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Vitamin D Status And Different Oxidative Stress Markers In Diabetic Patients.

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ABSTRACT

Vitamin D, a fat-soluble vitamin, plays a critical role in regulating plasma calcium concentration and may influence several non-skeletal medical conditions, including diabetes as well as other human diseases. The aim of this study was to determine the relationship between serum concentration of 25-hydroxy vitamin D 25 (OH) $D_3$and both of glycemic profile and some of oxidative stress markers in diabetic patients compared to healthy subjects. The study included 84 subjects aged between 40-60 years divided into two main groups: Type 2 Diabetes Mellitus (T2DM) (n=43) and healthy subjects (n=41). Further classification of each group was carried out based on 25 (OH) D_3status (deficient, insufficient and sufficient). Age was recorded and BMI was calculated. It was found that the prevalence of vitamin D deficiency was 44.18% and 56% for T2DM and control groups, respectively. Results indicated highly significant elevation in the mean values of FBG, HbA1c, FI, HOMA-IR, TG, MDA and AGEs in T2DM group compared to control group. Mean values of TAC, HDL were significantly decreased in T2DM compared to control group. Mean values of BMI, age, cholesterol, LDL and 25 (OH) D_3 were not significantly changed in T2DM compared to control group. In control group, non-significant correlations were obtained for 25(OH) D_3 with each tested parameter, however, in T2DM, 25(OH) D_3 showed significant correlations with cholesterol and LDL. This study has shown that dyslipidemia, poor glycemic control and increased oxidative stress markers were highly prevalent among diabetic subjects. Though vitamin D deficiency was prevalent in T2DM and non-diabetic control subjects, its relationship to glycemic parameters and oxidative stress markers could not be confirmed, demonstrating that improvement in vitamin D status was not the only factor responsible for better health of the individuals but lifestyle and dietary changes seem to play a role, which will improve the overall health including hemoglobin glycation and insulin resistance along with vitamin D levels.

Keywords: Vit D, oxidative stress, diabetes, markers

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INTRODUCTION

Vitamin D, a fat-soluble vitamin, plays a critical role in regulating plasma calcium concentration through effects on intestinal absorption and bone metabolism (Liu et al. 2010). There is mounting evidence suggesting that vitamin D may influence several non-skeletal medical conditions, including neuromuscular function, cancer, and a wide range of chronic diseases, including autoimmune diseases, atherosclerosis, obesity, cardiovascular diseases, diabetes, and associated conditions such as the metabolic syndrome and insulin resistance (1).Type 2 Diabetes Mellitus (T2DM) is a multifactorial disease characterized by chronic hyperglycemia, altered insulin secretion, and insulin resistance – a state of diminished responsiveness to normal concentrations of circulating insulin (2, 3).

Oxidation is a chemical process whereby electrons are removed from molecules and highly reactive free radicals are generated (4). Free radicals include reactive oxygen species (ROS) such as superoxide and hydroperoxyl and reactive nitrogen species (RNS) such as nitric oxide and nitrogen dioxide (5). There is a growing scientific and public interest in connecting oxidative stress with a variety of pathological conditions, including diabetes mellitus as well as other human disease. Oxidative stress has been implicated in the pathogenesis of type 2 diabetes and its complications (6).

Recent studies showed that vitamin D status, measured as plasma 25(OH)D, was inversely associated with markers of oxidative stress, such as urinary isoprostanes and serum lipid peroxides(7). However, some others found that 25-OH-D had no beneficial effect on antioxidant defense (8). The aim of this study was to determine the relationship between serum concentration of 25-hydroxy vitamin D (25-OH-D) and both of glycemic profile and some of oxidative stress markers in diabetic patients compared to healthy subjects.

