ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
Index Copernicus Value: 68 . 16
PubMed - National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 5, Issue –4, July - 2020, Page No. : 183 - 190

Status of oxidative stress markers in Type II Diabetes mellitus patients with Vitamin D deficiency

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Citation this Article: Dr Ayesha Juhi, Dr N. Balakrishna, "Status of oxidative stress markers in Type II Diabetes mellitus patients with Vitamin D deficiency", IJMSIR- July - 2020, Vol – 5, Issue - 4, P. No. 183 – 190.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: Diabetes and its related complications cause a major disease burden in India. Vitamin D in addition to bone metabolism has an essential role in maintain the glucose homeostasis in the body. Deficiency of vitamin D has been associated with increased risk of developing Type II diabetes mellitus. In Diabetes Mellitus, augmented oxidative stress is due to the enhanced free radical-generating process and/or impaired capacity of the antioxidant defence system to scavenge the excess free radicals which are induced by chronic hyperglycemia. Vitamin D is a potent membrane antioxidant, it prevents the lipid peroxidation of the cell membranes thereby preventing damage to the cells. Hence, this study was taken up with an objective to study the status of oxidative stress markers in diabetes mellitus patients with vitamin D deficiency.

Material and methods: This cross-sectional study was conducted on 55 newly diagnosed Type II diabetes mellitus patients of both the gender in the age group of 40- 80 years with the FBS of >125mg/dl. Biochemical parameters studied were HbA_1C, Vitamin D levels and oxidative stress markers MDA and TAC. Pearson's correlation analysis was performed to determine the relationships between oxidative stress markers, HbA_1C ,FBS and vitamin D variables. Regression model of oxidative variables with HbA_1C and FBS was used to study the relationships and how much percent variation explained in the model.

Results: Vitamin D levels and HbA_1C had highly significant ($p<0.01$) negative association ($r=-0.402$) between them. Vitamin D also had a highly significant ($p<0.01$) negative association with MDA ($r=-0.410$).HbA_1C had a negative correlation($r=-0.250$) with TAC but not statistically significant ($p>0.05$).HbA_1C had a very strong ($p<0.001$) positive relation with MDA ($r=0.598$).TAC had a highly significant negative correlation with FBS ($r=-0.374$) with a p value of 0.005.TAC had non-significant positive correlation with Vitamin D and also non-significant negative correlation with HbA_1C . The regression model showed that with every 1% increase in HbA_1C there was 44.6 nmol % increase in MDA levels. The model also expressed with every 1mg/dl of FBS increase there was 2.51µmol/lit decrease in

TAC.**Conclusion:** Vitamin D deficiency could be one the factors contributing to the development of hyperglycemia and progression into diabetes mellitus.Due to both conditions prevailing together could have lead to increased oxidative stress in the body. Hence it is very important to consider the Vitamin D levels in every diagnosed diabetes patient. Also, periodically monitor Vitamin D levels and include Vitamin D supplementation in their treatment plan to improve the glucose tolerance in diabetes type 2 patients to avoid oxidative stress injury.

Keywords: Vitamin D, HbA1C (glycosylated hemoglobin) , Diabetes mellitus type II,FBG (Fasting blood glucose) ,MDA (Malondialdehyde) , TAC (Total antioxidant capacity)

Introduction

According to World Health Organization (WHO) report, India is predicted to have 80 million diabetics by the year 2030. Diabetes and its related complications cause a major disease burden in India which is mainly due to intermediate hyperglycemia. Deficiency of vitamin D has been associated with increased risk of developing Type 2 diabetes mellitus. Vitamin D in addition to bone metabolism has an essential role in maintain the glucose homeostasis in the body[(1)]. Vitamin D deficiency may predispose to glucose intolerance, altered insulin secretion and type 2 diabetes [(2)]. It acts directly by activation of vitamin D receptor (VDR) or indirectly via calcemic hormones and even by inducing inflammation [(3).] Vitamin D is a potent antioxidant and anti inflammatory protective agent [(4)] .

