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Review

Vitamin D status and oxidative stress in diabetes mellitus

Wee Chee Lee[1], Siti Safiah Mokhtar[1], Seetha Munisamy[1], Sahran Yahaya[2], Aida Hanum Ghulam Rasool[1*]

[1] Pharmacology Vascular Laboratory, School of Medical Sciences, Universiti Sains Malaysia (Health Campus), 16150 Kota Bharu, Kelantan, Malaysia

[2] Department of Orthopedics, School of Medical Sciences, Universiti Sains Malaysia (Health Campus), 16150 Kota Bharu, Kelantan, Malaysia

Correspondence to: aida.rasool@yahoo.com, aidakb@usm.my

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Abstract: Diabetes mellitus is an epidemic that is gaining global concern. Chronic hyperglycemia in diabetes induces the excess production of free radicals. The deleterious effects of excess free radicals are encountered by endogenous antioxidant defense system. Imbalance between free radicals production and antioxidants defense mechanisms leads to a condition known as "oxidative stress". Diabetes mellitus is associated with augmented oxidative stress that induced micro- and macrovascular complications, which presents a significant risk for cardiovascular events. Low vitamin D levels in the body have also been reported to be associated with the pathogenesis of diabetes and enhanced oxidative stress. The article is to review available literature and summarize the relationship between oxidative stress and vitamin D levels in diabetes. We also review the effects of vitamin D analogs supplementation in improving oxidative stress in diabetics.

Key words: Vitamin D; Oxidative stress; Diabetes mellitus; Deficiency; Supplementation; Cardiovascular risk; Biomarkers.

Introduction

Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disorder, characterized by elevated blood glucose levels over a prolonged period, resulting from defective insulin production and/or insulin action (1). There are three major types of DM:

1. Type I diabetes (T1DM) is due to immune-mediated destruction of pancreatic β-cells that leads to insufficient insulin production;
2. Type II diabetes (T2DM) is due to cellular insulin resistance, whereby the targeted tissues are unable to properly respond to insulin; and
3. Gestational diabetes (GDM), which is glucose intolerance during pregnancy (2).

The worldwide prevalence of DM increases tremendously during the past decades. International Diabetes Federation (IDF) reported that the global prevalence of DM has increased from 285 million (6.6%) in year 2010 (3) to 425 million (8.8%) in year 2017. The figure is estimated to exceed 629 million (9.9%) in year 2045. IDF has also reported that diabetes causes death of 4 million individuals globally (10.7% of global all-cause mortality), and the treatment cost for diabetes constituted 727 billion USD (12.5% of global health expenditure) in year 2017 (4).

In Malaysia, the prevalence of DM increases from 11.6% in year 2006 (5) to 16.9% (3.5 million diabetic patients) in year 2017 (4). It is predicted to rise up to 21.6% in year 2020 with 4.5 million individuals having diabetes (6). Annual expenditure for diabetes treatment in Malaysia has been reported to be as high as RM20.9 billion in year 2015 (7). This constitutes a huge portion of the Malaysian national healthcare budget. In short, high prevalence of DM consumes large amount of global expenditure and will adversely impact on the nation's economy.

Long term augmented blood glucose level, known as chronic hyperglycemia, exposes an individual with DM to the significant risk of specific diabetes-related micro- and macrovascular complications. Microvascular complications include diabetic nephropathy (kidney damage), diabetic neuropathy (neural damage) and diabetic retinopathy (eye damage). On the other hand, macrovascular complications comprise of coronary heart disease, peripheral vascular disease and cerebrovascular disease (stroke) (8).

Both micro- and macrovascular complications contribute significantly to the rates of morbidity and mortality in DM (9), with approximately 65-75% of diabetic deaths being due to cardiovascular diseases (CVD) (10-11). Hence, the prevention of diabetic-related complications becomes the main objective in managing DM (9).

Vascular oxidative stress, in addition to chronic hyperglycemia is reported to be a key contributor in the pathogenesis of DM and its secondary complications (12). In DM, augmented oxidative stress is due to the enhanced free radical-generating process (13-14) and/or impaired capacity of the antioxidant defense system to scavenge the excess free radicals which are induced by chronic hyperglycemia (15).

Vitamin D deficiency was reported to be closely associated with enhanced vascular oxidative stress and increased risk of major CVD in diabetic populations (16). This review aims to explore the association between vitamin D levels and oxidative stress in diabetes. The

possible beneficial effects of vitamin D supplementation in correcting any vitamin D insufficiency leading to improvement in vascular oxidative stress, and the ability to reduce risk of diabetes and cardiovascular complications are also discussed.

