The Effect of Vitamin D Supplementation on The Outcome of Treatment with Bevacizumab in Diabetic Macular Edema: A Randomized Clinical Trial

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Abstract

Purpose: Concomitant vitamin D deficiency (VDD) is speculated to aggravate diabetic macular edema (DME). We aimed to determine the effect of hypovitaminosis D correction on the outcome of treatment with intravitreal bevacizumab (IVB) in DME eyes.

Methods: 83 eyes of 83 patients with DME were recruited and divided into three groups: normal vitamin D levels + IVB administration (Group 1), vitamin D insufficient/deficient + IVB administration (Group 2), and vitamin D insufficient/deficient + IVB administration + oral vitamin D supplementation (Group 3). Visual (corrected distance visual acuity, CDVA) and anatomical (central macular thickness, CMT) outcomes of intervention were evaluated 1, 3, and 6 months after the triple IVB injections were completed. Serum vitamin D levels were measured 1 and 6 months after the third IVB administration.

Results: In months 1, 3, and 6, after the basic triple IVB injection, visual acuity and CMT improved in all three groups, but improvements (both functional and anatomical) in groups 1 and 3 (sufficient vitamin D and corrected hypovitaminosis D) in month 6 were more significant than in group 2 (uncorrected hypovitaminosis D) ($p < 0.001$). The mean number of IVB injections during follow-up was 5.33 (range: 4-7), which did not differ between the groups.

Conclusion: Correction of vitamin D deficiency in DME patients with type 2 diabetes and vitamin D deficiency, in addition to IVB injections, may play a role in improving CDVA and CMT. However, this beneficial effect seems to be delayed by several months.

Trial registration: Iranian Registry of Clinical Trials (IRCT), IRCT20200407046978N1, registered on April 11, 2020, - retrospectively registered. <https://en.irct.ir/trial/46999>

1. Introduction

Diabetic retinopathy (DR) is a microvascular complication of diabetes mellitus and a leading cause of vision loss among working-age adults and the elderly (1). Diabetic macular edema (DME) can develop at any stage of DR, including mild, moderate, and severe non-proliferative DR (NPDR) and proliferative DR (PDR); it is characterized by retinal vessels leakage, decreased endothelial integrity, and exudative fluid accumulation in the macula (2). The inflammatory milieu in the diabetic eye and the vascular endothelial growth factor (VEGF) expression, with chronic hyperglycemia and advanced glycation end-products buildup in the background, significantly contribute to DME pathogenesis by promoting neovascularization and disrupting the blood-retinal barrier (BRB) (2–4).

A meta-analysis of observational studies on diabetic patients screened for DR has collectively demonstrated vitamin D deficiency (VDD) as a risk factor of developing DR (5). VDD has also shown an inverse association with DR severity (6,7). Several in vitro and in vivo studies have embarked on the anti-angiogenic and anti-inflammatory capabilities of calcitriol ($1\alpha,25(\text{OH})_2\text{D}_3$; the active metabolite of vitamin D) and its analogs in retinal tissue (8–12). Numerous randomized clinical trials supported a beneficial impact of vitamin D supplementation on endothelial function (13), and some studies have confirmed such

an impact in the setting of diabetes as well, both in vitro (14) and in vivo (15). Given the pivotal role of neovascularization and vascular abnormalization in DR progression and DME, the clinical efficacy of VDD correction as a low-cost, easily available co-intervention in DME eyes undergoing standard treatment with intravitreal anti-VEGF therapies appears an intriguing topic of investigation.

Our objective was to evaluate the visual and anatomical outcomes associated with vitamin D deficiency correction in DME eyes undergoing intravitreal bevacizumab (IVB) administration. Outcomes were measured for six months, to enable a more comprehensive appraisal of potential benefits associated with this co-intervention.

