and severity of diabetic retinopathy among patients with type 2 diabetes mellitus. These findings reflect a possible causality relationship between vitamin D deficiency and the development and progression of diabetic retinopathy. We recommend to validate our results in larger trials and to study the effect of vitamin D supplements on the prevention of DR.

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