Oxidative stress

Oxidative stress is a condition caused by the overproduction of reactive species, known as pro-oxidants and the incapability of the antioxidant defense system to scavenge these species (17). Highly reactive species are normally generated as a by-product of aerobic metabolism in the body (18). These reactive species at a certain amount is often necessary to maintain normal metabolic processes (19). However, the excess in radical species produced is likely to bring deleterious effects to the human body (20); thus they need to be sufficiently removed by the body's antioxidant defense system to maintain the homeostasis of the body system (15).

In diabetes, chronic hyperglycemia induces excessive formation of these reactive species that might diminish antioxidant activity, leading to the domination of oxidative stress (17,21). Oxidative stress then further propagates the production of more reactive species, subsequently causing the development of pathological conditions in DM and its secondary complications (12).

Highly reactive species and free radicals

In general, highly reactive species can be classified into reactive oxygen species (ROS) and reactive nitrogen species (RNS). Both species are divided into free radical or non-radical species. Free radical ROS comprise of superoxide anion ($\bullet O_2^-$), peroxyl ($\bullet RO_2$), hydroxyl radical (•OH) and hydroperoxyl ($\bullet HRO_2^-$) while non-radical ROS include hydrochlorous acid (HOCl) and hydrogen peroxide (H_2O_2). Free radical RNS include nitric oxide (•NO) and nitrogen dioxide ($\bullet NO_2^-$) whereas non-radical RNS comprise of nitrous oxide (HNO_2), peroxynitrite ($ONOO^-$), and alkyl peroxynitrates (RONOO) (22-23). The classification of highly reactive species is listed in Figure 1.

Free radicals are defined as any chemical entities that contain unpaired electrons in an atomic orbital. They are relatively unstable and highly reactive with short half-life (24). They behave as oxidants or reductants because

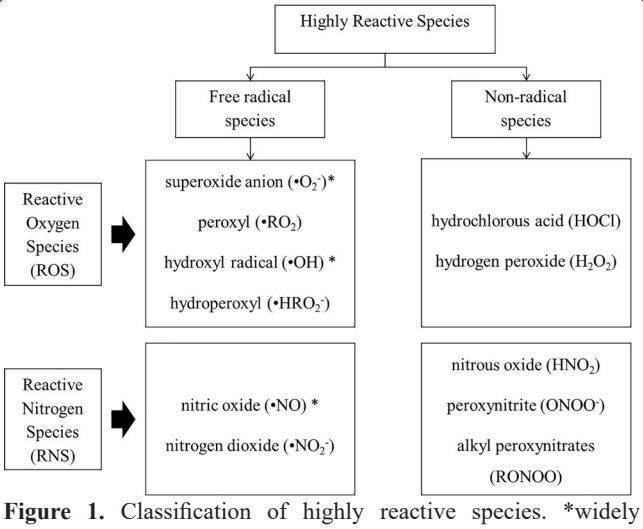

Figure 1. Classification of highly reactive species. *widely addressed in diabetes-induced cardiovascular complications.

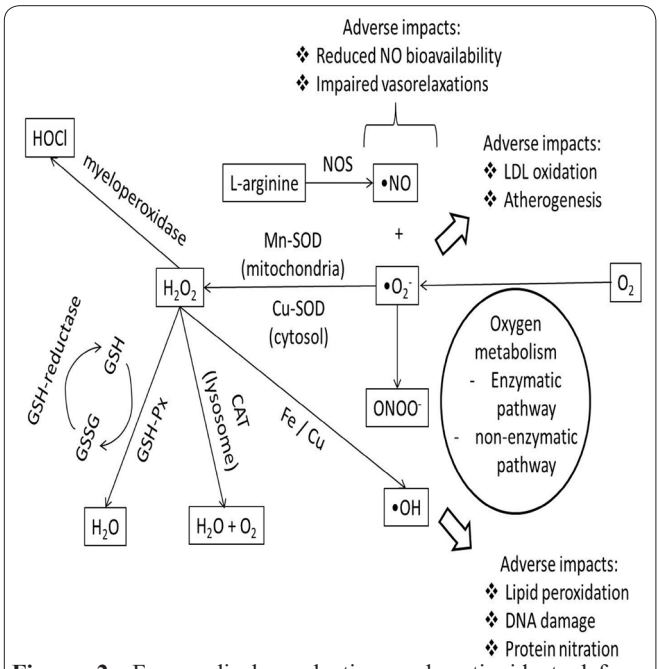

Figure 2. Free radical production and antioxidant defense mechanism. SOD: superoxide dismutase; CAT: catalase; GSH: reduced glutathione; GSSG: oxidized glutathione; GSH-Px: glutathione peroxidase, NOS: nitric oxide synthase; O_2: oxygen; $\bullet O_2^-$: superoxide anion; •OH: hydroxyl radical; HOCL: hydrochlorous acid; H_2O_2: hydrogen peroxide; •NO: nitric oxide; $ONOO^-$: peroxynitrite; H_2O: water; LDL: low-density lipoprotein; DNA: deoxyribo nucleic acid; Mn: manganese; Cu: copper; Fe: iron.

they tend to either accept an electron from or donate an electron to other molecules to achieve stable electron configuration (25).