2. Methods

2.1 Design

This study adheres to the CONSORT checklist for reporting randomized clinical trials (16). The present controlled randomized parallel clinical trial was conducted in the tertiary Retina and Vitreous Clinic of Labbafinezhad Hospital to compare the outcome of treatment with IVB in combination with vitamin D supplementation to IVB alone in eyes with diabetic macular edema. The study was conducted from May 2018 to February 2020 (~21 months). Patients with insufficient vitamin D levels were randomly assigned to two groups, one receiving vitamin D supplementation and standard IVB treatment and the other receiving only the IVB treatment; the allocation ratio was 1:1. The Ethics Committee of the Ophthalmic Research Center of Shahid Beheshti University of Medical Sciences approved the study protocol, and all participants signed an informed consent letter. The registry number of the present clinical trial is IRCT20200407046978N1.

2.2 Population and definitions

All adult (>18 years) patients with diabetic macular edema, admitted to the Retina and Vitreous Clinic, who required IVB administration, were assessed in terms of eligibility for inclusion in the trial. The inclusion criteria were: I) a central macular thickness (CMT, i.e., within the central 1mm of the macula) of 300 microns or above, as determined by optical coherence tomography (OCT) imaging (Spectralis OCT; Heidelberg Engineering, Heidelberg, Germany), and II) a corrected distance visual acuity (CDVA) of 8/10 (0.1 logMAR) or worse, in the affected eye. Patients were excluded in case of I) any previous ocular surgery (except a complication-free cataract surgery before the last six months), II) any previous intravitreal injections (any anti-VEGF agents, steroids, etc.), III) epiretinal membrane or vitreomacular traction, IV) history of retinal vascular accidents, V) history of uveitis, VI) pregnancy or breastfeeding, VII) consumption of vitamin supplements at the time of recruitment, VIII) failure to adhere to the vitamin D supplementation regimen, IX) severe vitamin D deficiency, X) any macular pathology other than diabetic macular edema, XI) hypercalcemia and hyperphosphatemia, and XII) serum creatinine levels above 3mg/dl. From each eligible individual, one eye was enrolled in this study.

Sufficiency, insufficiency, deficiency, and severe deficiency of serum 25(OH)D levels were defined as levels above 30 ng/ml, 20-30 ng/ml, 10-20 ng/ml, and below 10 ng/ml, respectively. DME was defined as a CMT of 300 microns or above.

2.3 Patient assignment and interventions

Before any therapeutic intervention, all participants were interviewed; demographic information and the complete history of diabetes mellitus, medications, and other comorbid disorders (e.g., hypertension, hyperlipidemia, ischemic heart disease, etc.) were obtained. All recruited eyes underwent complete ophthalmic examinations before the treatment initiation and upon each follow-up session, including CDVA assessment as well as anterior segment evaluation and tonometry using slit lamps. Further, a detailed fundus examination was performed using non-contact 78 D or 90 D lenses; CMT was also measured using Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany). An ophthalmologist graded the DR severity using three field fundus photographs (optic disc centered, fovea centered, and centered on the temporal edge of the macula) following the International DR Severity Scale (17). In addition, to perform urinalysis (U/A) and laboratory assessments on baseline serum vitamin D levels, fasting blood sugar (FBS), hemoglobin A1C (Hb1Ac), erythrocyte sedimentation rate, C-reactive protein, lipid profile (including triglyceride, total cholesterol, HDL, and LDL), and serum creatinine levels, urine and serum samples were collected from participants before treatment.

Participants with insufficient (10-30 ng/ml) and sufficient (>30 ng/ml) baseline serum 25-hydroxyvitamin D (25(OH)D) levels were divided into two groups. Patients with vitamin D insufficiency were randomly assigned (using a random number table) to two groups receiving the standard treatment with IVB or IVB combined with vitamin D supplementation. Thus, with an allocation ratio of 1:1:1, three groups were formed: 1) vitamin D sufficient group, 2) vitamin D insufficient group not receiving oral vitamin D supplements, and 3) vitamin D insufficient group receiving oral vitamin D supplements.

