Is there any correlation between vitamin D insufficiency and diabetic retinopathy?

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Abstract

• **AIM: To determine a relation between vitamin D level, which is an inhibitor of angiogenesis, and diabetic retinopathy and its risk factors**

• **METHODS: In a clinic–based cross sectional study two hundred and thirty –five type 2 diabetic patients older than 20y were selected. Patients were classified according to ophthalmologic examination as following: no diabetic retinopathy (NDR) (n=153), non –proliferative diabetic retinopathy (NPDR) (n=64) and proliferative diabetic retinopathy (PDR) (n=18). Study subjects were tested for fasting blood glucose, glycated hemoglobin A1C (HbA1C), lipid profile, microalbuminuria, HsCRP, IGF1, insulin (in patients without history of insulin taking) and 25 hydroxy vitamin D [25 (OH) D] levels. Vitamin D insufficiency was defined according to 25 (OH) D level less than 30 ng/mL. The relationship between diabetic retinopathy and serum 25 (OH) D insufficiency was evaluated.**

• **RESULTS: The prevalence of diabetic retinopathy was 34.8% in our patients. Long duration of diabetes, hypertension, poor glycemic control, diabetic nephropathy, hyperinsulinemia and insulin resistance were risk factors for diabetic retinopathy but 25 (OH) D level was not significant different between NDR, NPDR and PDR groups. Correlation between 25 (OH) D level and other known risk factors of diabetic retinopathy was not significant.**

• **CONCLUSION: This study did not find any association between diabetic retinopathy and its severity and vitamin D insufficiency. Vitamin D insufficiency is not related to risk factors of diabetic retinopathy.**

• **KEYWORDS:** diabetes; retinopathy; vitamin D; insulin resistance

INTRODUCTION

Diabetes mellitus (DM) type 2 is a tremendous health burden in worldwide and the middle east is expected to have highest prevalence of diabetes by 2030 [1]. The prevalence of DM in urban Iranian population aged ≥20y was 8.1% in 2008 [2]. Diabetes is the leading cause of blindness in individuals between age 20 to 65y and diabetic retinopathy is the commonest complication of DM. According to a population-based study in Iran, the prevalence of diabetic retinopathy was 37% among type 2 diabetic patients[3]. Diabetic retinopathy has a complex process. While it has been established that age, duration of diabetes, poor glycemic control, hypertension, smoking, inflammation, insulin resistance, hyperlipidemia, anemia, microalbuminuria and increased growth factors are associated with diabetic retinopathy, the pathophysiology of it is not completely established[4].

Vitamin D insufficiency is very common in the world. Different epidemiological studies implied that more than 30% of adult populations are at risk of vitamin D insufficiency[5]. Vitamin D insufficiency has been implicated in the pathogenesis and progression of diabetes and also correlated with obesity, insulin resistance, metabolic syndrome and cardiovascular disease [6-10]. Vitamin D deficiency may play a role in the pathogenesis of diabetic retinopathy. Vitamin D affects on insulin secretion and insulin sensitivity. It has anti-inflammatory and immunosuppressive effect and causes suppression in rennin-angiotensin system (RAS) [11,12]. In addition, this hormone has a potent inhibitory effect on angiogenesis, so it seems through several mechanisms vitamin D insufficiency exerts a role in the development and progression of diabetic retinopathy [13,14]. In this study, we examined the relationship between vitamin D insufficiency and diabetic retinopathy and its risk factors in type 2 diabetes.

SUBJECTS AND METHODS

The study protocol was approved by the local Ethics Committee (the research ethics committee of Mashhad

University of Medical Sciences), and was conducted according to the principles of Helsinki Declaration. Written informed consent was obtained from all patients. Patients who completed their comprehensive physical examination were included in the study.