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 macro vascular complications. Vascular oxidative stress, in addition to chronic hyperglycemia is reported to be a key contributor in the pathogenesis of Diabetes mellitus and its secondary complications [(5).]

In Diabetes Mellitus, augmented oxidative stress is due to the enhanced free radical-generating process and/or impaired capacity of the antioxidant defence system to scavenge the excess free radicals which are induced by chronic hyperglycemia [(6)].Diabetes mellitus is associated with the declined antioxidant capacity and increased production of ROS through increases of lipids, proteins and DNA oxidation products, glycated biomolecules, such as advanced glycation end products (AGEs), and advanced oxidation protein products[(7-9)].

Vitamin D is a potent membrane antioxidant , it prevents the lipid peroxidation of the cell membranes there by preventing damage to the cells.In a study conducted by Polidoro et al on human endothelial cell, found out that vitamin D is able to decrease the impairment after H_2O_2 mediated stress by the attenuation of anion superoxide yield and apoptosis [(10)].

Hypothesis of this study is due to the Vitamin D deficiency prevailing among Type II diabetes mellitus patients, oxidative stress markers are altered in their concentrations.

Hence this study was designed to study the status of oxidative stress markers in Type II Diabetes mellitus patients with Vitamin D deficiency.

Material And Methods

This cross-sectional study was conducted on 55 newly diagnosed Type II diabetes mellitus patients of both the gender in the age group of 40- 80 years. The study was conducted only after the clearance was obtained from the ethical committee of Apollo medical college, Hyderabad.

Inclusion and exclusion criteria

The inclusion criteria for the study subjects were newly diagnosed type 2 diabetics with Fasting blood glucose

≥126mg/dl who were unaware of their disease previously and complained symptoms of polyuria, polydypsia, polyphagia, loss of weight and increased fatigue. Subjects excluded were previously known diabetics, metabolic syndrome, polycystic ovarian disease, cardiovascular disorders, morbid obesity, endocrine disorders and any other condition which alters the glucose homeostasis. Patients on vitamin D supplementation were also excluded from the study. Written and informed consent was obtained from subjects before the sample collection.

Sample collection for biochemical tests

Venous blood samples were drawn in a fasting state from all the participants. Blood samples were left to clot at room temperature and centrifuged, and the serum was separated and stored at −20°C until further analysis of HbA_1C, Vitamin D levels and oxidative stress markers MDA and TAC.

Glycosylated hemoglobin (HbA_1c) was measured using Fully Automated H.P.L.C. using Biorad variant Turbo in units of percentage(%) .

Serum malondialdehyde (MDA) was estimated using Thiobarbituric acid reactive substances assay(TBARS) in units of (nmol %) [11].

Total Antioxidant status (TAC) was measured by FRAP (Ferric Reducing Antioxidant power) assay in units of µmol/litre [12].

Serum 25-OH vitamin D3 was estimated by Fully Automated Chemi Luminescent Imunno Assay analyzed on Siemens ADVIA centura in units of ng/mL. Serum 25-OH vitamin D3 level is a better indicator of vitamin D status than 1,25(OH)2D3, since the former has a slower rate of clearance than the later [13].

Statistical Analysis

The results were expressed as mean ± standard deviation. Normality of the variables was verified by one sample Kolmogorov-Smirnov test. Non parametric test of Spearman rank correlation and Mann-Whitney 'U' test was used when variables are violated the assumption of normality.

Independent 't' test was applied to compare the mean values of oxidative stress variables by Vitamin D levels (<10 &≥10) and HbA_1C (<6.5 & ≥6.5).

Pearson's correlation analysis was performed to determine the relationships between oxidative stress markers, HbA_1C and vitamin D variables.

Regression model of oxidative variables with HbA_1C and FBS was used to study the relationships and how much percent variation explained in the model.