MATERIALS AND METHODS

The study population included 84 subjects (43 men and 41 women) aged 40-60 years, subjects were randomly selected from outpatient clinics in King Abdulaziz University Hospital. Subjects were diagnosed by hospital physicians and were divided into two groups: control and T2DM, according to clinical and biochemical criteria of The American Diabetes Association (9).The T2DM group included 43 subjects meanwhile, the control group that was selected from the outpatient department included 41 subjects who were apparently healthy with normal glucose levels and normal lipid profiles. Further classification of each group was carried out based on 25 (OH) D_3 status; Deficient 25 (OH) D_3 subgroup: (control or T2DM with serum 25 (OH) D_3 level < 30 nmol/L), Insufficient 25 (OH) D_3 subgroup (control or T2DM with serum 25 (OH) D_3 level range from 30 to < 50 nmol/L and Sufficient 25 (OH) D3 subgroup: (control or T2DM with serum 25 (OH) D_3 level range from 50 - 80 nmol/L).

The study protocol was approved by the King Abdulaziz University Hospital Ethics Committee. All participants were asked to sign an informed consent prior to entering the study. All measurements were carried out at the laboratory of King Abdulaziz University Hospital.

Each participant was subjected to a single withdrawal of blood sample after overnight fasting. The blood sample was divided into two parts. First part was added to the EDTA tube for $HbA1_C$% measurement meanwhile, second part was added to a plain tube and allowed to clot for 30 minutes at room temperature and it was centrifuged at 3000 r.p.m for 10 minutes to separate serum. Sera were divided into aliquots to avoid freezing and thawing and were kept on -20°C pending assay. Sera were used for determination of: fasting blood glucose, fasting insulin, lipid profile parameters, vitamin D, Total antioxidant capacity, Malondialdhyde and Advanced glycation end products.

Instruments which were used in this study were: Dimension Xpand (Siemens) for determination of blood glucose, HbA_{1C} and lipid profile. Cobas e 601 (cobas) Immunoassay analyzer for determination insulin and vitamin D and Humareader plus, Human, GmbH, Germany for determination Total antioxidant capacity, Malondialdhyde and Advanced glycation end products.

Fasting blood glucose was determined by using a kit provided by Siemens Healthcare. Serum HbA1c was determined by using a kit provided by Siemens Healthcare Diagnostic Limited, UK. Insulin levels were determined using an electrochemiluminescence immunoassay technique employing a kit purchased from

Cobas. For lipid profile: Serum cholesterol was determined by using a kit provided by Siemens Healthcare, Serum triglyceride was determined by using a kit provided by Siemens Healthcare, Serum LDL Cholesterol was determined by using a kit provided by Siemens Healthcare, Serum HDL Cholesterol was determined by using a kit provided by Siemens Healthcare. Vitamin D levels were determined using an electrochemiluminescence immunoassay technique employing a kit purchased from Cobas. Total Antioxidant Capacitywas determined by using a kit provided by Cell Biolabs, Inc., USA. Malondialdehyde was determined by using a kit provided by Cell Biolabs, Inc., USA. Advanced glycation end products were determined by using a kit provided by Cell Biolabs, Inc., USA.

Statistical Analysis

Statistical analysis was performed with the Statistical Package for Social Sciences Software (SPSS Inc., USA) version 24. Data were expressed as means ± standard deviation (SD). Differences in means of study parameters between normal control and diabetic subjects were assessed using Independent-Sample T-Test. Pearson correlations were used to study the interrelationship between different parameters included in the study. A p-value of <0.05 was considered statistically significant.

RESULTS

The study included 84 subjects who were classified into two main groups: control (n=41) and T2DM (n=43). Mean values of body mass index, age, and multiple different blood glucose levels compared between control and T2DM groups are shown in Table (1). In addition, it shows mean values of lipid profile and 25 hydroxyvitamin D_3 in the studies groups. The mean value of Triglycerides significantly increased while the mean value of HDL significantly decreased inT2DM compared to the normal control group. On the other hand, the mean values of cholesterol, LDL and 25 hydroxyvitamin D_3 were not significantly changed in T2DM. Table 1 additionally demonstrates difference in mean values of total antioxidant capacity, malondialdehyde and advanced glycation end products between control and T2DM groups. Data obtained from this study revealed highly significant increased mean values of MDA and AGEs in T2DM compared to the control group. In contrast the mean value of TAC significantly decreased in T2DM compared to normal control group.

