The Relationship between Macrovascular Complications and Vitamin D Deficiency in Type 2 Diabetes Mellitus

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Abstract: The presence of hyperglycemia in individuals with Type 2 Diabetes Mellitus (T2DM) is associated with systemic complications within multiple organ systems. Specifically, patients with T2DM have an increased risk of developing macrovascular complications. Interestingly, patients with T2DM are often found to be deficient in vitamin D, a fat-soluble vitamin that not only plays a role in bone growth and gastrointestinal nutrient absorption, but insulin resistance as well. Thus, the purpose of this review is to summarize the literature that associates vitamin D deficiencies with macrovascular complications patients with T2DM. This review will also summarize developments in genetic testing for VDR mutations and their potential role in diabetes progression, as well as the effects of vitamin D supplementation in patients with T2DM.
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1. Introduction

Vitamin D isa group of fat soluble prohormones. In humans, vitamin D is Unique because it can be ingested as cholecalciferol (vitamin D3) or ergocalciferol (vitamin D2) it is found in fish, eggs, fortified milk, cod liver oil and supplements and the body can also synthesize it (form cholesterol) when sun exposure is adequate (hence its nickname, the "sun shine vitamin") insufficiency is defined as a concentration of 25-hydroxyvitamin D between 20-30ng/ml and deficiency below 20 ng/ml. Vitamin D has important actions on glucose metabolism. These include improved insulin exocytosis, direct stimulation of insulin receptor, improved uptake of glucose by peripheral tissuesand improving insulin resistance **1.**

It has got various effects like suppression of cell mediated immunity, regulation of cell proliferation, stimulation of neurotropic factors such as nerve growth factor, Glial cell line-derived neurotrophic factor, neurotropin, suppression of RAAS, reduction of albuminuria, immunomodulatory effects, and anti-inflammatory effects. Thus, vitamin D is implicated in many ways in the development of Type 2 diabetes mellitus and pathogenesis of vascular complication as macrovascular events included myocardial infarction, stroke, cardiovascular death, and coronary or carotid revascularization and microvascular events included retinopathy, neuropathy and nephropathy **2.** Recent stuies show that VDD may have a potential explanation for developing macrovascular complications in patients with T2DM as VDDmay have a role in CVD risk factors including hyperglycemia, insulin resistance, hypertension, dyslipidemia, obesity**,** DKD and atherosclerosis. Proposed mechanisms underlying the cardiovascular protective effects of VD in patients with DM include facilitation of the function of endothelial nitric oxide synthase**2,** and suppression of the RAAS system as experimental studies indicate that 1, 25 (OH) D participates in the regulation of renin-angiotensin axis by directly suppressing renin gene expression. Renin over-expression can be produced in wild-type mice by pharmacological inhibition of vitamin D synthesis, Blunting the adverse effects of AGEs on endothelial cells during hyperglycemia **3**, and prevention of foam cell formation in macrophages**4**, decreasing systemic inflammatory mediators of vascular disease and attenuating immune cells with anti-inflammatory properties and antagonism of prosclerotic effect on vascular smooth muscle cells related to secondary hyperparathyroidism**5.**

Vascular smooth muscle cells and endothelial cells express receptors for vitamin D and have the ability to convert circulating 25 (OH) D to 1, 25 (OH) D. Putative vascular effects of vitamin D are wide ranging and include modulation of smooth muscle cell proliferation, inflammation, and thrombosis. Interestingly, transgenic rats constitutively expressing vitamin D-24-hydroxlyase, the enzyme that catalyzes

the breakdown of 1 to 25 (OH) D, develop substantial atherosclerosis **6.**

Vitamin D deficiency triggers secondary hyperparathyroidism. Parathyroid hormone (PTH) promotes myocyte hypertrophy and vascular remodelling. Other studies suggest that PTH has a proinflammatory effect, stimulating the release of cytokines by vascular smooth muscle cells **7.**

It should be noted that high parathyroid hormone (PTH) levels are a hallmark of vitamin D deficiency and are known to be associated with myocardial hypertrophy and higher blood pressure levels. In addition, increasing evidence suggests that the mutual interplay between vitamin D, parathyroid hormone and aldosterone mediates cardiovascular damage independent of the RAAS **8.**