The results were considered significant at $P < 0.05$. The statistical calculations were done using Statistical Package for the Social Sciences version 21.

Table 1: Mean values of the different variables of the study

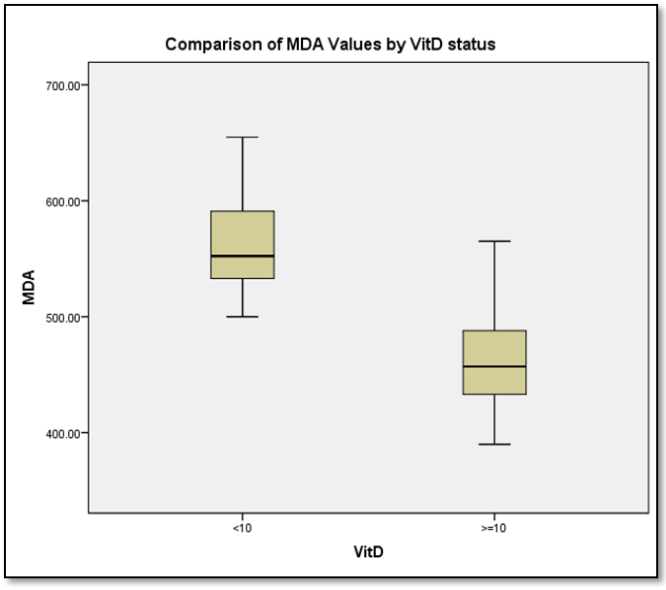
N=55	Mean	Std. Deviation
Age (years)	52.11	10.66
FBS(mg/dl)	166.69	20.513
VITD(ng/mL)	13.16	2.885
HBA_1C (%)	7.2709	0.69380
TAC(µmol/lit)	1218.9942	81.20716
MDA (nmol %)	481.4067	55.27940

Table 2: Mean and SD* values of Oxidative markers by Vitamin D and HbA_1C

Variables	Categories	N	MDA	TAC
VIT D	<10	6	564±53.8	1125±86.8
	≥10	49	471±46.7	1231±73.4
	Total	55	481±55.3	1219±81.2
	P value		<0.001	<0.01
HbA_1C	<6.5	5	453±27.6	1243±79.9
	≥6.5	50	484±56.7	1217±81.7
	Total	55	481±55.3	1219±81.2
	P value		>0.05	>0.05

* Provide absolute values of oxidative stress markers but inferences were drawn on non-parametric test of Mann Whitney 'U' test