In diabetes-induced cardiovascular complications, $\bullet O_2^-$, •OH and •NO are among the free radicals that are widely addressed in the literature (26). $\bullet O_2^-$ is produced by one electron reduction of molecular oxygen (O_2) in oxygen metabolism (Figure 2). It is the initial source that propagates the free radical chain reaction. Hydrogen peroxide, H_2O_2 which is produced from $\bullet O_2^-$ during dismutation, in the presence of transition elements such as copper and iron, might be converted to •OH (27). •OH is highly reactive and the most potent oxidant to attack most biological molecules, further propagating the chain reaction. $\bullet O_2^-$ might also react rapidly with •NO to produce cytotoxic $ONOO^-$. This pathological modification impairs the role of •NO to act as the mediator of vascular tone and inhibits its anti-proliferative property, leading to the pathogenesis of vascular dysfunction (22).

Metabolic abnormalities of DM induce mitochondrial overproduction of $\bullet O_2^-$, leading to uncontrolled elevation of free radicals (28). Free radicals attack important macromolecules in the body, leading to cellular damage and homeostatic disruption (29). Free radicals also lead to foam cell formation and atherosclerotic plaque by stimulating the low-density lipoprotein (LDL) oxidation (30), causing it to be taken up by scavenger receptors of macrophages (26). Besides the uncontrolled propagation of free radical chain reaction, free radicals also involve in the alteration of enzymatic antioxidant defense system by impairing glutathione metabolism and thus reducing the levels of antioxidant

(1). The reduced level of antioxidant makes the cell and tissue more prone to oxidative stress, causing oxidative damage, further exacerbating diabetes complications (31).

Antioxidant defense system and antioxidants

Antioxidant defense system plays a pivotal role to scavenge excess radical species and neutralize the toxicity arising from the elevated amount of reactive species. The system is generally divided into endogenous and exogenous antioxidants (32). Enzymatic endogenous antioxidants include superoxide dismutase, catalase and glutathione (oxidized/reduced) while exogenous antioxidants can be acquired from diet and supplements. The antioxidant defense mechanism is simplified in Figure 2. Vitamin C and vitamin E are among the most classic naturally occurring antioxidants that regulate oxidative stress in the pathogenesis of diabetes-related vascular complications.

Superoxide dismutase (SOD)

SOD is an important antioxidant enzyme in the regulation of oxidative stress in DM (16). It acts as a first line defense against reactive species to reduce oxidative stress and subsequently reduce the risk of cellular and histological injury (33). The different forms of SOD, manganese superoxide dismutase (Mn-SOD) in the mitochondria and copper superoxide dismutase (Cu-SOD) in the cytosol catalyze the dismutation of $\bullet O_2^-$ into less toxic O_2 and H_2O_2 (27). Alteration in the metabolic state of DM leads to diminished activity and level of SOD in diabetic organs, tissue and blood (34-35). Reduced SOD level elevates the production of reactive species, leading to augmented vascular oxidative stress and increased CVD risk in diabetics.

Catalase (CAT)

CAT is another important enzyme that metabolizes H_2O_2 into H_2O and O_2 in lysosome (26). Chronic hyperglycemia induces the augmented formation of H_2O_2 and down-regulates the gene expression of CAT (36), leading to the decreased production of this enzyme. As the major regulator in H_2O_2 metabolism, CAT enzyme deficiency causes cell injury mediated by the accumulation of H_2O_2 in diabetic model.

Glutathione (GSH)

GSH acts as direct scavenger and co-substrate for the enzyme glutathione peroxidase (GSH-Px). GSH-Px is one of the antioxidant enzymes in H_2O_2 metabolism that metabolizes H_2O_2 into H_2O and O_2 in mitochondria (26). Reduced GSH-Px expression has been reported in most diabetic models (37-38).

Vitamins

It is well established that vitamin C and E are non-enzymatic exogenous antioxidants against oxidative stress in the prevention of diabetes and its complications. These antioxidant vitamins neutralize free radicals directly and also interact in recycling processes to regenerate reduced forms of vitamins for further antioxidant actions (39).