All eyes underwent three monthly intravitreal injections of bevacizumab (Avastin®, Genentech/Roche, CA, USA) by ophthalmologists blinded to the patients' groups at the Vitreous and Retina Clinic of Labbafinezhad Hospital. Fundus examination and OCT imaging were repeated before each session. Patients in group 3 started taking oral vitamin D supplements along their IVB injection schedule. The supplementation regimen for patients with serum 25(OH)D levels of 10-20 ng/ml was eight weekly doses of 50000IU vitamin D₃ pearls (D-Vigel 50000 IU, Daana Pharmaceutical Company, Iran) for eight consecutive weeks followed by a maintaining daily dose of 800IU. Those with serum 25(OH)D levels between 20 ng/ml and 30 ng/ml received daily doses of 800IU. After achieving a sufficient vitamin D level (30 ng/ml), the daily 800IU dose was continued for both subgroups.

2.4 Outcomes

Patients were followed up for six months after the third IVB administration. The primary outcomes were functional improvements in vision, as evaluated by E-charts and expressed through changes in CDVA

logMAR and anatomical reductions in CMT, as measured by Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany). Comprehensive ophthalmic examinations were performed 1, 3, and 6 months after the third IVB injection. The secondary outcome was serum 25(OH)D level; it was assessed on months 1 and 6 after the third IVB injection. Upon follow-up sessions, additional IVB injections were given if needed. After the study was completed, patients in group 2 received vitamin D supplementation. Considering that patients with severe vitamin D deficiency were not enrolled, a nine-month delay in correcting vitamin D levels above 10 ng/ml was not considered harmful; patients in group 2 were thoroughly informed of and consented to this allocation.

2.5 Blinding

The ophthalmologist who injected the IVB were blinded to the randomized groups. The optometrists who performed the ophthalmic examinations were blinded to the patient's group and findings from the previous follow-up sessions.

2.6 Sample size and statistical analysis

With no similar previous study available when designing this study, we were to run an initial pilot study with 10 participants in each group and use the obtained data to determine the minimum sample sizes required. After the interim analysis of the initial pilot phase results, we estimated that a minimum of 20 patients was required for each group (one-tailed $\alpha = 0.05$; $1-\beta = 0.80$; the clinically acceptable margin for CMT changes = 50 microns; the standard deviation of CMT = 56 microns).

Descriptive statistics were expressed through mean, standard deviation (SD), median, range, frequency, and percentage. Analysis of inter-group differences was performed using Chi-square or ANOVA, where appropriate. Paired T-test was utilized to assess changes in DCVA, CMT, and serum 25(OH)D levels between different time points. All statistical analyses were performed using the SPSS software (V.25.0, IBM). P-values below 0.05 were considered statistically significant.

3. Results

3.1 Baseline demographic and clinical findings

A total of 102 patients screened for eligibility, and 91 who met the inclusion criteria were recruited; the recruitment took place from May 2018 to April 2019. 83 patients completed the trial, and eight were excluded from the analysis. Three patients discontinued participation in follow-up sessions for various reasons (one lost their interest in participation, one could not continue participation due to severe comorbid conditions, and one patient died). Five patients were excluded because their serum vitamin D levels did not achieve a sufficient level after the supplementation period (three because of non-adherence to the regimen and two because of unknown reasons). Eventually, 83 DME patients in three groups (29 in group 1, 26 in group 2, and 28 in group 3) were included in the analysis (shown in Fig. 1).

Demographic data of patients are presented in Table 1. The mean $\pm$ SD age (range) was 60 $\pm$ 8 (42-75); 66.2% of patients were male (female to male ratio ~ 1:2). No significant intergroup differences in age, sex, smoking status were observed ($p>0.05$). Data on patients' comorbidities, diabetes duration and medication, DR stage, previous ocular therapeutic interventions for DR or DME, FBS, and HbA1C are presented in Table 2. As interpreted from the table, no intergroup difference regarding the above-mentioned factors existed ($p>0.05$).

