Association of Vitamin D with oxidative stress in patients with Type 2 diabetes mellitus

Running title: Vitamin D and oxidative stress in patients with T2DM patients

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Abstract

Background: Type2 Diabetes mellitus (T2DM) is non communicable disease including hyperinsulinemia, insulin resistance, hyperglycemia and low-grade chronic inflammation associated with various micro and macro vascular complications. The present study was aimed to estimate the Vitamin D levels, total antioxidant capacity and Malondialdehyde (MDA) levels in T2DM patients compared with healthy volunteers, further to assess the vitamin D association between HbA1c, insulin resistance, total antioxidant capacity, MDA, lipid profile levels.

Methods: Seventy type 2 diabetic patients with age group of 35 to 50 years were selected and 70 healthy age matched subjects were selected as controls. Serum Vitamin D and Insulin was estimated by the enzyme-linked immunosorbent assay (ELISA), Glycosylated hemoglobin (HbA1C) was assessed by High-performance liquid chromatography (HPLC) method and other routine lipid profile investigations carried out by Beckman Coulter fully automated analyzer.

Results: Vitamin D levels were significantly decreased in type 2 diabetic patients. HbA1C and insulin resistance values are significantly increased in type 2 diabetic patients. Vitamin D levels negatively correlated with MDA, insulin resistance, HbA1c and positively correlated with total antioxidant capacity. But there is no significant correlative changes observed between lipid profile parameters.

Conclusion: Vitamin D deficiency may be one of the vital risk factor responsible for increased oxidative stress in T2DM patients. Regular monitoring and supplementation of vitamin D beneficial for reduction of oxidative stress and vascular complications in T2DM patients.

Keywords: Type 2 Diabetes Mellitus (T2DM), Vitamin D (Vit D), Insulin Resistance Evaluation (IR)

Introduction

Diabetes mellitus is a non-communicable disease concerned as global health burden, further it was estimated in 2019 as 9 th largest cause of mortality and prevalent in all income levels (1-3). Vitamin D reported as to regulate the cellular proliferation, differentiation, and immune modulation (4). Further several studies demonstrated, it was also recognized as anti-rachitic action of vitamin D protection against several diseases, like diabetes, hypertension, cardiovascular, autoimmune and dermatological diseases and cancer (5-6). Research studies states higher vitamin D levels lower risk of insulin resistance, when the body is unable to respond to or use the insulin that it's producing. Vitamin D metabolites stimulates the immune response in children and adults, having a crucial role in anti-infective defense (7).

Oxidative stress develops when the proportional rate of free radical generation exceeds than compared to the antioxidant defense systems due to the toxic effects of free radicals (8,9). Oxidative stress is potent risk factor for vascular complications in diabetes and as well as insulin resistance, inducing pathophysiologic changes in diabetes mellitus (10-12). So the aim of the study was to find out the VitD, total antioxidant capacity, MDA levels in T2DM patients and their association with HbA1c, Insulin resistance and lipid profile parameters.

Methods

A case control study with Seventy T2DM patients of both sexes with 35-50 years of age group on oral hypoglycemic drugs, attending Government general Hospital attached to Siddhartha Medical College, Vijayawada, Andhra Pradesh, India, were selected for present study in period of August 2022 to April 2023. Patients on insulin, thyroid disorders, Smokers, Alcoholics, Tobacco chewers, other active infective diseases, neoplastic disorders, liver dysfunction, history of myocardial infarction, stroke, and occlusive peripheral vascular disease were excluded. Seventy healthy age and sex match subjects were selected as control. The informed consent was obtained from all the study subjects and the study was approved by the Institutional Human Ethics Committee (IHEC) (IEC- SMCGGH/2024/AP/018). Experiments were done in accordance with Helsinki

Fasting blood samples were obtained from the subjects and centrifuged at 2000×g for 10 min. Samples were analyzed for glucose, lipid profile (Total Cholesterol, HDL-C, LDL-C, triglycerides) using by Beckman Coulter fully automated analyzer. Vitamin D and Insulin was assessed by Enzyme Linked Immuno Sorbent Assay (ELISA), HbA1c assayed by HPLC method. The total antioxidant capacity was estimated by Benzie et al. method (1996) (13), and lipid peroxidation (MDA) was estimated by Mahfouz et al. (1986) method (14) by spectrphotometry. Homeostasis model assessment for insulin resistance evaluation (HOMA-IR) was calculated by fasting plasma insulin × glucose/22.5 (15).