This cross sectional study conducted in Mashhad, Iran. A total of 235 type 2 diabetic patients older than 20y were recruited consecutively from an outpatient diabetes clinic. The exclusion criteria were recent history of acute illness, chronic liver or renal disease, pregnancy, lactation, history of cancer, malnutrition, hyperparathyroidism or hypoparathyroidism, nephrolithiasis and who were taking any drugs known to influence on 25 hydroxy vitamin D [25 (OH) D] level such as multivitamins, anticonvulsants, glucocorticoids and rifampin. Age, sex, duration of diabetes, history of hypertension and antihypertensive therapy, history of hyperlipidemia and lipid lowering agents and all medications were recorded. The presence and severity of retinopathy was evaluated by fundoscopic examination by an expert ophthalmologist. Patients were classified according to ophthalmologic examination as following: no diabetic retinopathy (NDR), non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Blood pressure was measured on the right arm in a sitting position after at least 20min of rest with a mercury sphygmomanometer. Hypertension was defined as systolic blood pressure $\geqslant$ 130 mm Hg or diastolic blood pressure $\geqslant$ 85 mm Hg or history of hypertension and receiving antihypertensive drugs. Blood samples were drawn in the morning after an overnight fast. Fasting plasma glucose was measured by the glucose oxidase method (Human, Germany). Total cholesterol, triglyceride (TG), and high density lipoprotein (HDL) were measured by enzymatic method (Parsazmon Karaj, Iran). Low density lipoprotein (LDL) was calculated according to Friedwall formula [LDL= total cholesterol- (HDL+TG/5)]. Glycated hemoglobin A1C (HbA1C) was assessed by Column chromatography (Biosource kit, Barcelona, Spain). Urinary albumin was measured as the albumin to creatinine ratio (ACR) in a morning sample. Urine albumin in spot urine was measured by immunoturbidometry assay (Parsazmon, Karaj, Iran). Urine creatinine was measured by enzymatic colorimetric assay. Serum high sensitive C-reactive protein (HsCRP) concentration as a marker of inflammation was measured by immunoturbidimetry assay (Parsazmon, Karaj, Iran). Insulin level was assessed in non insulin treated patients by immunoradiometric assay and insulin resistance was estimated with the homeostasis model assessment-insulin resistance (HOMA-IR) index (fasting blood sugar×insulin/22.5). Serum concentration of 25 (OH) D was measured by RIA method (Biosource Europ, Nivelles, Belgium). Vitamin D

Table 1 Baseline characteristic of patients (*n*= 235)

Characteristics	$\bar{x} \pm s$
Male (%)	30.2
Age (a)	54.8±9.4
Body mass index (kg/m^2)	27.6±4.6
Duration of diabetes (a)	7.52±6.1
Waist circumference (cm)	98.4±12.7
Systolic blood pressure (mm Hg)	125.2±20.1
Diastolic blood pressure (mm Hg)	76.9±11.7
FBS (mg/dL)	173.8±70.3
HbA1C (%)	8.7±1.9
Total cholesterol (mg/dL)	175.9±39.8
Triglyceride (mg/dL)	168.1±115.4
HDL cholesterol (mg/dL)	41.9±7.0
LDL cholesterol (mg/dL)	102.9±29.8
Creatinine (mg/dL)	0.97±0.2
Ca (mg/dL)	9.4±0.4
P (mg/dL)	3.7±0.6
PTH (pg/mL)	61.0±15.9
25 (OH) D (ng/mL)	9.8±8.5
Urine albumin to creatinine ratio (micg/mg)	47.9±78.0
Glumerolar filtration rate (mL/min)	83.1±26.4
IGF1	104.4±44.5
High sensitive C-reactive protein	3.0±3.7
Hypertension (%)	56.7
Smoking (%)	5.2
History of macrovascular disease (%)	26.2
Retinopathy (%)	35% (21.8% non-proliferative retinopathy, 7.7% proliferative retinopathy and 7.1% macular edema)

FBS: Fasting blood glucose; HbA1C: Glycosylated hemoglobin; LDL: Low density lipoprotein; HDL: High density lipoprotein; PTH: Parathyroid hormone; 25(OH) D: 25 hydroxy vitamin D.

insufficiency was defined according to 25 (OH) D level less than 30 ng/mL. Statistical analyses were performed by SPSS, version 11.5. Data was expressed as mean and standard deviation (SD). Non-normal variables were compared by Mann-Whitney U test between groups. Other variables were tested by either one-way ANOVA or Student's t -test. Categorical variables were compared by the Chi-squared test. Variables with a statistically significant difference between the patients with and without diabetic retinopathy were evaluated by multiple logistic regressions with forward stepwise analysis to identify independent risk factors for diabetic retinopathy. A P value <0.05 was considered significant.