The molecular structure of vitamin C offers electron donating and accepting potential to be involved in redox reaction, thus it acts as a reducing agent in free radical-mediated oxidation processes to efficiently scavenge free oxygen radicals (40). Vitamin E is a fat-soluble vitamin that can protect cell membrane from oxidative damage by discontinuing a potentially destructive series of oxidative chain reactions on the structural and functional components of cells and vessel walls (41). Vitamin E has proved to be effective in preventing glucose -induced lipid peroxidation and other free radical-driven oxidative events. It can also prevent LDL oxidation and reduce oxidized LDL uptake which will subsequently lead to foam cell and atherosclerotic plaque formation, by downregulating the protein expression of scavenger receptor (42).

The supplementation of vitamin E (alone or in combination) produces a significant impact on the parameters of antioxidant status, particularly in plasma antioxidant capacity and enzyme concentrations. Unlike vitamin E, vitamin C contributes in neutralizing the radical form of other antioxidants to regain their antioxidant ability. Hence, intervention using vitamin C did not show impactful outcome compared to those using vitamin E. However, intervention using the combination of vitamin C and E showed promising results in increasing GSH and SOD activity (43-44).

Considerable efforts have been put into studying the antioxidant action of vitamin D in the past decade. Vitamin D may be regarded as an antioxidant in terms of their homologous structure to cholesterol. Wiseman *et al.* (1993) demonstrated that vitamin D acts as a membrane antioxidant in view of its ability to inhibit iron-induced lipid peroxidation of brain liposomes (45). Moreover, vitamin D has been reported to attenuate oxidative stress by up-regulating antioxidant enzymes and suppressing elevated lipid peroxidation (37,46).

Oxidative stress biomarkers

The reactions of free radicals and antioxidants occur instantaneously; hence it becomes a major problem to perform direct measurement on oxidative stress (47). Thus, the indirect way to evaluate oxidative stress is through observable biomarkers (48). The effectiveness of antioxidant defense system to counteract elevated amounts of free radicals can be measured by the levels of the endogenous antioxidant enzymes. Meanwhile, excess production of free radicals can be determined by measuring the products produced as the result of oxidative damage caused by these species.

Oxidative damage comprises of cellular protein glycation, membrane lipid peroxidation and the damage to nucleic acids (49). Among the oxidative damage, augmented lipid peroxidation has been reported to be closely associated with chronic hyperglycemia in DM (50). DM alters the lipid profile of the cells by removing hydrogen from lipids to produce $\bullet HRO_2^-$ when attacked by $\bullet RO_2$, further propagating the free radical pathway (29). Furthermore, the natural presence of multiple bonds in polyunsaturated fatty acids in cell membrane makes them more susceptible to free radicals for lipid peroxidation.

$\bullet HRO_2^-$ can exert direct toxicity on the cells; it can be degraded to •OH, or to react with transition metals (such as iron or copper) to form stable aldehydes (30), such as malondialdehyde (MDA) to consequently damage cell

membranes (51). A stable MDA has been documented as a primary biomarker (52) to evaluate lipid peroxidation, mostly studied with thiobarbituric acid reactive substances (TBARS) assays (53). Elevated TBARS and MDA levels in plasma, serum, and other tissues in diabetic patients suggest that peroxidative injury may be involved in the development of diabetes complications (12,50).

Vitamin D

Vitamin D is classically known for its role as an important hormone in mineral homeostasis and maintenance of musculoskeletal health (54). However, vitamin D also possesses antioxidant properties as potent as, or even better than the classical antioxidant vitamin E (45,55-57). Furthermore, vitamin D has also been discovered to be a potent hormone that exerts significant biological actions, such as induction of cell differentiation, reduction in inflammation and immunomodulation (58).

Vitamin D is a fat-soluble vitamin, whereby more than 90% are obtainable by cutaneous production from sunlight exposure while only approximately 10-20% is obtained by dietary intake. There are two major forms of vitamin D, which differ chemically only in their side chains. Ergocalciferol (vitamin D_2) is synthesized by ultraviolet irradiation of plant sterols (ergosterol) and invertebrates while cholecalciferol (vitamin D_3) is photosynthesized endogenously when solar ultraviolet B radiation with wavelength of 280-320 nm strikes human epidermis. Irradiation stimulates non-enzymatic photolytic conversion of pro-vitamin D (7-dehydrocholesterol) to pre-vitamin D, thereafter undergoes thermal isomerization into vitamin D_3 (54). Vitamin D_3 is also naturally present in food especially from animal sources, such as oily fish, fortified dairy products and animal fats.