Table 1

Demographic information of patients

Parameter		Group			p-value
		Group 1 (vitamin D>30ng/ml)	Group 2 (vitamin D: 10-30ng/ml)	Group 3 (vitamin D: 10-30ng/ml + vitamin D supp.)	
Age		59.97 $\pm$ 7.89	59.46 $\pm$ 8.76	59.57 $\pm$ 7.00	0.969 [†]
Sex	Male (%)	17 (58.6)	15 (57.7)	18 (64.3)	0.863*
	Female (%)	12 (41.4)	11 (42.3)	10 (35.7)	
Eye	OD (%)	13 (44.8)	16 (61.5)	17 (60.7)	0.363*
	OS (%)	16 (55.2)	10 (38.5)	11 (39.3)	
Smoking	No (%)	15 (51.7)	15 (57.7)	11 (39.3)	0.382*
	Yes (%)	14 (48.3)	11 (42.3)	17 (60.7)	
Total no.		29	26	28	

Footnotes: [†], ANOVA test; *, Chi-square test

Table 2

Data on previous ocular treatments, diabetes duration and medication, comorbid conditions, DR stage, fasting blood sugar, and HbA1C.

Parameter		Group			P-value
		Group 1 (vitamin D>30ng/ml)	Group 2 (vitamin D: 10-30ng/ml)	Group 3 (vitamin D: 10-30ng/ml + vitamin D supp.)	
DM Medication	Ins (%)	2 (6.9)	1 (3.8)	1 (3.6)	0.851*
	Met (%)	14 (48.3)	10 (38.5)	11 (39.3)	
	Ins + Met (%)	13 (44.8)	15 (57.7)	16 (57.1)	
DR Stage	Mild NPDR (%)	7 (24.1)	6 (23.1)	3 (10.7)	0.786*
	Moderate NPDR (%)	8 (27.6)	6 (23.1)	11 (39.3)	
	Severe NPDR (%)	9 (31.0)	8 (30.8)	9 (32.1)	
	PDR (%)	5 (17.2)	6 (23.1)	5 (17.9)	
HTN	No (%)	7 (24.1)	7 (26.9)	3 (10.7)	0.281*
	Yes (%)	22 (75.9)	19 (73.1)	25 (89.3)	
HLP	No (%)	4 (13.8)	3 (11.5)	5 (17.9)	0.798*
	Yes (%)	25 (86.2)	23 (88.5)	23 (82.1)	
CVA	No (%)	25 (86.2)	24 (92.3)	26 (92.9)	0.641*
	Yes (%)	4 (13.8)	2 (7.7)	2 (7.1)	
IHD	No (%)	18 (62.1)	17 (65.4)	15 (53.6)	0.655*
	Yes (%)	11 (37.9)	9 (34.6)	13 (46.4)	
Prev. IVB	No (%)	12 (41.4)	7 (26.9)	7 (25.0)	0.347*
	Yes (%)	17 (58.6)	19 (73.1)	21 (75.0)	
Prev. IVT	No (%)	23 (79.3)	20 (76.9)	21 (75.0)	0.927*
	Yes (%)	6 (20.7)	6 (23.1)	7 (25.0)	
Prev. PRP	No (%)	25 (86.2)	20 (76.9)	23 (82.1)	0.671*
	Yes (%)	4 (13.8)	6 (23.1)	5 (17.9)	
Prev. MPC	No (%)	24 (82.8)	22 (84.6)	23 (82.1)	0.969*
	Yes (%)	5 (17.2)	4 (15.4)	5 (17.9)	
DM Duration		9.38 ± 4.7	8.81 ± 4.04	9.36 ± 4.21	0.861†

HbA1C	8.04 ± 0.72	8.12 ± 0.64	8.25 ± 0.61	0.499 [†]
FBS	160.28 ± 20.85	162.42 ± 21.2	166.07 ± 21.75	0.586 [†]

Footnotes: [†], ANOVA test; *, Chi-square test

Abbreviations: DM, diabetes mellitus; Ins, insulin; Met, metformin; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative retinopathy; Supp., supplementation; HTN, hypertension; HLP, hyperlipidemia; CVA, cerebrovascular accident; IHD, ischemic heart disease; IVB, intravitreal bevacizumab; IVT, intravitreal triamcinolone acetonide; Prev., previous; PRP, panretinal photocoagulation; MPC, macular photocoagulation; HbA1C, hemoglobin A1C; FBS, fasting blood sugar