Vitamin D deficiency has been associated with higher blood pressure levels, which was already shown in most, but not all, prospective studies, as well as meta-analyses of observational studies **9.** An RCT in 130 hypertensive patients who were supplemented with 3000 IU of vitamin D or placebo over 20 weeks during winter in Denmark. They found a non-significant reduction of BP in the results of 24-h ambulatory blood pressure monitoring (ABPM) (−3 mmHg, $p = 0.26$/−1 mmHg, $p = 0.18$). Interestingly, when only vitamin D-insufficient patients were analyzed, with 25(OH)D levels below 32 ng/mL, ($n = 92$), systolic and diastolic BP levels in 24-h ABPM were significantly lowered (−4 mmHg, $p = 0.05$/−3 mmHg, $p = 0.01$) in the therapy group compared to placebo**10.**

Some observational studies indicate an association of vitamin D deficiency with lower high-density lipoprotein (HDL) and higher triglycerides, as well as higher apolipoprotein E levels**11**.

Towards this, a large prospective evaluation of vitamin D levels and blood lipids showed a significant association of lower vitamin D levels with hypercholesterinemia **12.** However, it should be acknowledged that the results on vitamin D and blood lipids are inconsistent and could be confounded by the above-mentioned link of vitamin D and obesity **13**. clinical studies that have evaluated the effect of vitamin D supplementation on blood lipids in some RCTs yielded conflicting evidence. They showed rather inconsistent findings with the majority of the studies reporting on no significant effect on blood lipids when vitamin D supplementation was compared to placebo **13**

Cross-sectional observational studies have confirmed that lower vitamin D levels are associated with endothelial dysfunction and arterial stiffness due to loss of the vasoprotective action of vitamin D which may be mediated by increasing nitric oxide production, inhibiting foam cell formation, and reducing the expression of adhesion molecules in endothelial cells**14.** This is in line with reports from cross-sectional observational studies, which showed that lower vitamin D levels are associated with endothelial dysfunction, as well as increased arterial stiffness **15.** Several, but not all, observational studies that have been published indicated that low vitamin D levels are associated with higher incidence of macrovascular disease espetially cardiovascular events. Even asymptomatic coronary artery disease was associated with lower vitamin D levels in high risk type 2 diabetic patients **16.** In an observational study of patients in the 5-year Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial, low blood 25(OH)D concentrations were associated with an increased risk of macrovascular disease events in type 2 diabetes**17.** another observational study found that lower 25-OHD_3 levels predicted incident CAC and inanother prospective study concluded that VDD independently predicted prevalence and development of CAC, a marker of coronary artery plaque burden, in individuals with type 2 diabetes**18.**

The Framingham Offspring Study assessed subjects with no prior diagnosis of cardiovascular disease. Subjects with severe vitamin D deficiency (25(OH)D <10 ng/mL) experienced a hazard ratio of 1.80 for developing some first cardiovascular event 5 years after follow-up compared with subjects with higher levels of 25(OH)D (>15 ng/mL) **19.** In some study of 1739 Framingham offspring participants without prior cardiovascular disease and found that low 25(OH)-vitamin D levels (< 15 ng/mL) were significantly associated with an increased incidence of a first cardiovascular event during the mean 5.4 years of follow-up. Among diabetics in this cohort, low 25(OH)-vitamin D levels were significantly more common than non-diabetics (11% vs 7) **19.** In an observational study showed associations of VDD with cardiovascular burden and survival in diabetic patients, the potential mechanisms by which treatment of vitamin D can lead to improved survival and lower cardiovascular events reflect the diverse actions of vitamin D in the body**20**.

A similar association with vitamin D deficiency was also demonstrated in the Honolulu Heart Program, which studied 7385 men over a 34-year period **20.**

However, it should be noted that not all single studies reported on a significant association between low 25(OH)D levels and increased risk of CVD. some other data in the ACS patients without stratification for T2DM have not shown an association between vitamin D deficiency with markers of inflammation **21.** Likewise, another study evaluating ACS patients with very low serum 25OHD concentrations, from 6 ng/mL to 17 ng/mL (lower to upper quartile), showed no association with the extent of coronary lesions.