Comparison between the mean levels of all parameters included in the study as regards to 25 hydroxyvitamin D_3 status

According to 25 (OH) D_3 levels, control and T2DM subjects were classified into 3 subgroups as follows:

- Deficient subgroup: 25 (OH) D_3< 30 nmol/L.
- Insufficient subgroup: 25 (OH) D_3 30-<50 nmol/L.
- Sufficient subgroup:25 (OH) D_3 50-80 nmol/L.

Results obtained from deficient subgroup

The results in Table 2 displays the mean values of FBG, HbA1C, FI and HOMA-IR in deficient T2DM subgroup, which shows a significant increase relative to their matched values in the control subgroup with deficient 25 (OH) D_3. In the T2DM deficient subgroup, mean values of TG and LDL increased and the mean value of HDL decreased compared to control subgroup with deficient 25 (OH) D_3, however these results were not significant. Meanwhile, both of the two subgroups had similar values of cholesterol and 25 hydroxyvitamin D_3.

Significant elevation in mean values of MDA and AGEs was noted in T2DM deficient subgroup, while TAC showed a non-significant difference between the same two subgroups.

- T2DM: type 2 diabetes mellitus and BMI: body mass index.
- FBG: fasting blood glucose, HbA1C: Glycated hemoglobin FI: fasting insulin and HOMA-IR: Homeostasis model assessment insulin resistance.

TG: triglycerides, LDL: low density lipoprotein, HDL: high density lipoprotein and 25 (OH) D_3: 25 hydroxyvitamin D_3.

- TAC: total antioxidant capacity, MDA: malondialdhyde and AGEs: advanced glycation end products.

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Results obtained from insufficient subgroup

As seen in Table 3 above, the insufficient T2DM subgroup reported significantly higher mean values of FBG, HbA1C, FI, HOMA-IR and TG in comparison to control subgroup. T2DM group also reported slightly higher mean values of cholesterol, LDL and 25 (OH) D3 with a slight decrease of HD. Furthermore, results showed a significant increase in mean values of MDA and AGEs for insufficient T2DM subgroup compared to control subgroup.

Results obtained from sufficient subgroup

Tables 4 below shows slightly higher TG values, meanwhile mean values of cholesterol, LDL, and HDL were slightly lower in T2DM subgroup with sufficient 25 (OH) D3 compared to control sufficient subgroup. On the other hand, highly significant elevation could be noted in the mean values of FBG, HbA1C, FI, HOMA-IR, MDA and AGEs for sufficient T2DM subgroup compared to control sufficient subgroup. The mean values of 25 (OH) D3 and TAC were almost the same in these two subgroups.

Table 1: Mean levels of Age, BMI, different blood glucose levels and Homeostasis model assessment in control and T2DM groups

Groups / Parameters	Control n =41	T2DM n =43	P value
Age (year) $\bar{X}$± SD Range	 46.83 ± 6.62 40-60	 47.91 ± 6.19 40-60	 >0.05
BMI (Kg/m2) $\bar{X}$ ± SD Range	 27.7 ± 5.4 21.43 - 40.05	 29.0 ± 6.5 21.60 - 54.43	 >0.05
FBG (74-106 mg/dl)* $\bar{X}$± SD Range	 101±10.29 48-127	 169±57.01 99-325	 0.000
HbA1C (4.3-6%)* $\bar{X}$ ± SD Range	 5.81 ± 0.36 5.1-6.6	 8.21 ±1.90 4.9-13.5	 0.000
FI (2.6-24.9 mIU/L)* $\bar{X}$± SD Range	 12.62 ± 5.95 4.25- 27.2	 29.34 ± 26.10 2.31 - 89.13	 0.000
HOMA-IR $\bar{X}$ ± SD Range	 3.23 ± 1.74 0.95- 8.19	 11.70 ± 0.87 0.83- 44.60	 0.000
Cholesterol (< 200 mg/dl) * $\bar{X}$± SD Range	 191.83 ± 34.20 107-262	 190.93± 39.91 119-263	 >0.05
TG (< 150 mg/dl) * $\bar{X}$ ± SD Range	 128.20 ±42.05 72-239	 159.63 ± 71.07 60-358	 0.016
LDL (< 99 mg/dl) * $\bar{X}$± SD Range	 113.56 ± 27.96 54-177	 112.84 ±37.29 46-189	 >0.05
HDL (40 -60 mg /dl) * $\bar{X}$ ± SD Range	 56.27 ±22.28 23-160	 47.63 ±12.33 21-80	 0.030
25 (OH) D3 (50-80 nmol/L) * $\bar{X}$ ± SD Range	 34.29 ± 22.97 7.5-81.28	 37.99 ± 22.90 7.5-85	 >0.05