3.2 Serum vitamin D levels

In groups 2 and 3, 19 (73.1%) and 20 (71.4%) patients had insufficient (20-30 ng/ml) serum 25(OH)D₃ levels, and 7 (26.9%) and 8 (28.6%) had vitamin D deficiency (serum 25(OH)D₃ levels between 10 and 20 ng/ml), respectively. No intergroup difference was observed in this regard (p>0.05). Mean (±SD) serum vitamin D level at the baseline in group 1 was higher than the other two groups (43.86 ± 6.06 in group 1 versus 17.38 ± 4.59 in group 2 and 16.61 ± 4.35 in group 3).

Serum vitamin D levels in groups 1 and 2 were not changed throughout the follow-up; mean (±SD) serum vitamin D levels in group 3 (receiving vitamin D supplementation) showed significant increases on both checkpoints (one month and six months after the third IVB injection) (p<0.001). Hypovitaminosis D in group 3 was corrected within a month of follow-up (41.68 ± 6.09) (Table 3).

Table3- Mean and Mean changes of serum vitamin D levels on the baseline and one and six months after the third IVB administration.

Time	Parameter	Group			P-value*	Pairwise comparison†
		Group 1 (vitamin D>30ng/ml)	Group 2 (vitamin D: 10-30ng/ml)	Group 3 (vitamin D: 10-30ng/ml + vitamin D supp.)		
Baseline	VitD	43.86 ± 6.06	17.38 ± 4.59	16.61 ± 4.35	<0.001	1-2, 1-3
M1	VitD	43.93 ± 5.79	17.54 ± 4.47	41.68 ± 6.09	<0.001	1-2, 2-3
	VitD change	0.07 ± 2.28	0.15 ± 2.05	25.07 ± 6.43	<0.001	1-3, 2-3
	<i>P-WITHIN</i>	<i>0.872</i>	<i>0.706</i>	<i><0.001</i>		
M6	VitD	44.66 ± 6.62	17.38 ± 4.49	62.32 ± 9.14	<0.001	All
	VitD change	0.79 ± 2.77	0 ± 1.83	45.71 ± 8.35	<0.001	1-3, 2-3
	<i>P-WITHIN</i>	<i>0.134</i>	<i>>0.999</i>	<i><0.001</i>		

*, ANOVA test; †, pairwise T-test with Bonferroni; M, month; supp., supplementation; VitD, vitamin D.

3.3 Visual outcomes

Table 4 and Figure 2.a present the mean (±SD) CDVALogMAR values at the baseline and upon each follow-up ophthalmic examination one, three, and six months after the third IVB injection. Mean (±SD) baseline CDVALogMAR measures were similar in all groups ($p>0.05$). At any given interval (baseline – month 1; month 1 – month 3; month 3 – month 6), visual improvements were significant in all groups ($p<0.001$). No intergroup differences were observed regarding the mean changes of CDVALogMAR measure at the first two follow-up checkpoints, i.e., months 1 and 3. However, mean CDVALogMAR changes between the third and sixth months in groups 1 and 3 were significantly greater than in group 2, comprising DME patients with insufficient/deficient vitamin D levels whose hypovitaminosis D was not corrected. The mean (±SD) change in CDVALogMAR values in group 3, who received IVB + vitamin D supplementation, six months after the third IVB injection was -0.2 ± 0.06 , equivalent to two lines of improved visual acuity in clinical terms.

Table 4

Mean and Mean changes in CDVALogMAR values on the baseline, one, three, and six months after the third IVB administration.