RESULTS

Baseline clinical and paraclinical characteristic of patients is shown in Table 1. The prevalence of diabetic retinopathy was 34.8% (27.3% NPDR, 7.5% PDR). Comparison of laboratory

Table 2 Comparison of clinical and paraclinical characteristics among non-diabetic retinopathy (NDR), non proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR) $\overline{x} \pm s$

Variables	NDR	NPDR	PDR	P
Age (a)	54.2±10.3	56.6±7.1	57.9±7.3	0.11
Male (%)	17.64	20	12.86	0.21
Duration of diabetes (a)	3.2±4.2	7.4±6.0	9.2±7.2	<0.001
Body mass index (kg/m^2)	27.5±4.7	27.8±4.2	27.5±4.1	0.95
Systolic blood pressure (mm Hg)	122.7±18.9	136.9±23.0	139.1±18.0	0.004
Diastolic blood pressure (mm Hg)	76.2±11.7	76.2±12.1	83.4±10.0	0.04
Cholesterol (mg/dL)	174.3±39.4	179.7±44.1	171.9±33.5	0.64
Triglyceride (mg/dL)	169.9±124.7	169.4±108.9	149.5±80.4	0.75
LDL (mg/dL)	101.5±29.2	105.0±33.5	104.0±27.9	0.75
HDL (mg/dL)	41.7±6.2	43.0±9.6	40.7±4.0	0.35
FBS (mg/dL)	164.0±59.2	191.0±87.3	178.7±80.2	0.05
HbA1C	8.4±1.8	9.3±1.5	9.6±2.1	0.001
Creatinine (mg/dL)	0.9±0.2	0.9±0.3	1.15±0.3	<0.001
Urine albumin to creatinine ratio (micg/mg)	31.9±34.7	72.7±122.2	96.0±119.8	<0.001
25 (OH) D (ng/mL)	10.3±9.4	9.0±7.0	10.1±6.8	0.7
IGF1	107.9±43.8	93.8±42.0	99.8±44.0	0.16
HsCRP	2.8±3.7	3.8±3.9	3.0±3.8	0.27
Insulin	10.9±5.1	8.6±3.7	18.6±15.6	<0.001
HOMA-IR	1.6±0.8	1.4±0.7	2.9±2.1	<0.001

FBS: Fasting blood glucose; HbA1C: Glycosylated hemoglobin; LDL: Low density lipoprotein; HDL: High density lipoprotein; 25(OH) D: 25 hydroxy vitamin D; HsCRP: High sensitive C-reactive protein; HOMA-IR: Homeostasis model assessment of insulin resistance; *P*<0.05 is considered significant; ANOVA test was used for analysis of normal distribution variable and Kruskal Wallis for non normal distribution variable.

and clinical parameters between patients without diabetic retinopathy, NPDR and PDR is shown in Table 2. According to this table, long duration of diabetes, hypertension, poor glycemic control, diabetic nephropathy, hyperinsulinemia and insulin resistance were risk factors for diabetic retinopathy but 25 (OH) D level was not significant different between groups. Prevalence of vitamin D insufficiency in our patients was 93.3%. Correlation between 25 (OH) D level and other variables was not significant. According to vitamin D status, we stratified patients to vitamin D insufficient and sufficient patients and all parameters compared with other in two groups (Table 3). Based on this classification, vitamin D insufficiency was not related to risk factors of diabetic retinopathy. Finally, we divided 25 (OH) D levels into quartiles. Clinical and paraclinical laboratory characteristics of all patients according to 25 (OH) D quartiles are illustrated in Table 4. With respect to this table, there was not any significant difference in known risk factors of diabetic retinopathy in different levels of 25 (OH) D levels. The results of stepwise multiple logistic regressions showed that HbA1C and duration of diabetes are only independent predictive factors for diabetic retinopathy.

DISCUSSION

To our knowledge, this is the first study to evaluate an association between vitamin D insufficiency and known risk factors of diabetic retinopathy and its severity. In our study, there was no association between vitamin D insufficiency and