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TAC m mol /L $\bar{X}$± SD Range	 0.62±0.26 0.40- 1.71	 0.51±0.10 0.13 - 0.65	0.008
MDA µmol/L $\bar{X}$ ± SD Range	 3.01 ± 1.05 1.25 - 5.16	 6.02 ±1.83 2.67 - 10.32	0.000
AGEs µg/ml $\bar{X}$± SD Range	 7.74 ± 0.87 6.33 - 10.66	 11.98 ± 2.67 7.02 - 17.03	0.000

*values between brackets indicates the normal range for each parameter.

Table 2: Difference in mean levels of multiple variables between control and T2DM groups with deficient 25 (OH) D3.

Groups Parameters	Control n =23	T2DM n =19	P value
FBG (74-106 mg/dl)* $\bar{X}$± SD Range	 101.39 ± 9.66 85-122	 176.37 ± 59.75 99-325	0.000
HbA1C (4.3-6%)* $\bar{X}$ ± SD Range	 5.81± 0.36 5.10-6.30	 8.31 ± 1.96 5.2-11.5	0.000
FI (2.6-24.9 mlU/L)* $\bar{X}$± SD Range	 12.72 ± 6.51 4.59-27.20	 29.84± 30.15 3.96-89.13	0.025
HOMA-IR $\bar{X}$ ± SD Range	 3.26±1.89 1.08-8.19	 12.58 ±13.04 1.51-44.61	0.006
Cholesterol (< 200 mg/dl)* $\bar{X}$± SD Range	 199.30 ±26.81 150-262	 208.32± 36.02 132-263	> 0.05
TG (< 150 mg/dl)* $\bar{X}$ ± SD Range	 137.74± 47.67 72-239	 165.16 ± 87.36 60-358	> 0.05
LDL (< 99 mg/dl)* $\bar{X}$± SD Range	 118.35± 25.30 75-177	 130.00± 33.04 58-189	> 0.05
HDL (40 -60 mg /dl)* $\bar{X}$ ± SD Range	 54.96± 14.94 31-92	 47.16± 11.91 33-68	> 0.05
25 (OH) D3 (50-80 nmol/L)* $\bar{X}$ ± SD Range	 18.00 ± 6.63 7.5-29.1	 17.62± 6.53 7.5-27.42	> 0.05
TAC mmol /L $\bar{X}$± SD Range	 0.66 ± 0.33 0.4-1.72	 0.51 ± 0.11 0.14-0.62	> 0.05
MDA µmol/L $\bar{X}$ ± SD Range	 2.99 ± 1.16 1.25-5.16	 6.54 ± 1.75 3.18-10.04	0.000
AGEs µg/ml $\bar{X}$± SD Range	 7.82± 0.69 6.78-9.2	 12.12 ± 2.88 7.02-16.99	0.000

*values between brackets indicates the normal range for each parameter.

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Table 3: Difference in mean levels of multiple variables between control and T2DM groups with insufficient 25 (OH) D3.