ALso, study examined patients undergoing angiography, but did not associate coronary lesions with vitamin D deficiency. Similarly, hypovitaminosis D has been independently associated with carotid artery intimal-medial thickening, a harbinger of cerebrovascular and cardiovascular events **22.** another study showed in a meta-analysis of seven studies, including 47,809 individuals and 926 cerebrovascular events that, under consideration of established cardiovascular risk factors, the risk for cerebrovascular disease was significantly lower in subjects with high 25(OH)D levels compared to those with insufficient vitamin D status**22.**

With regards to cerebrovascular disease, in a large population-based prospective study in Copenhagen, an increase risk of symptomatic ischemic stroke was observed with decreasing plasma 25(OH)D concentrations **22.** another study suggest that low vitamin D levels are a risk factor for the occurrence of strokes. Another meta-analysis reported on similar results when comparing low *versus* high vitamin D levels, with an RR for strokes of 1.52 in the lowest *versus* the highest 25(OH)D group. In Indian Subjects Vitamin D deficiency was also associated with acute myocardial infarction (MI) **23**. In a study of 250 stroke patients in India, a deficiency of 25(OH)D was independently associated with ischemic stroke, especially large artery atherosclerosis and cardioembolic stroke and studies are needed to establish if vitamin D supplementation reduces the risk of ischemic stroke in the general population **23**. When reviewing these above mentioned meta-analyses, it has to be kept in mind that these observations could also be influenced by confounding factors, such as reduced mobility and physical activity in chronically ill patients, therefore leading to reduced sunlight exposure and lower vitamin D levels. Other confounders, such as increased age or higher rate of obesity, as well as PTH, renin, calcium and phosphorus, cannot be ruled out with certainty, although they are included as possible confounders in most trial analyses **15.**

Polymorphisms in the VDR gene, namely, *Taq*I*, Bsm*I*, Apa*I, and *Fok*I, have been identified. Unfortunately, there have been variable results regarding the possibility that polymorphisms in the VDR gene influence T2DM progression. The inconsistent results are mostly likely due to the fact that the VDR gene is large (75kb) and the fact that different ethnic groups appear to have varying allele frequencies thus making it difficult to isolate and study specific mutations in the gene **20.** Vitamin D receptors have a broad tissue distribution that includes vascular smooth muscle, endothelium, and cardiomyocytes. In vitro, activated 1, 25-dihydroxyvitamin D (1, 25-OH D) directly suppresses renin gene expression, regulates the growth and proliferation of vascular smooth muscle cells and cardiomyocytes, and inhibits cytokine release from lymphocytes. Studies in knockout mice confirm that the absence of vitamin D receptor activation leads to tonic upregulation of the renin-angiotensin system, with the development of hypertension and left ventricular hypertrophy **24.** anotherstudy genotyped patients with Type I diabetes and found that the BsmI genotype of the VDR gene had a significant association with diabetic nephropathy. This finding was expected because Type I diabetes is a genetically inherited disease, but researchers wanted to further investigate genetic polymorphisms for T2DM, which is an adult onset, non-genetic disease. To do so, researchers started looking at single nucleotide polymorphisms (SNPs) in the gene that encodes for the VDR, and they tried to categorize these SNPs depending on the ethnicity of the patients, as SNPs can vary depending on ethnicity. The results were conflicting: The FokI polymorphism was strongly associated with T2DM in an Asian population, whereas another study found no association between FokI and T2DM complications in a Polish Caucasian population **25.** Other researchers analyzed VDR mutations in 264 T2DM patients from the United Arab Emirates. They found that T2DM patients with the TaqI mutation had higher LDL and total cholesterol levels compared to T2DM patients who had the BsmI mutation. They were even able to specify that the AG and GG genotypes within the TaqI group had a stronger association with increased LDL and total cholesterol level**3.** In general, the patients with more severe disease progression in their foot ulcers were more likely to be carriers of the T allele in their VDR gene. Additionally, the frequency of the T allele was higher in patients with elevated levels of TBARS**2.**

Thus, the presence or absence of specific genotypes within the VDR gene in patients with T2DM may be predictive of a patient's risk for experiencing more aggressive vascular disease progression. Scientists should continue to collaborate and investigate which specific mutations in the VDR are predictive of vascular complications, and how dependent these mutations are on ethnicity**14.**

2. Patient and Methods

This is a cross sectional study that was performed between Jan 2017 to Jan 2018 at the department of Internal Medicine, Damanhour Medical National Institute. Patients were briefed by the details of the procedures recruited after informed consent. This study will be conducted on (100) subjects divided into two groups **Group (A):** (80) patients with type 2 diabetes mellitus as follow Forty patients (40) with evidences of macrovascular complications as subgroup

(A1) Forty patients (40) without evidences of macrovascular complications as subgroup (A2) **Group (B):** (20) non-diabetic subjects with age and sex matched as control group.