presence of diabetic retinopathy and its severity. In addition, we did not find any relation between vitamin D insufficiency and some of known risk factors (duration of diabetes, poor glycemic control, hypertension, inflammation, insulin growth factor and nephropathy) of diabetic retinopathy. These results are contrary with other studies in this regard. For the first time in 1999, Aksoy *et al* [15] found that severity of diabetic retinopathy was negatively related with serum level of 1, 25 dihydroxy vitamin D [1, 25 (OH)$_2$D$_3$]. This result is supported by other studies of adults with type 2 diabetes and children and adolescents with type 1 diabetes [16,17]. A causal role of vitamin D deficiency in the development and progression of diabetic retinopathy supported by the role of vitamin D on angiogenesis. In biological models, it seems that higher levels of vitamin D is associated with lower rate of angiogenesis[18,19]. Vitamin D also decreases vascular endothelial growth factor (VEGF) and also replication of vascular smooth muscle cells[20]. It reduces vascular mitogenic response to stimulatory factors like thrombin and platelet-derived growth factor (PDGF). Vitamin D even prevents negative effects of advanced-glycosylation-end (AGE) products on endothelial cells [21]. Vitamin D decreases lymphocyte proliferation and cytokine production. It seems to down regulates NF (nuclear factor) - Kβ activity and increases IL10 [22]. These changes lead to a cytokine profile; which is less inflammatory. Vitamin D decreases the production and activity of tissue matrix metalloproteinase (MMPs) as enzymes that involved

Table 3 Baseline clinical and laboratory characteristics of patients in accordance with levels of 25 hydroxy vitamin $\bar{x}\pm s$

Variables	25 (OH) D<30 ng/mL	25 (OH) D>30 ng/mL	P
Age (a)	54.4±9.54	60.4±11.2	0.06
Sex (M/F)	60/160	5/10	0.74
BMI (kg/m^2)	27.8±4.75	26.2±3.56	0.28
Systolic blood pressure (mm Hg)	123.9±20.1	125.4±16.3	0.08
Diastolic blood pressure (mm Hg)	76.4±12.1	75.4±5.2	0.78
FBS (mg/dL)	173.0±65.0	177.0±97.3	0.87
HbA1C (%)	8.62±1.87	8.68±2.28	0.91
Cholesterol (mg/dL)	174.7±39.4	180.6±34.8	0.61
Triglyceride (mg/dL)	170.8±114.6	142.4±65.9	0.39
LDL (mg/dL)	102.3±29.3	110.0±32.3	0.38
HDL (mg/dL)	42.0±7.34	42.3±3.4	0.90
Creatinine (mg/dL)	0.96±0.23	0.95±0.18	0.88
Urine albumin to creatinine ratio (micg/mg)	48.1±78.0	45.1±67.7	0.89
GFR	85.6±26.3	73.1±29.4	0.15
IGF1	105.1±44.8	121.3±46.3	0.23
HsCRP	3.2±3.8	4.5±4.2	0.27
Insulin	10.8±5.9	10.3±4.4	0.81
HOMA-IR	1.7±0.9	1.5±0.6	0.67

FBS: Fasting blood glucose; HbA1C: Glycosylated hemoglobin; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; GFR: Glumerolar filtration rate; HsCRP: High sensitive C-reactive protein; HOMA-IR: Homeostasis model assessment of insulin resistance; $P<0.05$ is considered significant; ANOVA test was used for analysis of normal distribution variable and Kruskal Wallis for non normal distribution variable.

Table 4 Comparison of clinical and paraclinical characteristics according to quartiles of serum 25 hydroxy vitamin D levels

Variables	Quartile 1 25(OH)D (0.5-5.71) ng/mL	Quartile 2 25(OH)D (5.72-7.25) ng/mL	Quartile 3 25(OH)D (7.26-9.74) ng/mL	Quartile 4 25(OH)D (>9.74) ng/mL	P
Age	53.6± 9.53	53.2± 8.1	58.3±11.2	59.3±11.2	0.02
BMI (kg/m^2)	27.7±4.39	28.1± 5.16	27.2±4.8	26.4±3.4	0.66
Systolic blood pressure (mm Hg)	123.1±22.8	123.9±17.9	125.5±19.3	125.0±15.6	0.94
Diastolic blood pressure (mm Hg)	75.8±13.5	77.0±10.6	76.3±12.3	75.8±5.1	0.96
FBS (mg/dL)	174.3± 72.8	171.3± 58.8	176.1±62.1	179.6±93.7	0.97
HbA1C (%)	8.7± 1.84	8.4±2.03	8.68±1.66	8.7±2.2	0.82
Cholesterol (mg/dL)	170.1±36.8	178.4±39.0	176.1±45.4	182.3±33.9	0.57
Triglyceride (mg/dL)	153.6±103.6	190.8±132.6	165.0±96.0	150.6±69.7	0.25
LDL(mg/dL)	99.0±28.4	104.7±25.3	102.6±35.9	114.0±34.2	0.36
HDL (mg/dL)	42.1±5.37	40.7± 6.2	44.1±11.1	42.6±3.5	0.14
Creatinine (mg/dL)	0.97±0.25	0.93±0.21	0.98±0.25	0.95±0.17	0.72
Urine albumin to creatinine ratio (micg/mg)	48.6±76.1	43.1±52.1	55.0±114.9	48./3±55.6	0.90
GFR	84.2±20.7	90.1±30.3	80.7±28.2	72.9±27.9	0.15
IGF1	104.3±42.8	105.9±47.4	106.9±44.6	116.2±48.1	0.85
Insulin	11.6±7.9	10.7±4.0	9.5±4.3	10.3±4.4	0.49
HOMA-IR	1.9±1.3	1.6±0.6	1.4±0.6	1.5±0.6	0.14
HsCRP	3.3±3.6	3.6±3.9	2.4±4.1	4.4±4.1	0.32
Sex (male %)	37.1%	31.4%	22.2%	9.3%	0.92
Hypertension (%)	34.35%	40.45%	19.0%	6.2%	0.58