Parameters \ Groups	Control n =8	T2DM n =12	P value
FBG (74-106 mg/dl)*			0.006
$\bar{X}$± SD	102.75 ± 14.08	167.08 ± 56.73	
Range	84-127	112-273	
HbA1C (4.3-6%)*			0.007
$\bar{X}$ ± SD	5.89 ± 0.45	8.08 ± 2.29	
Range	5.40-6.60	4.90-13.50	
FI (2.6-24.9 mlU/L)*			0.035
$\bar{X}$± SD	13.03 ± 5.83	26.06 ± 18.05	
Range	4.62-19.50	10.87-72.40	
HOMA-IR			0.005
$\bar{X}$ ± SD	3.42 ±1.82	10.13 ± 6.64	
Range	0.96-6.12	4.33-25.36	
Cholesterol (< 200 mg/dl)*			> 0.05
$\bar{X}$± SD	174.50 ±39.20	186.75 ±35.37	
Range	121-217	129-251	
TG (< 150 mg/dl)*			0.034
$\bar{X}$ ± SD	127.13 ±31.07	168.83 ±44.41	
Range	91-179	70-224	
LDL (< 99 mg/dl)*			> 0.05
$\bar{X}$± SD	101.00 ±30.99	111.67 ±33.98	
Range	54-14	47-168	
HDL (40 -60 mg /dl)*			> 0.05
$\bar{X}$ ± SD	48.75± 12.48	43.42 ±12.01	
Range	28-65	21-63	
25 (OH) D3 (50-80 nmol/L)*			> 0.05
$\bar{X}$ ± SD	36.54 ± 5.32	41.68 ± 5.54	
Range	30.80-45.70	31.85-49.34	
TAC mmol /L			> 0.05
$\bar{X}$± SD	0.59 ± 0.15	0.48 ± 0.12	
Range	0.44-0.90	0.15-0.64	
MDA µmol/L			0.001
$\bar{X}$ ± SD	3.03 ± 0.94	5.76 ±1.83	
Range	1.91-4.91	2.62-10.32	
AGEs µg/ml			0.001
$\bar{X}$± SD	7.60 ± 0.67	11.76 ± 3.02	
Range	6.33-8.50	7.58-17.03	

*values between brackets indicates the normal range for each parameter.

Table 4: Difference in mean levels of multiple variables between control and T2DM groups with sufficient 25 (OH) D3.

Parameters \ Groups	Control n =10	T2DM n =12	P value
FBG (74-106 mg/dl)*			0.002
$\bar{X}$± SD	99.50 ± 9.11	162.33 ± 56.60	
Range	85-112	102-288	
HbA1C (4.3-6%)*			0.000
$\bar{X}$ ± SD	5.76 ± 0.31	8.17 ± 1.49	
Range	5.40-6.20	6.20-10.80	
FI (2.6-24.9 mlU/L)*			0.039
$\bar{X}$± SD	12.05 ± 5.18	31.82 ± 27.73	

ISSN: 0975-8585

Range	4.25-32.20	2.31-89	
HOMA-IR $\bar{X}$ ± SD Range	2.99 ± 1.42 1.04-6.24	11.85± 9.41 0.83-30.76	0.008
Cholesterol (< 200 mg/dl)* $\bar{X}$± SD Range	188.50 ±42.70 107-254	167.58±39.64 119-241	> 0.05
TG (< 150 mg/dl)* $\bar{X}$ ± SD Range	107.10 ±28.72 77-165	141.67±65.68 61-260	> 0.05
LDL (< 99 mg/dl)* $\bar{X}$± SD Range	112.60 ±31.04 56-165	86.83± 33.47 40-146	> 0.05
HDL (40 -60 mg /dl)* $\bar{X}$ ± SD Range	65.30±37.33 23-160	52.58 ±12.55 31-77	> 0.05
25 (OH) D3 (50-80 nmol/L)* $\bar{X}$ ± SD Range	69.95± 10.97 54.20-81.30	69.15± 12.91 55.85-85	> 0.05
TAC mmol /L $\bar{X}$± SD Range	0.56 ± 0.15 0.40-0.84	0.52 ± 0.08 0.38-0.65	> 0.05
MDA µmol/L $\bar{X}$ ± SD Range	3.02 ± 0.96 1.45-4.52	5.46 ± 1.98 2.74-9.04	0.002
AGEs µg/ml $\bar{X}$± SD Range	7.67 ±1.36 6.33-10.70	11.98 ± 2.09 8.90-15.63	0.008

*values between brackets indicates the normal range for each parameter.