Vitamin D is biologically inert and needs to be biologically activated via two hydroxylation processes in the body before being utilized for biological actions. Vitamin D is absorbed in the small intestines, transported via the lacteal system and conveyed via the lymphatic system into the venous circulation (59). Vitamin D is then bound to vitamin D-binding protein (DBP), and is transported to the liver via the blood circulation in this bound form (60). In the liver, hepatic cells transform vitamin D into 25-hydroxyvitamin D, 25(OH)D (calcidiol) in the presence of vitamin D 25-hydroxylase enzyme. In the kidney, 25(OH)D is then metabolized either intrarenally by 25(OH)D-1α-hydroxylase enzyme or intracellularly at extra-renal sites in a variety of cells/tissues to the physiologically active vitamin D, 1,25-dihydroxyvitamin D, $1,25(OH)_2D$ (calcitriol) (59,61). $1,25(OH)_2D$ entered the bloodstream to act as the primary steroid hormone in mineral and skeleton homeostasis. The process of vitamin D synthesis and metabolism is illustrated in Figure 3.

Vitamin D status and supplementation

Instead of $1,25(OH)_2D$, serum 25(OH)D is the most common determinant of vitamin D level in the body (62). This is in view of its longer circulating half-life of 15 days (63); it is also the primary circulating form of vitamin D (64). The level of serum 25(OH)D reflects the precise storage amount of vitamin D (both by solar-

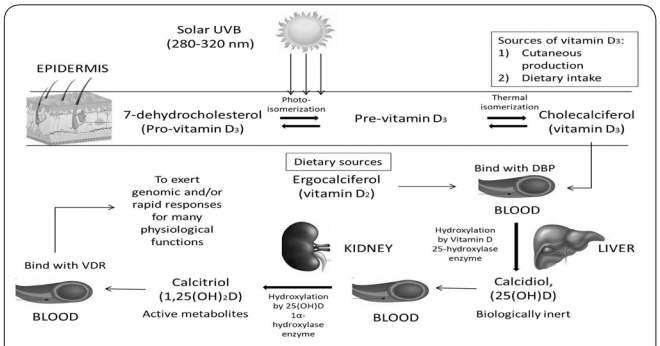

Figure 3. Vitamin D synthesis and metabolism. UVB: ultraviolet B radiation; DBP: vitamin D-binding protein; 25(OH)D: 25-hydroxyvitamin D; $1,25(OH)_2D$: 1,25-dihydroxyvitamin D; VDR: vitamin D receptor.

activated cutaneous production and long period dietary intake) in the human body. The status of vitamin D (deficiency, insufficiency and sufficiency) in the body can be defined after measurement of serum 25(OH)D. The Endocrine Society defines vitamin D insufficiency as serum 25(OH)D between 50–74 nmol/L, serum 25(OH)D of less than 50 nmol/L as vitamin D deficiency (65) while vitamin D sufficiency as serum 25(OH)D of at least 75 nmol/L. The optimal range of 75-125 nmol/L is needed to optimize intestinal calcium absorption and to cover all physiological functions of vitamin D (66).

Hypervitaminosis D

Hypervitaminosis D, or commonly known as vitamin D toxicity, occurs when excess pharmacologic doses of vitamin D is taken, translating to a large increase in circulating 25(OH)D concentration, that is beyond DBP binding capacity. Excessive sun exposure does not lead to hypervitaminosis D as pre-vitamin D_3 will undergo photo-degradation to inactive sterols in the skin (54). The main clinical consequences of vitamin D toxicity are hypercalcemia and other symptoms including hypercalciuria, ectopic calcifications, hyperphosphatemia, kidney stones, polyuria and polydipsia, hypertension, anorexia, nausea, vomiting and constipation (62). Although it is uncommon, hypervitaminosis D has been reported in multiple age groups and from multiple causes, including manufacturing errors (67), errors in milk fortification (68), incorrect dosing from liquid preparations (69) and ingestion of mega doses of vitamin D supplements (70). There are studies which had reported an increase in cancer incident rates and mortality risks at both low and high levels of serum 25(OH)D (71-72). Nimesh *et al.* (2015) reported that a one-year old child developed acute hypertension and severe hypercalcemia due to vitamin D toxicity after high doses of oral calcitriol supplementation (70). Gallagher (2016) also showed that the administration of annual bolus doses of vitamin D at concentration of 300,000 IU or 500,000 IU resulted in the increased risk of falls and fractures (73). Therefore, until further evidence is available, a reasonable upper limit for 25(OH)D level at 125 nmol/L (50 ng/mL) is suggested in elderly individuals (73), infants (74) and healthy young adults (75).