FBS: Fasting blood glucose; HbA1C: Glycosylated hemoglobin; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; GFR: Glumerolar filtration rate; HsCRP: High sensitive C-reactive protein; HOMA-IR: Homeostasis model assessment of insulin resistance; $P<0.05$ is considered significant; ANOVA test was used for analysis of normal distribution variable and Kruskal Wallis for non normal distribution variable.

in the breakdown of atherogenic plaques and induction of thrombosis[23].

Poor glycemic control is a known risk for the development and progression of diabetic retinopathy. There are strong evidences that an optimal amount of vitamin D concentration is necessary for efficient insulin secretion and function[24-26]. It is possible that vitamin D insufficiency in diabetic patients adversely affects the glycemic control and aggravates diabetic retinopathy. We could not find any relation between 25 (OH) D and fasting blood glucose and HbA1C level.

Some studies have been reported an association between insulin resistance and hyperinsulinemia and diabetic retinopathy [27,28]. We found a significant difference in insulin resistance and hyperinsulinemia according to severity of retinopathy. Vitamin D deficiency can increases insulin resistance by the effects on insulin receptors, inducing of systemic inflammation and obesity. We tried to show an association between vitamin D insufficiency and insulin resistance as a risk factor for diabetic retinopathy but our results did not suggest this association.

Older age is additional risk factor for diabetic retinopathy. In our results, patients with PDR were older age than patients with NPDR and NDR but differences were not significant. In our patients, serum level of 25 (OH) D increased according to age that this result is similar with some other studies[16,29].

Similar with other studies we found a significant difference in systolic and diastolic blood pressure in retinopathic patients compared with patients without retinopathy. Vitamin D is a negative regulator of rennin biosynthesis [30]. This effect has beneficial impacts on blood pressure control. We did not show any association between vitamin D insufficiency and blood pressure.

Another pathophysiologic mechanism for diabetic retinopathy is inflammation[31]. We chose HsCRP as a marker of inflammation. In our study there was no significant difference in HsCRP level in patients with and without diabetic retinopathy. Vitamin D has anti-inflammatory and immunosuppressive effects[32,33]. In previous study, we found a negative significant correlation between vitamin D level and HsCRP[34]. However, in current study there was no significant difference in mean of HsCRP level according to vitamin D status of patients.

There are several evidences that IGF1 play a role in the diabetic retinopathy [35,36]. Our data did not confirm the role of IGF1 as a contributing factor for diabetic retinopathy. Insulin-like growth factor I components may play a role in the regulation of vitamin D and also active form of vitamin D may regulate multiple insulin-like growth factor binding protein genes [37,38]. we expected to see a relation between IGF1 , vitamin D levels and retinopathy but in our cross sectional study , we did not find this relation.

There are several possible explanations for why we saw a different result in the association between vitamin D insufficiency and diabetic retinopathy and its risk factors than what others have reported. Difference in sample population tested, biochemical methods that used, exclusion of patients with diabetes and chronic kidney disease, high percentage of vitamin D insufficiency in our patients and possible difference in vitamin D receptor polymorphism in our population could play a role.

There were some limitations in our study. The cross sectional design of this study, absence of information about dietary intake and outdoor exposure time in patients and low sample size are limitations for this study.

In conclusion, decreased serum vitamin D levels did not show increased risk for diabetic retinopathy and its risk factors. Larger studies are needed to confirm these findings.

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