DISCUSSION

In the present study showed that the BMI was non–significantly increased in T2DM as compared to the control group. Luca and Iordache (2013) consider obesity as a strong risk factor for T2DM and its complications and this may explain and support the significant positive correlation between BMI and Insulin in the present study for T2DM. No association between vitamin D and BMI could be observed in both T2DM and control groups included in this work. Agreeing, Sebekova et al. (2015) also did not reveal such association. Meanwhile, a meta-analysis of 21 studies reported that each unit of increase in BMI (kg/m2) is associated with 1.15% lower plasma concentrations of 25(OH)D (10). Likewise, further data suggests that reduction of weight and consequently of fat in overweight and obese subjects was not found to be associated with significant changes in white adipose tissue or circulating vitamin D3 levels (11). This could be explained by that vitamin D accumulates in fat cells which probably results in its trapping in that site consequently causing a decrease in vitamin D levels.

Results in the current study indicated highly significant elevation in the mean values of FBG, HbA1c, FI and HOMA-IR in T2DM group compared to normal control. On the other hand the mean values of 25(OH) D was not significantly changed in T2DM group compared to control group.

Further classification of normal and diabetic groups was carried out based on 25(OH) D status (deficient, insufficient and sufficient). The results obtained showed that in all these three classes the diabetic patients had significant elevated levels of FBG, HbA1c, FI and HOMA-IR and comparable values of 25(OH) D compared to non-glycemic control. In addition pearson's correlation between different parameters in T2DM and control groups of the present study could not show any association between vitamin D values and any of the glycemic parameters which may be due to the small size in number of subjects.

The evidence so far available on the role of vitamin D in the pathogenesis of T2DM is inconclusive. An association between low vitamin D status and T2DM have been reported in several cross-sectional studies (12, 13), as well as prospective cohort studies (14-16). Two recent large prospective cohort studies provided evidence of an inverse association between serum 25(OH)D and markers of glucose homeostasis (15)and risk of T2DM (16) ,indicating a possibility of a causal relationship. Nonetheless, vitamin-D supplementation was not found to be effective in reducing HbA1c, as stated by Melville (2013) (17). In contrast, as with the present work, other studies reported no association between 25(OH)D levels and the incidence of T2DM (18), hemoglobin glycation(19) and glycemic control (20).

Activation of vitamin D receptors and calcium homeostasis have been reported to be possibly affected by impaired pancreatic-β cell function and insulin resistance in T2DM (21). This has been confirmed by a couple of in vitro studies, suggesting its role in improving insulin sensitivity and secretion (20-22). On the other hand, Fonseca (2009) (23) explained that the β-cell reserve attenuates with the progression in the duration of T2DM, but Shethet al. 2015(19)could not observe significant association between HbA1c and HOMA-IR with 25(OH)D taken into account with the duration of T2DM.

A study conducted in Australia in 2014 by Elkassabyet al. (2014) (24) observed a transient improvement in glycaemia in T2DM with the intake of oral vitamin D3 supplements without change in either HbA1c or beta cell function, meanwhile concluded that high doses have little or no therapeutic benefit. A similar study from United Arab of Emirates (UAE) done by Sadiya A.et al. (2014) (25) has also reported no significant change in HbA1c levels after six months of supplementation with vitamin D3 in vitamin D-deficient obese T2DM patients of an Emirati population. Furtherly confirming this, Kampmannet al. (2014) (26) showed that improvement in vitamin D status may increase insulin secretion but did not improve insulin resistance and HbA1c in patients with T2DM. This is in agreement with our findings that vitamin D levels did not show any significant association with HOMA-IR status in T2DM cases as well as control subjects. This is possibly because the inflammatory mechanisms are highly stimulated by the diabetic milieu or the β-cell dysfunction, and insulin resistance is more severe and less reversible by extended duration of diabetes as explained by Luo et al. (2009) (20). On the other hand, Aksuet al. (2013) (27) found that serum vitamin D levels increase after the correction of hyperglycemia and HbA1c, suggesting a bidirectional biological relationship between blood glucose level and vitamin D metabolism.