Statistical analyses have been carried by SPSS 25.0. Values were expressed as mean ± standard deviation by t-test, p value < 0.05 was considered statistically significant. The Pearson correlation test was used for correlation analysis.

Results

Table 1. Comparison of Baseline parameters in control and T2DM Patients

Parameters	Controls (n=70)	T2DM patients (n=70)	P-value
Age	41.7±3.9	43.1±4.7	0.07
Body mass index (BMI)	24.5±1.3	26.9±3.6 [a*]	0.001*
Waist/Hip ratio	0.92±0.03	0.92±0.06	0.08
Systolic BP (mmHg)	112.9±7.1	125.5±15.5 [a#]	0.04 [#]
Diastolic BP (mm Hg)	75.1±3.4	81.7±7.5 [a#]	0.05 [#]
Duration DM (years)	-	6.1±2.0	NA

Data are expressed as mean ±SD, * **P** <0.001, # **P** <0.05 was considered statistically significant

Table 2. Comparison between fasting plasma glucose, and postprandial glucose, Lipid profile, HbA1c, Insulin, HOMA-IR, Vitamin D, Total antioxidant capacity, MDA levels in controls, T2 DM patients

Parameters	Controls (n=70)	T2 DM patients (n=70)	P-value
Fasting plasma glucose (mg/dl)	89.3±10.2	178.7±7.9	0.001*
Post prandial plasma glucose (mg/dl)	110.3±9.4	210.1±23.6	0.001*
Total cholesterol	181.5±8.9	223.5±16.9	0.04#
Serum triglycerides (mg/dl)	112.6±15.7	201.8±12.8	0.03#
HDL cholesterol (mg/dl)	43.1±8.5	35.6±5.8	0.03#
LDL cholesterol (mg/dl)	122.6±16.2	172.8±16.7	0.001*
HbA1c %	5.3±0.8	9.6±1.9	0.001*
Insulin (µ IU/ml)	8.0±1.6	17.8±3.2	0.001*
HOMA –IR	1.8±0.15	6.1±2.1	0.001*
Vitamin D (ng/ml)	35±2.9	20.8±4.8	0.001*
Total antioxidant capacity (µmol/l)	436.4±25.7	348.4±25.7	0.001*
MDA (µmol /l)	2.8±0.5	5.3±1.7	0.001*

Data are expressed as mean ±SD, * **P** <0.001, # **P** <0.05 was considered statistically significant

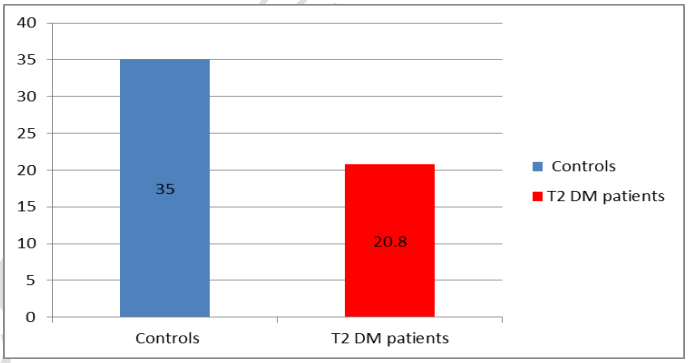

Figure 1. Vitamin D levels in controls and T2 DM patients

Table 3: Correlation between Vitamin D and measured parameters in T2 DM subjects and control group