The inclusion criteria:

- Type 2 diabetes mellitus more than 6 months.
- Age from 30-50.

Exclusion criteria:

- Patients with type 1diabetes mellitus.
- Less than 6 months' duration.
- Receiving vitamin D treatment.
- Patients with disorders affecting vitamin D metabolism such as chronic renal failure, chronic liver disease, hyperparathyroidism, malabsorption syndrome, etc.
- Patients receiving drugs affecting vitamin D or Ca metabolism such as calcium supplement, vitamin D, heparin and diuretics.
- Immobilized patients.
- Uncooperative patient.
- Patient who refuse to give written consent for participation in the study.
- Malnourished patient.
- Presence of any malignant conditions.

After written informed consent was obtained, a well-designed questionnaire was used to collect the data of the recruited patients retrospectively. The questionnaire included socio-demographic characteristics such as age, gender All biochemical assays was carried out by the same team of laboratory technicians using the same method, throughout the study period. The samples were assayed for Fasting blood sugar, Postprandial blood sugar, HbA1c %, Liver function tests, Renal function tests, Microalbuminuria, Total serum cholesterol, HDL-C, LDL-C, Serum triglycerides, Assessment of serum 25-OHD_3 by the electrochemiluminescence immunosay (ECLIA),

Echocardiography assessment of left ventricular mass, Assessment of ankle brachial index using hand-held Doppler and Doppler Carotid Artery to determine the carotid intima media thickness.

Statistical analysis:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

3. Results

This study was conducted on 100 subjects included 28 males and 12 females in the group of patients with T2DM with macrovascular complications (group A1), 24 males and 16 females in the group of patients with T2DM without macrovascular complications (group A2) and 13 males and 7 females in the control group (group B)

The mean age of the studied subjects was 43.85 ± 4.87 years in the group of patients with T2DM with macrovascular complications (group A1), 46.40 ± 4.01 years in the group of patients with T2DM without macrovascular complications (group A2), and 43.10 ± 4.64 years in the control group (group B).

The mean duration of diabetes in the group of patients with T2DM with complications (group A1) was 7.65 ± 3.10 years and 8.43 ± 2.94 years in the group of patients with T2DM without complications (group A2).

The mean FPG was 217.30 ± 38.50mg/dl in the group of patients with T2DM with macrovascular complications (group A1), 147.65 ± 21.60 mg/dl in the group of patients with T2DM without macrovascular complications (group A2) and 96.30 ± 9.01mg/dl in the control group (group B). The mean FPG was statistically significant different between the two groups of patients with T2DM ($p=<0.001$) and also it was statistically significantly higher in the group of patients with T2DM (group A) than the mean FPG in the control group (group B) ($p<0.001^*$).

The mean PPG in the group of patients with T2DM with macrovascular complications (group A2) was 394.67 ± 72.85 and 283.82 ± 35.52 in the group of patients with T2DM without macrovascular complications (group A2) with statistically significant difference between the two groups ($p<0.001$). and also, the mean PPG in the control group (group B) was 114.85 ± 12.90 which was statistically significantly lower than the mean PPG in the group of patients with T2DM ($p<0.001$).

The mean HbA1C percentage in the group of patients with T2DM with macrovascular complications (group A2) was 10.63 ± 2.07% and 7.44 ± 0.64 % in the group of patients with T2DM without macrovascular complications (group A2) with statistically significant difference between the two groups ($p<0.001$). and also, the mean HbA1C percentage in the control group (group B) was 4.99 ± 0.25 which was statistically significantly lower than the mean HbA1C in the group of patients with T2DM ($p<0.001$)

The mean value of HDL-C was 46.80 ± 6.91mg/dl in the group of patients with T2DM with macrovascular complications (group A1) and 51.85 ± 12.23 mg/dl in the group of patients with T2DM without macrovascular complications (group A2) with statistically significant difference between the two groups ($p=0.027$) and also the mean value of HDL-C in the control group (group B) was 70.55 ± 10.56

mg/dl which was statistically significantly higher than the mean value of HDL-C in the group of patients with T2DM (group A) (p<0.001).