In this study, the mean value of triglycerides was significantly increased while the mean value of HDL was significantly decreased in T2DM compared to control group. In contrast Thanpariet al. (2015) (28) demonstrated that HDL-C level was raised significantly in T2DM. Maharjanet al. (2008) (29) reported that there was no significant difference between HDL-C in control and diabetes. The raised HDL levels should protect the diabetic patients from atherosclerotic complications because it can play a role in reverse cholesterol transport, and it also carries cholesterol from the atherosclerotic plaques to the liver for removal. So, it is considered to be a protective factor to the development of atherosclerosis (30).

Upon further classification of normal and diabetic groups as regard to vitamin D status, it was found that the prevalence of vitamin D deficiency was 44.18% and 56% for T2DM and control groups respectively and vitamin D sufficiency and insufficiency were 55.81% for T2DM and 43.90% for control. The present study displayed that there was a significant negative correlation between vitamin D and cholesterol and LDL in diabetic patients. Meanwhile, the relationship between vitamin D and each of BMI, cholesterol, triglycerides and LDL in the control group was non-significant.

The inverse correlations of total cholesterol and LDL with vitamin D noted in this study are consistent with other studies that evaluated cardiovascular risk in T2DM patients from sunny regions (31, 32). Perhaps lipid levels might be the intervenient variable that explains the link between hypovitaminosis D and cardiovascular disease in patients with T2DM (33). However, the literature on this subject is not homogeneous and interventional studies have failed to demonstrate that raising the levels of vitamin D resulted in improvement in the lipid profile of T2DM patients (34-36).Ford et al. (2005) (37) found a negative association between serum levels of 25(OH) D and TG in patients with hypertriglyceridemia. However, this relationship was not observed in HDL levels in healthy subjects. Rejnmarket al. (2009) (38) reported that serum concentration of triglycerides was inversely associated with 25(OH)D in healthy postmenopausal women who had been treated with either 40 mg/day Simvastatin or a placebo for one year. In contrast, Chiu et al. (2004) (39) showed no relationship between serum levels of 25(OH) D and TG or HDL cholesterol in healthy subjects.

The biological mechanism explaining both direct and indirect effects of vitamin D on modifying the lipid profile may occur through regulatory action that increases the activity of lipoprotein lipase in adiposity (40). The effect of vitamin D on serum lipids could also be via suppression of the PTH secretion as PTH has been reported to reduce lipolysis at least in vitro (41). In addition, vitamin D could affect the serum lipids through an increased calcium level which may reduce hepatic TG formation and/or secretion (42, 43). Furthermore, vitamin D may have an effect both on insulin secretion and insulin sensitivity and thereby indirectly influencing lipid metabolism (44).

Lots of evidences link Reactive Oxygen Species (ROS) and oxidative stress to the pathogenesis of T2DM and development of complications (45). Several studies have shown elevated extra and intracellular glucose concentrations resulting in elevated oxidative stress among diabetic animals and individuals (46), and ROS can contribute to the development and progression of diabetes and related complications by directly damaging DNA, proteins, and lipids or indirectly activating a number of cellular stress-sensitive pathways to induce damage to tissues such as islet β cells (47).

Vitamin D status was inversely associated with markers of oxidative stress, such as urinary isoprostanes and serum lipid peroxides (48). However, some others found that 25-OH-D had no beneficial effect on antioxidant defense (49).

The results obtained from this study revealed highly significant decrease in the mean value of TAC accompanied by a highly significant increase in the mean values of MDA and AGEs in T2DM as compared to control group. The same trend of decrease and increase were also noted in these parameters for the three subgroups (deficient, insufficient and sufficient).