Parameters	Correlation Coefficient-r -T2 DM group	P-value	Correlation Coefficient-r - Control group	P-value
Total antioxidant capacity	0.378	0.04*	0.342	0.04*
MDA	-0.413	0.01*	-0.378	0.01*
HbA1c	-0.378	0.02*	-0.321	0.02*
HOMA-IR	-0.407	0.01**	-0.413	0.01**
Cholesterol	0.227	0.07	0.187	0.07
TG	0.178	0.06	0.121	0.06
HDL-C	-0.215	0.07	-0.201	0.07
LDL-C	0.300	0.06	0.282	0.06

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

Discussion

T2DM is associated with insulin resistance, a defective response to physiological or increased exogenous or endogenous insulin concentration, that leads to hyperglycemia and hyperinsulinism. Insulin, not only regulates the metabolism and also acts as a growth factor. Hyperinsulinism stimulates abnormal activation of multiple cellular signaling cascades, strengthening growth factor-dependent cell proliferation (16,17). In the present study we observed significant reduction of Vit D levels in type 2 diabetic patients compared with healthy controls. Studies reported that Vit D may have pivotal role on insulin sensitivity through a different mechanism, including an increase in the transcriptional activation and expression of insulin receptor genes, promoting basal and insulin-stimulated glucose oxidation, and thereby improving insulin sensitivity (18,19). Nazarian S et.al reported that Vitamin D3 supplementation beneficial to improve insulin sensitivity in subjects with impaired fasting glucose levels (20).

The present study also stating that Vit D levels show negative correlation with insulin resistance, HbA1c. One of the experimental studies revealed Vit D levels can promote the synthesis and secretion of insulin in the pancreas of mice (21). Vitamin D restores glucose-stimulates insulin secretion by through promoting β-cell survival by modulation of cytokines (22,23). Further insulin secretion is also influenced by calcium concentration and flux through the β-cells (24). Vitamin D regulates the function of calbindin, a systolic calcium-binding protein found in pancreatic β-cells, and acts as a modulator of depolarization-stimulated insulin secretion via regulation of intracellular calcium. PTH, which has its concentration regulated by vitamin D, is associated with insulin synthesis and secretion in the pancreas (25). That means decreased Vit D levels is one the confounding factor for increased insulin resistance and elevated HbA1c levels in diabetic patients.

The present study shows enormous oxidative stress in diabetic patients by through the assessment of total antioxidant capacity and lipid peroxidation (MDA) assessment. Vit D levels were positively correlated with total antioxidant capacity and negatively correlated with MDA levels. One of the studies demonstrated that deficiency of Vit D causes insulin resistance by causing oxidative stress in hepatocytes (26). Vit D could play a role to increase of some anti-inflammatory cytokines, whiling decreasing the production of some pro-inflammatory cytokines. Depletion of Vit D, stably silencing 1α (OH)ase in L02 hepatocytes led to significant reactive oxygen species and reactive nitrogen (ROS and RNS) production, as well as subsequent p53-p21 activation and DNA damage (27-30). So, the decreased Vit D levels are crucial for promoting oxidative stress in diabetic patients.

Conclusion

Vitamin D deficiency may be one of the vital risk factors responsible for increased oxidative stress in T2DM patients. Regular monitoring and supplementation of vitamin D beneficial for reduction of oxidative stress and vascular complications in T2DM patients.

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Ethical statement

Institutional Human Ethics Committee (IHEC) (IEC- SMCGGH/2024/AP/018).

Conflicts of interest

Multi-Disciplinary Research Unit (MRU) and Department of Biochemistry Siddhartha Medical college, Vijayawada

Author contributions

Balu Mahendran Kanumuru: Data collection and processing, Manuscript preparation. Sridevi Nutakki: Study design, Validation, Resources and formal analysis, Statistical analysis.

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