Time	Parameter	Group			P-value*	Pairwise comparison [†]
		Group 1 (vitamin D>30ng/ml)	Group 2 (vitamin D: 10-30ng/ml)	Group 3 (vitamin D: 10-30ng/ml + vitamin D supp.)		
Baseline	CDVALogMAR	0.68 ± 0.16	0.72 ± 0.14	0.71 ± 0.14	0.642	
M1	CDVALogMAR	0.6 ± 0.16	0.62 ± 0.14	0.6 ± 0.13	0.877	
	CDVALogMAR change	-0.08 ± 0.06	-0.1 ± 0.04	-0.1 ± 0.02	0.148	
	<i>P-WITHIN</i>	<0.001	<0.001	<0.001		
M3	CDVALogMAR	0.55 ± 0.19	0.55 ± 0.16	0.55 ± 0.14	0.997	
	CDVALogMAR change	-0.13 ± 0.07	-0.17 ± 0.05	-0.16 ± 0.04	0.057	
	<i>P-WITHIN</i>	<0.001	<0.001	<0.001		
M6	CDVALogMAR	0.5 ± 0.17	0.58 ± 0.14	0.51 ± 0.14	0.097	
	CDVALogMAR change	-0.18 ± 0.03	-0.14 ± 0.05	-0.2 ± 0.06	<0.001	1-2 , 2-3
	<i>P-WITHIN</i>	<0.001	<0.001	<0.001		

*, ANOVA test; [†], pairwise T-test with Bonferroni; CDVA, corrected distance visual acuity; CDVALogMAR, CDVA as expressed through the logarithm of the minimum angle of resolution; M, month; supp., supplementation.

3.4 Anatomical outcomes

Table 5 and Figure 2.b present the CMT measures at the baseline and upon each follow-up ophthalmic examination one, three, and six months after the third IVB injection. Mean baseline CMT in groups 2 and 3 was higher than in group 1 (with sufficient baseline vitamin D levels); this intergroup difference was statistically significant only between groups 1 and 2 (p<0.001). All groups showed significant reductions in CMT at any given interval (baseline – month 1; month 1 – month 3; month 3 – month 6) (p<0.001). Mean CMT reduction in group 1 was more prominent than in groups 2 and 3 on the first and second follow-up examinations (months 1 and 3) (baseline – month 1, p=0.015; month 1 – month 3, p=0.002). However, the mean CMT changes between the third and sixth months in groups 3 and 1 were significantly greater than in group 2. At the last follow-up, in the sixth month, the mean CMT measures in groups 1 and 3 were significantly lower than in group 2 (384.59 ± 58.89 and 414.46 ± 55.71, respectively, versus 460.85 ± 43.6; p<0.001). OCTs from different time points pertaining to a patient in group 3 are presented in Figure 3.

Table 5

Mean and Mean changes in CMT on the baseline, one, three, and six months after the third IVB administration.

Time	Parameter	Group			P-value*	Pairwise comparison [†]
		Group 1 (vitamin D>30ng/ml)	Group 2 (vitamin D: 10-30ng/ml)	Group 3 (vitamin D: 10-30ng/ml + vitamin D supp.)		
Baseline	CMT	466.83 ± 57	527.46 ± 42.3	500.61 ± 56.01	<0.001	1-2
M1	CMT	427.83 ± 56.4	476.46 ± 44.37	449.07 ± 55.18	0.004	1-2
	CMT change	-39 ± 20.41	-51 ± 17.84	-51.54 ± 15.12	0.015	1-2, 1-3
	<i>P-WITHIN</i>	<0.001	<0.001	<0.001		
M3	CMT	410.34 ± 55.53	455.12 ± 46.61	429.04 ± 60.21	0.013	1-2
	CMT change	-56.48 ± 14.58	-72.35 ± 20.55	-71.57 ± 20.36	0.002	1-2, 1-3
	<i>P-WITHIN</i>	<0.001	<0.001	<0.001		
M6	CMT	384.59 ± 58.89	460.85 ± 43.6	414.46 ± 55.71	<0.001	1-2, 2-3
	CMT change	-82.24 ± 11.43	-66.62 ± 14.34	-86.14 ± 18.36	<0.001	1-2, 2-3
	<i>P-WITHIN</i>	<0.001	<0.001	<0.001		

*, ANOVA test; [†], pairwise T-test with Bonferroni; CMT, central macular thickness (expressed in microns); M, month; supp., supplementation.