Studies conducted among different populations have also showed a decreased level of total antioxidants in type 2 diabetes than controls (50- 53). In addition, a study conducted among Egyptians consisting of 50 type 2 diabetes patients have concluded that the depletion of total antioxidant capacity is associated with diabetic complications (54).On the contrary, a recent study conducted in Romania has reported an increase in total antioxidants when compared to controls. The compensatory response from the endogenous antioxidants might contribute to such increase in total antioxidant activity in diabetes mellitus (55).

The significant elevation noted in the mean value of MDA in T2DM as compared to control in the present work was in line with many previous studies (51, 53, 56, 57) which all of them observed an association between lipid peroxidation and T2DM.

Hyperinsulinemia in diabetes increases the activity of the enzyme fatty acyl coenzyme A oxidase, which initiates β- oxidation of fatty acids, resulting in lipid peroxidation (58). Oxidative stress arises because of excessive production of ROS and impaired antioxidant defense mechanisms. It has been suggested that ROS induce membrane lipid peroxidation and that the toxicity of generated fatty acid peroxides are important causes of cell malfunction (59). Interaction between ROS with lipoproteins (LDL and HDL) leads to their hydro peroxidation. Due to this interaction and interaction between free radicals and polyunsaturated fatty acids, MDA levels were increased in the plasma (60).

The present study indicated the absence of correlation between MDA and any of glycemic control parameters nor oxidant-antioxidant parameters. Kassim (2011) (61) found that HbA1c was associated with MDA and SOD, but Vessbyet al. (2002) (62) have not observed such a correlation.

T2DM group in the present study were having more highly significant mean value of AGEs than control group. This was in concordance with Bucala and Cerami (1992) (63) and Šebekováet al (2015) (64). AGEs may directly contribute to induction or aggravation of diabetes causing progressive insulin secretory defects and pancreatic beta cell deaths (65) and by enhancing insulin resistance via decreased biological activity of glycated insulin (66). The presence of high significant correlation between AGEs and each of FBG, HbA1c and insulin observed in diabetic group and between AGEs and each of FBG and HbA1c in control group for present study may support and clarify the mechanism of action of AGEs. These finding are compatible with

those of Aouacheriet al. (2015) (6) that correlate HbA1c with oxidative stress, and with Goodarziet al. (2008) (67) that support the correlation between the degree of hyperglycemia and oxidative stress.

In in vitro studies it was shown that the deleterious effects of AGEs modified albumin on endothelial cells could be prevented by incubation with calcitriol, the active form of vitamin D (68). In diabetic rats administration of vitamin D reduced systemic oxidative stress and the deposition of AGEs in the aortic wall (69). On the other hand,Sabekovaet al. (2015) (70) suggested that in diabetic subjects hypovitaminosis D is not associated with enhanced AGE accumulation and sufficient vitamin D levels are not linked with a lower AGE accumulation. Taken together in the current study the association between vitamin D and each of MDA and AGEs could not be observed.

This study had some limitations. First, the sample size was small; therefore, it may decrease the possibility of the study to be able detect a significant association between parameters and may limit the generalizability of these findings. Second, data about financial income, sun exposure or other characteristics could not be collected. Third, a random measurement error may be arising by the use of a single measurement of glycemic and oxidative stress markers.

CONCLUSION

This study has shown that dyslipidemia, poor glycemic control and increased oxidative stress markers were highly prevalent among diabetic subjects. Though vitamin D deficiency was prevalent in T2DM and non-diabetic control subjects, its relationship to glycemic parameters and oxidative stress markers could not be confirmed, demonstrating that improvement in vitamin D status was not the only factor responsible for better health of the individuals but lifestyle and dietary changes seem to play a role, which will improve the overall health including hemoglobin glycation and insulin resistance along with vitamin D levels.

RECOMMENDATIONS

Efforts should be made in the area of glycemic control, lipid lowering and life style modifications to reduce the risk of diabetes complications. In addition, more longitudinal studies focusing on the effects of vitamin D supplementation on oxidative stress markers are needed to elucidate a potential relationship between vitamin D status and oxidative stress markers accumulation and their interaction in potentiating of toxic effects.

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