Discussion

Vitamin D status and diabetes mellitus

In the development of adverse cardiovascular events,

especially in DM, low vitamin D level is suggested as one of the risk factors (59). This is supported by statistics showing high prevalence of vitamin D insufficiency and deficiency occurring in the diabetic population; 91.1% in India (76), 73.6% in Saudi Arabia (77) and 43% in Malaysia (78).Studies have shown that higher vitamin D levels were associated with 40% lower risk of T2DM in women while subjects that developed diabetes had lower vitamin D levels (serum 25(OH)D < 50 nmol/L) compared to non-diabetics (79). This suggested that low circulating vitamin D concentrations played a significant role in the pathogenesis of DM. This might occur via decreased pancreatic insulin release, underscored insulin resistance, reduced insulin sensitivity and deteriorated glucose tolerance (80). This is supported by Wang's study, whereby the incidence of adverse CVD was doubled in individuals with low vitamin D levels (serum 25(OH)D <37.5 nmol/L) in a 6-year-period study (16).

Moderate or severe vitamin D deficiency also increases the activities of glutathione-dependent enzymes (16), resulted in overproduction of ROS in diabetics (81). Vitamin D supplementation might be an alternative way to improve the vitamin D levels in the body, when there is insufficient cutaneous production or dietary intake. Vitamin D_3 supplementation is 87% more effective in raising serum 25(OH)D level compared to vitamin D_2 as it is converted 500% faster to calcitriol, the biologically active hormone (82). Vitamin D_3 is also the natural form of vitamin D produced by our body which is more potent in raising and maintaining vitamin D concentrations in the circulatory system longer, and produces 2-3 fold greater storage of vitamin D. Besides, the action of vitamin D_3 is approximately three times more effective because it binds with high affinity to DBP in plasma, thus it can stay longer in the circulation (59).

Vitamin D status and oxidative stress

In some studies involving human and animal models of diabetes or vitamin D deficiency, supplementation with vitamin D showed reduction in the levels of oxidative stress (37-38,83-84). Calcitriol supplementation had been shown to improve SOD activities and reduce ROS production in renal arteries of hypertensive patients as well (81).

In diabetic rats, high level of serum TBARS was significantly reduced nearly to control values after the treatment with vitamin D_3 (83). In asymptomatic vitamin D-deficient subjects, supplementation with vitamin D_3 (300,000 IU monthly for 3 months) significantly decreased serum TBARS similar to that of basal TBARS in control group (84). In subjects with T2DM, treatment with vitamin D_3-fortified doogh (500 IU twice a week for 12 weeks) showed reduction in MDA and increased GSH levels (37). However, treatment with vitamin D_3 capsules (50,000 IU twice) did not affect the biomarker of oxidative stress (MDA and GSH levels) in women with GDM (85). Similarly, Efterkhari *et al.* (2014) reported that there was no significant change in MDA levels in T2DM subjects after receiving calcitriol supplementation (0.5 µg/day for 12 weeks) (86). Another study in T2DM vitamin D-deficient patients reported that vitamin D_3 treatment (5,000 IU/day for 12 weeks) did not significantly affect SOD levels (87).

The difference in findings between these studies might be due to: 1) vitamin D dosage and study duration; 2) the status of vitamin D in the chosen diabetes population studies; and 3) the type of vitamin D analog used in the study. No improvement to oxidative stress markers was observed in the study by Yiu *et al.* (2013) despite increase in serum 25(OH)D levels. The vitamin D_3 dose given for only a short duration of treatment (12 weeks) may not be adequate to sufficiently increase the already low baseline serum vitamin D levels to therapeutic levels in their study subjects (87). No effect of vitamin D_3 supplementation on oxidative stress markers in women with GDM might be caused by insufficient dosage of vitamin D_3 given to the study subjects, which was 50,000 IU per capsule given twice throughout the study period of 6 weeks (85). The type of vitamin D analog used for the treatment was also a critical indicator. Vitamin D_3 performs better than vitamin D_2 as an effective supplement because non-active vitamin D metabolites might face impairment in their conversion to the active metabolite, especially in patients with kidney problems. This results in reduction of serum vitamin D concentrations in the circulation that is able to improve oxidative stress in diabetics. A limitation to the interpretation of the effect of vitamin D supplementation on oxidative stress is, some articles did not report baseline levels of vitamin D and oxidative stress biomarkers of enrolled patients before supplementation. These parameters should be measured to display better outcome on the beneficial effect of vitamin D supplementation, and also to eliminate the impact of confounding variables that might interfere with the results.

A study reported on the potential of vitamin D_3 supplementation either as a preventive measure or therapeutic strategy in diabetic rats. Diabetic rats received 5000 IU/kg bw/day vitamin D_3 supplement by gastric gavage before and after alloxan-induction respectively for 2 months as preventive and therapeutic groups of diabetes. The results showed that administration of vitamin D_3 in both groups (preventive and therapeutic groups) enhanced hepatic and renal activity of SOD, CAT and GSH-Px, as well as reduced lipid peroxidation as indicated by decreased TBARS level compared to untreated diabetic rats. However, vitamin D might play a better role in regulating oxidative stress prior to the development of diabetes as shown by the better significant improvement in the preventive group compared to the therapeutic group of diabetic rats (38). The relationship between vitamin D interventions and oxidative stress biomarkers in animal and human studies is summarized in Table 1.