The mean value of LDL-C was 166.25 ± 43.35 mg/dl in the group of patients with T2DM with macrovascular complications (group A1) and 106.33 ± 29.86 mg/dl in the group of patients with T2DM without macrovascular complications (group A2) with statistically significant difference between the two groups (p<0.001) and also the mean value of LDL-C in the control group (group B) was 78.85 ± 16.17 mg/dl which was statistically significantly lower than the mean value of LDL-C in the group of patients with T2DM (group A) (p<0.001).

The mean value of TC was 244.88 ± 42.55 mg/dl in the group of patients with T2DM with macrovascular complications (group A1) and 191.63 ± 24.62 mg/dl in the group of patients with T2DM without macrovascular complications (group A2) with statistically significant difference between the two groups (p<0.001). and also, the mean value of TC in the control group (group B) was 163.20 ± 17.13 mg/dl which was statistically significantly lower than the mean value of TC in the group of patients with T2DM (group A) (p<0.001).

The mean value of TG was 170.50 ± 40.13 mg/dl in the group of patients with T2DM with macrovascular complications (group A1) and 161.85 ± 45.59 mg/dl in the group of patients with T2DM without macrovascular complications (group A2) with no statistically significant difference between the two groups (p=0.326). on the other hand, the mean value of TG in the control group (group B) was 69.0 ± 16.43 mg/dl which was statistically significantly lower than the mean value of TG in the group of patients with T2DM (group I) (p<0.001).

The mean value of serum 25-OHD_3 was 11.68 ± 1.74 ng/ml in the group of patients with T2DM with macrovascular complications (group A1) which was statistically significantly lower than the mean value of serum 25-OHD_3 in the group of patients with T2DM without macrovascular complications (group A2) which was 34.06 ± 6.46 ng/ml (p<0.001). Also, the mean value of serum 25-OHD_3 was statistically significantly lower in the group of patients with T2DM (group A) than the mean value of serum 25-OHD_3 in the control group (group B) which was 39.75 ± 3.43 ng/ml (p<0.001).

The mean value of c-IMT was 0.93 ± 0.02 mm in the group of patients with T2DM with macrovascular complications (group A1) which was statistically significantly higher than the mean value of c-IMT in the group of patients with T2DM without macrovascular complications (group A2) which was 0.68 ± 0.03 mm (p<0.001). Also, the mean value of c-IMT was statistically significantly higher in the group of patients with T2DM (group A) than the mean value of c-IMT in the control group (group B) which was 0.59 ± 0.06 mm (p<0.001)

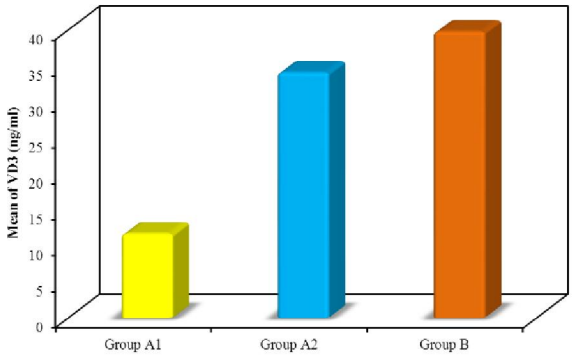

Figure (1): Comparison between the three studied groups according to VD3 (ng/ml).

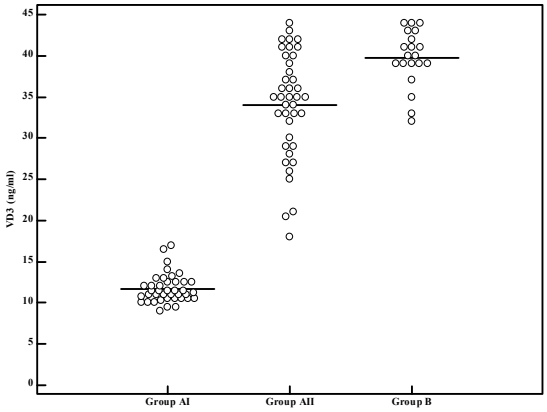

Figure (2): Comparison between the three studied groups according to VD3 (ng/ml).