Graph showing the comparison of MDA and Vitamin D with <10ng/mL and ≥10ng/mL



Graph showing the comparison of TAC and Vitamin D with <10ng/mL and ≥10ng/mL

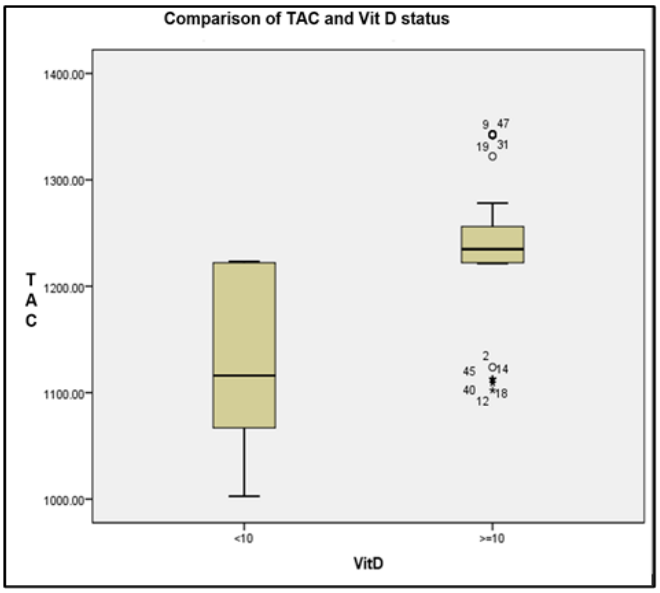


Table 3: Pearson correlation between the various variables

Correlations						
N=55		FBS (mg/dl)	VITD (ng/mL)	HbA_1C (%)	TAC (µmol/lit)	MDA (nmol %)
FBS	Pearson Correlation	1	-.452**	.860**	-.374**	.522**
	Sig. (2-tailed)		.001	.000	.005	.000
VIT D	Pearson Correlation	-.452**	1	-.402**	.121	-.410**
	Sig. (2-tailed)	.001		.002	.380	.002
HbA_1C	Pearson Correlation	.860**	-.402**	1	-.250	.598**
	Sig. (2-tailed)	.000	.002		.065	.000
TAC	Pearson Correlation	-.374**	.121	-.250	1	-.213
	Sig. (2-tailed)	.005	.380	.065		.119
MDA	Pearson Correlation	.522**	-.410**	.598**	-.213	1
	Sig. (2-tailed)	.000	.002	.000	.119	

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

Table 4: Regression model of oxidative stress variables with Vitamin D,HbA1c and FBS

Outcome Variable	Regression Model	R^2	F	P
MDA	234.0-4.0 VitD+44.6 HbA1c*-0.14 FBS	39.3	10.98	0.000
TAC	1426.6-1.58 Vit D+31.7 HbA1c-2.51FBS*	16.2	3.30	0.028

* Significant variables

Results

Mean age of the study subjects was about 52 years. Mean values of MDA by levels of Vitamin D was compared and statistically significant ($p<0.001$). High vitamin D levels have significantly ($p<0.001$) lower values of MDA (471) compared to low vitamin D status (564). Mean values of TAC was comparable across Vitamin D levels which showed that higher is the vitamin D levels higher is the TAC (1231).

FBS was negatively correlated with Vitamin D levels (r=-0.452) with highly significant value of 0.001. Relationship of FBS and HbA_1Cwere positively

(r=0.860) and significantly (p<0.001) related. FBS and TAC have high significant (p<0.01) negative relation (r=-0.374) between them and FBS was highly significant (p<0.001) positive relation with MDA (r=0.522).

Vitamin D levels and HbA_1C had highly significant (p<0.01) negative association (r=-0.402) between them. Vitamin D also had a highly significant (p<0.01) negative association with MDA (r=-0.410).

HbA_1C had a negative correlation(r=-0.250) with TAC but not statistically significant (p>0.05).HbA_1C had a very strong (p<0.001) positive relation with MDA (r=0.598).

TAC had a highly significant negative correlation with FBS (r=-0.374) with a p value of 0.005.TAC had non-significant positive correlation with Vitamin D and also non significant negative correlation with HbA_1C

Multiple linear regression model shows that HbA_1C(regression Coefficient:β=44.6) was alone significantly (p<0.001) related to MDA and the model was significant (p<0.001). Out of 38.3% variation explained these variables with MDA ,HbA_1C alone explained 35.7%. Similarly, FBS(regression Coefficient:β=-2.51) is negatively related with TAS and these variables explained 16.2 % only .

The regression model showed that with every 1% increase in HbA_1C there was 44.6 nmol % increase in MDA levels. The model also expressed with every 1mg/dl of FBS increase there was 2.51µmol/lit decrease in TAC.

Discussion

From the above results, it is observed that more is the level of FBS and HbA_1C among newly diagnosed diabetes mellitus patients lesser is the vitamin D concentration. This finding suggests that Vitamin D deficiency could be one of the factors for developing diabetes mellitus in them. This finding is congruent with the findings of the study conducted by Samiramiss Ghavam et al[14].

Decreased levels of Vitamin D could be one contributing factor for the causation of hyperglycemia in them. The mechanism of action of vitamin D in type II diabetes is thought to be mediated not only through regulation of plasma calcium levels, which regulate insulin synthesis and secretion, but also through a direct action on pancreatic Beta-cell function[15].