Vitamin D status and atherosclerosis

Epidemiological studies demonstrate that low levels of vitamin D is a risk factor for development of atherosclerosis (88). Vitamin D deficiency correlates with endothelial dysfunction, vascular smooth muscle cell (VSMC) proliferation and migration, augmented systemic inflammation, increased intima-media thickness (IMT) and enhanced oxidative stress in the development of atherosclerosis (16,89-91).

Endothelial dysfuntion is the precursor of early atherosclerosis development, which is due to the imbalance in production of endothelium-derived relaxing and

Table 1. Relationship between vitamin D interventions and oxidative stress biomarkers in animal and human studies.

Animal model/ Population studied	Intervention/ Treatment	Summary of findings	References (historical sequences)
Animal study			
Alloxan-induced diabetic adult male Wistar rats n = 10	Calcitriol (5,000 IU/kg bw) Once daily for 4 weeks Gastric gavage Study duration: 2 months i) preventive group: 15 days before alloxan injection ii) therapeutic group: 15 days after alloxan injection	• Marked elevated of SOD, CAT and GSH-Px in hepatic and renal tissues of both treatment groups. • Activity of SOD, CAT and GSH-Px in hepatic and renal tissues was significantly higher in the preventive group than the therapeutic group. • Reduced TBARS levels in hepatic and renal tissues of both treatment groups. • Reduction in TBARS is more significant in the preventive group than the therapeutic group.	Hamden K *et al.*, 2009 (38)
STZ-induced diabetic adult male Wistar rats n = 6-8	Cholecalciferol (12 µg/kg bw) Once daily for 14 days Oral administration Study duration: 15 days	• Significant elevated levels of TBARS in liver of diabetic group were significantly reversed to near control value in treatment group. • Significant decrease in SOD and GSH-Px gene expression in liver of diabetic group were reversed nearly to control level for treatment group.	George N *et al.*, 2012 (83)
Animal model/ Population studied	**Intervention/ Treatment**	**Summary of findings**	**References (historical sequences)**
Human Study			
Asymptomatic vitamin D-deficient subjects baseline level of plasma 25(OH)D < 25 nmol/L n = 23	Vitamin D_3 (300,000 IU) Once monthly for 3 months Intramuscular injection	• Low baseline plasma 25(OH)D levels were significantly increased after treatment. • High basal TBARS levels were decreased significantly after treatment, similar to that of basal TBARS in control group.	Tarcin O *et al.*, 2009 (84)
Pregnant women with GDM baseline level of serum 25(OH)D < 75 nmol/L n = 27	Cholecalciferol capsules (50,000 IU) Twice (baseline and on 21st day) Oral administration Study duration: 6 weeks	• Serum 25(OH)D increased significantly. • Plasma TAC levels were not affected. • No significant change in total GSH. • Significant decrease in fasting plasma glucose, serum total and LDL-cholesterol concentrations.	Asemi Z *et al.*, 2013 (85)
T2DM patients baseline level of serum 25(OH)D < 75 nmol/L n = 50	Vitamin D_3 tablets (5,000 IU) Once daily for 12 weeks Oral administration	• Significant increases in serum vitamin D levels. • No significant change in SOD levels.	Yiu *et al.,* 2013 (87)
Hyperlipidemia T2DM patients unknown baseline vitamin D levels n = 35	Calcitriol capsules (0.5 µg) Once daily for 12 weeks Oral administration	• Significant reduction in basal MDA levels in both placebo and treatment group. • The alteration in MDA levels was not significant between the groups.	Eftekhari *et al.*, 2014 (86)
T2DM subjects baseline level of serum 25(OH)D < 40 nmol/L n = 50	Vitamin D_3-fortified doogh (500 IU) Twice a week for 12 weeks Oral administration	• Serum 25(OH)D increased significantly. • Reduction in serum MDA levels. • Increased GSH levels.	Shab BS *et al.*, 2015 (37)

STZ: streptozotocin; bw: body weight; SOD: superoxide dismutase; CAT: catalase; TAC: total antioxidant capacity; MDA: malondialdehyde; GSH-Px: glutathione peroxidase; TBARS: thiobarbituric acid reactive substances; T2DM: type 2 diabetes mellitus; GDM: gestational diabetes mellitus; 25(OH)D: 25-hydroxyvitamin D.