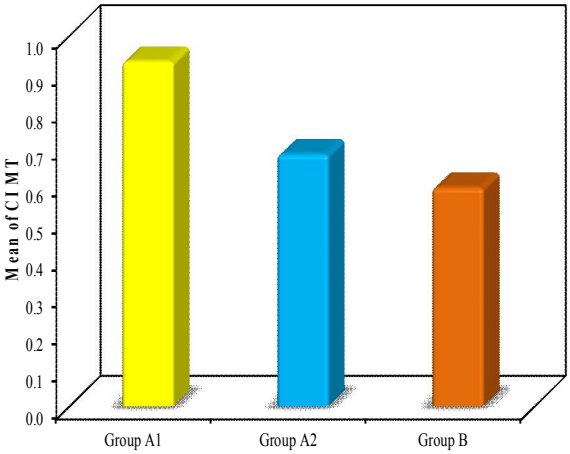

Figure (3): Comparison between the three studied groups according to CIMT.

The mean value of ABI was 0.64 ± 0.07 mm in the group of patients with T2DM with macrovascular

complications (group A1) which was statistically significantly higher than the mean value of c-IMT in the group of patients with T2DM without macrovascular complications (group A2) which was 1.12 ± 0.10 mm (p<0.001). Also, the mean value of ABI was statistically significantly higher in the group of patients with T2DM (group A) than the mean value of ABI in the control group (group B) which was 1.11 ± 0.08mm (p<0.001).

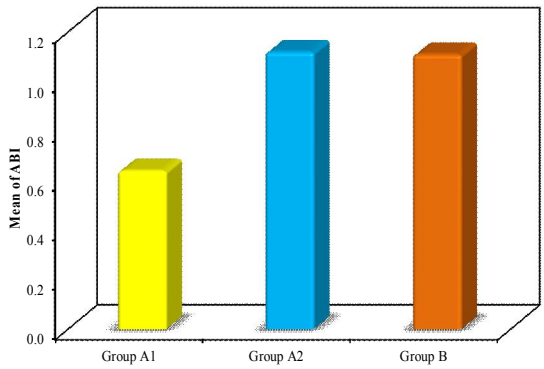

Figure (4): Comparison between the three studied groups according to ABI.

The mean value of LVM was 255.42 ± 29.59 mm in the group of patients with T2DM with macrovascular complications (group A1) which was statistically significantly higher than the mean value of LVM in the group of patients with T2DM without macrovascular complications (group A2) which was 156.83 ± 20.71 mm (p<0.001). Also, the mean value of LVM was statistically significantly higher in the group of patients with T2DM (group A) than the mean value of LVM in the control group (group B) which was 155.40 ± 27.11mm (p<0.001).

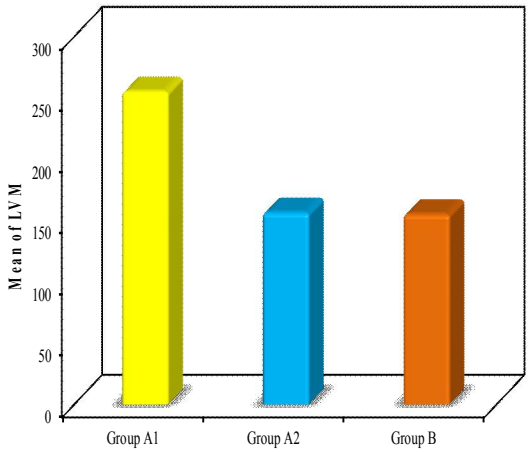

Figure (11): Comparison between the three studied groups according to LVM.

25-OHD_3 serum level was significantly negatively correlated to FPG, HbA1c, cIMT and LVM, while there was no statistically significant correlation between age, diabetes duration, HDL-C, LDL-C, total cholesterol, triglycerides, Urea, Creatinine, Micro albuminuria, ALT, AST and ABI.