In diabetes, oxidative stress is caused by both an increased formation of plasma free radicals and a reduction in antioxidant defences. Hyperinsulinemia and hyperglycemia may enhance the production of free radicals and induce oxidative stress[16].

Some experimental studies showed that Vitamin D may have antioxidant properties [17,18] . At physiological concentrations, vitamin D plays a very important role in protecting cells from oxidative stress and cellular damage through a mechanism involved in reducing the plasma level of MDA and increasing the total antioxidant capacity in the plasma as explained by Bhat and Ismail, Foroozanfard et al [19,20].

MDA has been documented as a primary biomarker to evaluate lipid peroxidation, mostly studied with thiobarbituric acid reactive substances (TBARS) assay [21] .

TAC is an indicator of all oxidative stress agents and the defensive effects of antioxidants can be used as the first step to evaluate a patients' health status [22].

As per the results of this study in presence of vitamin D deficiency among the diabetics, the oxidative stress markers MDA and TAC both are altered significantly. It was observed that there was significant inverse correlation between Vitamin D and levels of MDA.

With the vitamin D value less than 10 ng/dl the MDA was very high when compared to more than or equal to 10 ng/dl.

The correlation results showed significant positive correlation with HbA_1C with MDA, higher is the HbA_1C levels more is the MDA levels in the study subjects. The regression model also suggested the same that HbA_1C which is a better indicator of glucose control in the blood had a significant relation with levels of MDA among diabetic subjects.

FBS levels also had a significant inverse relation with TAC levels, higher was the FBS lesser were the TAC levels which was also explained in the regression model.

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 [23].

According to few studies, in T2DM, the prevalence of vitamin D deficiency is 20% higher than in non-diabetics, and low vitamin D levels nearly double the relative risk of developing CVD, and predict increased risk of all-cause and cardiovascular mortality compared to vitamin D normal levels in diabetic patients[24,25].

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 defence system , predisposing to the development of diabetes-related cardiovascular incidents and its complications, leading to morbidity and mortality[26].Vitamin D deficiency was reported to be closely associated with enhanced vascular oxidative stress and increased risk of major CVD in diabetic populations [27].

According to the present study the Type II Diabetes mellitus patients have low levels of Vitamin D. And also we can conclude that vitamin D among them could be one of the factor for their hypergylcemia. They also had high levels of MDA and low TAC which could be mainly due to their low vitamin D levels, which is a potent antioxidant in the body.

Oxidative stress occurring due to chronic hyperglycemia is the contributor in the pathogenesis of diabetes-related micro and macro vascular complications among diabetics. With Increased formation of oxidative free radicals generating process and further more reduced antioxidant defence system to scavenge the excess free radicals which are induced by chronic hyperglycemia and also Vitamin D deficiency would make the situation more worse leading to more serious life-threatening complications among diabetes mellitus patients.

Strength: The study focuses on studying two important oxidative stress markers MDA and TAC among diabetes mellitus type II who were newly diagnosed who were also vitamin D deficient. This study would provide an insight to the further research how would the levels of oxidative stress markers change with Vitamin D supplementation and what dose is an optimal to bring about significant change among diabetics.

Limitation: Sample size of the study was very less, looking into the burden of the disease larger sample size should have been chosen.

Conclusion

Vitamin D levels have to be monitored and maintained within optimal levels in the type II diabetes mellitus patients as it plays a very important role in the regulation of glucose metabolism and also a potent antioxidant. In diabetics there is increased production

of free radicals and impaired antioxidant scavenging system and upon which Vitamin D deficiency co-exists, there would more chances of developing both micro and macro vascular complication leading to severe complications. Periodic monitoring and supplementation of vitamin D among diabetes mellitus Type II patients will reduce not only the burden of diabetes but also the burden of other diseases linked to this disease.

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