contracting factors. Vitamin D exerts direct vasoprotective effects against endothelial dysfunction by enhancing endothelial-dependent vasorelaxation and inhibiting vasocontraction (92-93). Vitamin D improves the bioavailabity of endothelial nitric oxide (NO), the potent vasorelaxing factor and inhibitor of platelet and leucocyte aggregation and adhesion. This occurs via direct enhancement of transcriptional regulator of endothelial nitric oxide synthase (eNOS) (94) and/or exert effect on phosphatidylinositol 3 kinase in endothelial cell (EC), which activates eNOS to catalyse the production of NO from L-arginine (95-96). By inducing NO production, vitamin D able to stimulate EC proliferation and inhibit apoptosis (97). Vitamin D also induces the production of prostacyclin in VSMC through the cyclooxygenase (COX) pathway, which prevents thrombus formation, cell adhesion and VSMC proliferation (98-99). Besides that, vitamin D interferes with calcium influx into the EC (100), decreases the expression of COX-2 and downregulates the expression of prostaglandin receptors to reduce the production of vasoconstrictors (101).

IMT, which is a reflection of atherosclerotic burden was reported to be negatively related to serum 25(OH)D concentration (90). There was marked increase in carotid artery IMT in T2DM patients with low vitamin D levels compared to those with higher vitamin D levels. T2DM patients who developed carotid plaque has significantly lower 25(OH)D concentration compared to T2DM patients without carotid plaque (102). Vitamin D and its analogue suppress the mechanisms that leads to increased IMT and vascular calcification by inhibiting the over-expression of multiple adhesion molecules on EC (91) and the accumulation of plaque lipid in VSMC (103).

Vitamin D also has anti-inflammatory properties. It may suppress the production and release of several pro-inflammatory (91) and increase the production of anti-inflammatory cytokines (104). Vitamin D downregulates the inflammatory process by limiting the major role of T-helper 1 in pro-atherogenic response and shifting the T-cell response from T-helper 1 to T-helper 2 to limit the pro-atherogenic response (105). Vitamin D displays anti-atherogenic properties through an endoplasmic reticulum (ER) stress-dependent mechanism. ER stress is a functional switch that controls macrophage differentiation which may have a role in atherosclerotic plaque regression in diabetics (106). Vitamin D acts as ER stress reliever to prevent foam cell formation during macrophage differentiation, reduce macrophage infiltration and migration and stimulate an anti-atherogenic macrophage phenotype, thus reducing vascular inflammation and complications in T2DM patients. Vitamin D also reverses atherogenic cholesterol metabolism deposition by preventing the progression of macrophage cholesterol uptake and promoting cholesterol egression in macrophages from T2DM patients (107).

In order to counteract the dominant effect of oxidative stress in human endothelial cells, vitamin D analogs act as a negative endocrine regulator of the renin-angiotensin system by reducing the renin synthesis (108). Vitamin D also inhibits the augmented production of ROS, especially superoxide anion (100) through the authophagic and survival pathways (109). Vitamin D also improves SOD, CAT, GSH-Px in enhancing antioxidant defense mechanism and reduces lipid peroxidation that will lead to oxidative cell damage (37-38,83-84).

Conclusion

The relationship between vitamin D levels and oxidative stress in diabetes has been quite well studied. Low vitamin D levels substantially impaired insulin and glucose metabolism which may contribute to the pathogenesis and development of DM. Chronic hyperglycemia in DM further disrupted the homeostasis between the generation of radical species and the effectiveness of enzymatic antioxidant defense system (47), predisposing to the development of diabetes-related cardiovascular incidents and its complications, leading to morbidity and mortality.

Optimal control of blood glucose level might be able to slow down diabetes complications, although it is not able to completely prevent diabetes complications. Thus, evidences on the ability of antioxidants to regulate oxidative stress in diabetes are compelling and suggests potential additional treatment strategy to reduce cardiovascular risk and complications in diabetes. In view that vitamin D supplementation is able to improve many cardiovascular biomarkers in both diabetic (78, 110) and non-diabetic (111) population with vitamin D deficiency, it may be a potential measure in regulating oxidative stress underlying diabetic complications. By correcting the vitamin D levels as shown by accumulating evidences in epidemiology studies, enhanced oxidative damage and reduced antioxidant activities might be reversed or at least improved in diabetic population. Even though clinical trials conducted to date failed to provide adequate support for the implementation of vitamin D supplement in diabetes treatment, but they did show improvement on certain biomarkers for cardiovascular health. Thus, using vitamin D supplement in diabetes treatment, especially in vitamin D-deficient and insufficient patients, definitely deserves further assessment and consideration.

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Conflict of interests

None.

Authors' contribution

C. L. WEE – drafting the article
S. S. MOKHTAR – proofread and revision of the article
S. MUNISAMY – gathering information to prepare draft
S. YAHAYA – outline the design and critical revision of the article
A. H. G. RASOOL – conception and final revision/approval of the article.

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