4. Discussion

There are relatively a small number of studies which compare VD serum level in patients with macrovascular complications to those without macrovascular complications.

In an observational study of patients in the 5-year Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial, low blood 25(OH)D concentrations were associated with an increased risk of macrovascular and microvascular disease events in type 2 diabetes. A 25(OH)D concentration <20 ng/mL had a higher cumulative incidence of macrovascular and microvascular events than those with levels >20 ng/mL **26.** another study conducted a study which included 200 patients with T2DM and without a history of CAD. The results showed that severe VDD was associated with asymptomatic CAD**22.**

The Framingham Offspring Study assessed subjects with no prior diagnosis of cardiovascular disease. Subjects with severe vitamin D deficiency {25(OH)D <10 ng/mL} experienced a hazard ratio of 1.8 [95% confidence interval (CI), 1.05–3.08] for developing a first cardiovascular event 5 year after follow-up compared with subjects with higher levels of 25(OH) D (>15 ng/mL) **25.** In the Health Professionals FollowUp Study, men without prior cardiovascular disease and vitamin D levels of <15 ng/mL showed a twofold increase in the rate of myocardial infarction **13.** A cross-sectional study found an inverse relationship between lower levels of vitamin D and increased risk of all-cause and cardiovascular mortality**11.** another study found that diabetic patients with vitamin D deficiency had increased prevalence of coronary artery disease (CAD) when examined using coronary angiograph and this finding was consistent for both male and female patients**27**. Also, the results of another cross-sectional study revealed that decreased VD levels were associated with increased c-IMT in patients with T2DM.**10.**

Similarly, in a case-control study of 390 patients with T2DM and 390 age and sex-matched controls reported that hypovitaminosis D was highly prevalent in adults with T2DM and was strongly and independently associated with increased c-IMT**28.** Another study analyzed the IMT of the carotid artery in patients with T2DM found that 130 diabetic patients with vitamin D deficiency had significantly greater IMT compared to 260 diabetic patients who were not

vitamin D deficient. In the data analysis, the researchers also excluded patients who were taking statin medications yet the results remained statistically significant. Thus, the researchers demonstrated that vitamin D deficiency has a strong association with increased cIMT and atherosclerosis development**11.** In addition, studies involving Amish, European–American, and Italian participants revealed that 25(OH)D concentrations are inversely correlated with subclinical atherosclerosis, as measured by carotid atherosclerotic plaque or carotid intima-media thickness (cIMT)**17.** another study found that 130 diabetic patients with vitamin D deficiency had significantly greater cIMT compared to 260 diabetic patients who were not vitamin D deficient**25.**

In addition, a study showed that severe 25(OH)D deficiency is correlated with higher in-hospital mortality among patients presenting with acute coronary syndromes**22.** another study established that VDR knockout mice have elevated blood pressure, cardiac hypertrophy, and elevated activation of the renin–angiotensin aldosterone system (RAAS), which can be reversed with an angiotensin-converting enzyme (ACE) inhibitor**29.**

Another population-based study in patients with type 2 diabetes and elevated urinary albumin excretion rate showed that severe 25(OH)D deficiency (plasma level <12.5 nmol/L) is significantly associated with subclinical atherosclerosis (defined as elevated coronary calcium score ≥400)**1.**

A 20-year retrospective study has found that the incidence of DM complicated with cardiovascular diseases reduced by 33% in population with 800 IU vitamin D and 1200mg calcium of daily intake compared to those with 400 IU vitamin D and 600mg calcium of daily intake, which suggests that vitamin D supplementation might become an effective measurement to prevent the occurrences of T2DM complicated with macrovascular diseases **23.** In case control study of 390 cases (T2D) and 390 controls (normoglycaemic) concluded that vitamin D deficiency (defined as serum 25(OH)D (>37 nmol/l) is more pronounced in patients with T2D compared to controls and showed a strong and independent association with increased cIMT **30.**

In contrast, a published cohort study failed to demonstrate significant association between 25(OH) D deficiency and future development of CHD events **31.** Also, against to our study, another study included 1,125 patients with history of prior myocardial infarction didn't show a significant association between serum 25(OH)D level and incidence of secondary myocardial infarction **17.**