3.5 IVB administrations

As discussed earlier, after completing the triple IVB regimen, patients underwent additional IVB injections during the 6-month follow-up period, when necessary. The mean±SD (median, range) number of IVB injections in the entire study population was 5.33±0.96 (5, 4-7). No intergroup difference was noted in this regard (5.14±0.79 [5, 4-6] in group 1; 5.81±1.17 [6, 4-7] in group 2; and 5.07±0.77 [5, 4-6] in group 3; p>0.05); therefore, a confounding effect of additional IVB injections is unlikely, given their equal distribution across the groups.

4. Discussion

Findings from the present study are as follows: I) DME eyes with hypovitaminosis D gained less therapeutic benefits from IVB treatment than those with sufficient vitamin D levels, both functionally and anatomically, when followed up for six months, II) the unfavorable effect of hypovitaminosis D could be some rebound of DME and/or break in the gradual improvement, six months after the anti-VEGF treatment, rather than early improvement inhibition, and III) correction of hypovitaminosis D in DME patients provides better treatment outcomes, that is comparable to DME patients with normal vitamin D levels, both functionally and anatomically, when monitored for six months.

4.1 DME: current therapeutic challenges

DME is a microvascular complication of diabetes. DME disturbs the central vision and accounts for most cases of visual loss in diabetic patients (18). The estimated prevalence of DME in diabetic patients was around 4% in the United States and showed ethnic variations (19). Neovascularization and vascular abnormalization are key contributors to DME pathogenesis; VEGF can induce retinal angiogenesis and phosphorylation of occludin, ultimately leading to increased endothelial leakage and fluid accumulation (4). Thus, anti-VEGF therapies such as ranibizumab, aflibercept, and bevacizumab are currently the mainstay of DME treatment (20). However, even these treatments fail to bring about visual improvement in a significant proportion of patients (2). Explanations may include the contribution of downstream pathways of VEGF, with delayed upregulation, that may not be inhibited by anti-VEGF treatments alone (e.g., Rho/ROCK signaling pathway)(21), VEGF-independent pro-inflammatory cytokines, and glial activation (2).

4.2 VDD: implications in DR and DME

Overall, the underlying pathogenesis in DME is multifactorial and appears to depend on a complex interaction between several risk factors. One such factor that is indeed the subject matter of this study may be VDD. Several studies have investigated the association of VDD with DR occurrence or severity; the results, however, have been inconsistent. The absence of a decisive conclusion on the matter encouraged Zhang et al. to conduct a meta-analysis of the published data until that time (2017) (5). They found the overall OR of DR in patients with VDD versus those with optimal vitamin D levels to be 1.27 (95% CI, 1.17–1.37; P=0.001). Further elaborating on the impact of altered levels of vitamin D function on DR susceptibility, a recent meta-analysis demonstrated that particular vitamin D receptor (VDR) gene variations influence the risk of DR in diabetic patients, namely the Apal and FokI VDR gene variants (22).

4.3 VDD correction as a promising co-intervention in DME

Some known anti-angiogenic and anti-inflammatory effects of vitamin D (12,23) and the extensive expression of VDR in the retina (24) may imply its deficiency as a contributor to the risk and/or severity of DR and DME. The anti-angiogenic functions of vitamin D in the setting of retinal neovascularization are suggested to be VDR-dependant (9). Chiang et al. showed that $1\alpha,25(\text{OH})_2\text{D}_3$ and MART-10 (its newly synthesized analog with no hypercalcemia induction) could repress a downstream pathway of VEGF-A (i.e.,

ERK1/2) in human umbilical vein endothelial cells, responsible for the migration and invasion of these cells, in vitro and in vivo; MART-10 showed a higher potency. Also, both inhibited an autocrine positive feedback loop of VEGF-A expression, further supporting their anti-angiogenic effects (11). Moreover, in the setting of DR, vitamin D has shown protective measures against high-glucose-induced pro-inflammatory cytokines expression and attenuated the production of reactive oxygen species, both in vitro and in vivo (25).