In addition, and in contrast to our study, a published cohort study failed to demonstrate significant association between 25(OH)D deficiency and future development of CHD events. It included 936 veterans with type 2 diabetes (96.9% men, mean age of 59.7 ± 8.4 years). Almost 50% of the participants undergone intensive glucose-lowering therapies. After adjustment for age, therapeutic regimen, and prior CHD events, no difference was observed between 25(OH)D quartiles regarding the occurrence of CHD events during 3.7 years of follow-up **20.**

One RCT mega-study of vitamin D (and calcium) supplementation and CVD risk was from the Women's Health Initiative, in which 36, 282 postmenopausal women received either calcium (1000 mg daily) and vitamin D3 (400 IU daily) or placebo and it found no reduction was observed in MI or CHD death (hazard ratio, 1.04; 95% CI, 0.92–1.18]**13.** In European study**,** elderly individuals receiving a daily equivalent dose of 800 IU of vitamin D did not have improved cardiovascular survival compared to controls and another study has found no correlation between serum 1,25(OH)2D levels and coronary calcification among 50 patients undergoing coronary angiography**32.**

Regards to cerebrovascular disease, in a large population-based prospective study in Copenhagen, an increase risk of symptomatic ischemic stroke was observed with decreasing plasma 25(OH)D concentrations **29.** A similar association with vitamin D deficiency and CAD was also demonstrated in the Honolulu Heart Program, which studied 7385 men with type 2DM over a 34-year period **23.** and in a study of 250 stroke patients in India, a deficiency of 25(OH)D was independently associated with ischemic stroke, especially large artery atherosclerosis and cardioembolic stroke **9.**

In our current study, the mean value of ABI was 0.64 ± 0.07 mm in the group of patients with T2DM with macrovascular complications (group A1) which was statistically significantly higher than the mean value of ABI in the group of patients with T2DM without macrovascular complications (group A2) which was 1.12 ± 0.10 mm ($p<0.001$). Also, the mean value of ABI was statistically significantly higher in the group of patients with T2DM (group A) than the mean value of ABI in the control group (group B) which was 1.11 ± 0.08mm ($p<0.001$).

A number of studies have found an association between low 25-OHD_3 levels and the prevalence of PAD in the general population and in a systematic review which included 13 articles that have shown a significant association between VD and PAD and demonstrated that populations with lower VD levels were more likely to develop PAD in a graded manner. Higher amputation rates were also observed among patients with PAD and lower VD levels **16.**

Similarly, another study reported that individuals with VDD (<20 ng/mL) and PAD had a significantly

higher amputation rate compared with those who were not defined as VD deficient and among 143 patients, the patients having PAD (ABI < 0.9) had significantly lower levels of 25-OHD_3 as reported in an observational cross-sectional study **33.** another study analyzed data from 4,839 participants of the NHANES 2001–2004 to evaluate the relationship between 25-OHD_3 and PAD (defined as an ankle-brachial index <0.9) found that low serum 25-OHD_3 levels were associated with a higher prevalence of PAD and the association between 25-OHD_3 and PAD was similar in all sub-groups including those with T2DM**22.** In a cross-sectional study of 1028 patient with T2DM, concluded that low serum 25-OHD3 levels were significantly associated with a higher prevalence of PAD in T2DM patients < 65 years of age and comparably, the serum level of 25$(OH)D_3$ in T2DM group was lower than the one in control group, and it was the lowest in T2DM with PAD group in a case-control study conducted by **11.**

5. Conclusion

From the results of the present study, the following can be concluded:

Patients with T2DM have significantlylower serum level of 25$(OH)D_3$ compared to their age- and sex-matched controls.

Patients with T2DM with macrovascular complications have further lower levels of 25-OHD_3 than uncomplicated T2DM patients.

25$(OH)D_3$ serum level was significantly negatively correlated with HbA1c, FBG, LVM and cIMT.

Patients with T2DM have higher c-IMT and LVM compared to control subjects and those with macrovascular complications have further higher c-IMT than uncomplicated patients.

Recommendations

- The role of VD in the development of T2DM and its macrovascular complications as cardiovascular disease, cerebrovascular disease and peripheral arterial disease should be examined by more studies including larger numbers of patients and further prospective clinical studies by the treatment with VD.

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