4.4 Clinical efficacy: the available data and future research directions

The probable effects of VDD and its correction on treatment outcomes in DME patients have not been previously investigated in clinical settings, except for one recent study by Karimi et al. (26). They incorporated a somewhat similar methodological approach to ours; the main difference was their post-treatment monitoring period being one month, while patients in our study were followed up for six months, and treatment outcomes were evaluated on months 1, 3, and 6. They found that vitamin D supplementation for DME cases with hypovitaminosis D could not significantly improve the outcome of treatment with IVB one month after the third IVB injection (26). Our results also confirm this point; no extra therapeutic gain was detected with VDD correction one month after the third round of IVB. However, in the sixth month, a significant visual and anatomical superiority was observed in cases with normalized vitamin D levels over uncorrected VDD cases. This issue highlights the importance of more extended follow-up periods in the framework of future studies on this matter. Another strength of our study was that the efficacy of the implemented vitamin D supplementation protocols was confirmed one month after the final basic IVB injection and at the end of the follow-up.

As Figure 2 represents, the improvement rates from baseline to month 3 were comparable in all groups. Only was it during the third to sixth month that DME subjects with uncorrected VDD showed a reduced rate of improvement. The reason for this delayed difference between VDD and non-VDD subjects is unknown to us. Should future trials confirm a similar delay, a few explanations may be suggested and need to be attested, for example, potential yet unidentified roles of VDD in VEGF-independent DME pathomechanisms; such roles may have been masked within the temporal proximity of anti-VEGF treatment by significant bevacizumab-associated therapeutic effects. Another explanation may be a negatively modifying effect of VDD on glycemic control in DME patients that may take longer than three months to affect the complicated retinal pathologies in those patients. VDD can have direct and/or indirect deleterious effects on insulin secretion and action; it may also be associated with unfavorable upregulation of diabetes-associated systemic inflammation (27). Although results from several studies on the effects of VDD correction on HbA1c levels and insulin resistance in diabetic patients have been conflicting, there seems to be a modest beneficial effect, collectively, especially within a short-term follow-up period (<6 months)(28).

4.5 limitations

The hypothesis mentioned above brings us to a limitation of the present study, i.e., we did not assess the VDD correction effects on serum glycemc indices, such as HbA1c and FBS. Another limitation could be our relatively small sample size.

5. Conclusion

In conclusion, our findings suggest a beneficial effect of VDD correction in DME eyes undergoing anti-VEGF therapies in maintaining the visual acuity improvement and CMT decreases associated with these treatments during six months. Future studies with larger sample sizes and more extended follow-ups that also take changes in glycemc indices over time into account are warranted.

Declarations

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Competing interests

The authors declare that they have no competing interests.

Availability of data and material

The data supporting the findings of this study are available from the corresponding authors, Hosein Nouri and Sepehr Roozdar, upon reasonable request.

Code availability

Not applicable

Authors' contributions

SF and MS conceptualized and designed this study; SF, MS, and SR conducted the interventions, and SR acquired and interpreted the data; SHA and HN contributed to the data interpretation and drafted the manuscript. All authors have approved the final version of the manuscript.

Ethics approval

The ethics committee of Shahid Beheshti University of Medical Sciences approved the protocol of this study (ethics committee reference number: IR.SBMU.MSP.REC.1397.553).

Consent to participate

All patients signed informed consent letters for participating in this study.

Consent for publication

Not applicable

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Figures

Figure 1

CONSORT flow diagram of the present study

Figure 2

The mean CDVALogMAR (a) and CMT(b) measures of each group at different time points. Abbreviations: CDVA, corrected distance visual acuity (LogMAR); CMT, central macular thickness (in millimeters); M, month

Figure 3

optical coherence tomography of a macula from a patient in group 3; a) pronounced macular edema with intraretinal and subretinal fluid accumulation, at baseline (CMT: 694 microns); b, c, and d) showing significant anatomical improvement a month after the third bevacizumab administration (b), maintained throughout the third (c) and sixth months (d), following correction of the patient's hypovitaminosis D (CMTs: 243, 229, and 220 microns, respectively)