Vitamin D, Oxidative Stress and Cognition in Diabetes Mellitus

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Abstract

Introduction: Diabetes is a very common disease that affects almost all body systems. Of late recent studies have determined that vitamin D deficiency can cause several diseases such as diabetes, cardiovascular complications etc. Oxidative stress is an imbalance between free radicals and antioxidants in the body. Vitamin D is an antioxidant and its deficiency can cause oxidative stress .Thus vitamin D deficiency by itself and by causing oxidative stress can increase risk of developing diabetes

Hyperglycemia can affect cognition. Oxidative stress too can affect cognition. Studies done studying the role of vitamin D affecting cognition especially in diabetics are very few.

Aim: To determine the role of vitamin D in affecting cognition in Diabetic patients. To determine the correlation between vitamin D, oxidative stress and cognition in diabetes .

Materials and Method:

Comparative cross sectional study

100 Diabetes patients were studied.

Exclusion Criteria: Hypertensives, alzheimers disease, dementia, bone diseases, epileptics, taking calcium or vitamin d supplements, hypo or hyperparathyroidism, thyroid disorders.

After obtaining ethical approval from the institution a structured questionnaire was given to all.

Fasting blood glucose levels was determined by GOD-POD Method.

Hba1c was determined by Immunoturbidometry.

Vitamin D was determined by ELISA method.

Oxidative stress (malonylaldehyde) was measured by manual TBARS(Thiobarbituricacid reactive substances method)

Cognition was assessed using Montreal cognitive assessment questionnaire.

All tests were carried out at the central lab of Sree Balaji Medical College and Hospital,Chennai.

Results were analysed using Microsoft excel.

Results: A negative correlation was seen between vitamin D and MOCA scores.

A positive correlation was seen between MDA and MOCA scores.

Conclusion: Vitamin D deficiency causes oxidative stress and affects cognition in diabetics. Vitamin D supplementation can be considered to reduce oxidative stress and thus improve cognition in diabetes mellitus patients.

Keywords: *Diabetes, oxidative stress, vitamin D, Montreal cognitive assessment.*

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Introduction

Diabetes is a very common disease that affects almost all body systems. Of late recent studies have determined that vitamin D deficiency can cause several diseases such as diabetes, cardiovascular complications etc. Oxidative stress is an imbalance between free radicals and antioxidants in the body. Vitamin D is an antioxidant and its deficiency can cause oxidative stress.[1] Thus vitamin D deficiency by itself and by causing oxidative stress can increase risk of developing diabetes

Hyperglycemia can affect cognition. Oxidative stress too can affect cognition. Studies done studying the role of vitamin D affecting cognition especially in diabetics are very few.

Diabetes is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Recent studies have shown that diabetic patients are having low level of cognitive function in the form of mild cognitive impairment (MCI) which can lead to dementia and Alzheimer's disease.

MCI is diagnosed when there is:

(1) Evidence of memory impairment.

(2) Preservation of general cognitive and functional abilities.

(3) Absence of diagnosed dementia

Study Design:

Comparative cross sectional study

No of Patients data Collected = 100 Nos

Diabetes Patients with no cognitive impairement data Collected = 43 Nos

Diabetes Patients with mild cognitive impairement data Collected = 57 Nos

Materials and Method

Ethical approval was obtained from the institution ethical committee.

5ml of blood was taken in fasting state from the diabetes patients and subjected to following tests.

Plasma Glucose Level Estimation:

GOD – POD Method:

Principle: Glucose is oxidized by glucose oxidase (GOD) to produce gluconate and hydrogen peroxide. The hydrogen peroxide is coupled oxidatevely with 4 amino- antipyrene (4-AAP) and phenol in the presence of peroxidase (POD) to yield a red quinoeimine dye that is measured at 505nm .The concentration of glucose is proportional to the absorbance at 505nm.

$$Glucose + 2H_2O + O_2 \qquad Gluconate + H_2O_2$$

$$2H_2O_2 + 4\text{-}AAP + Phenol \qquad Quinoeimine\ Dye$$
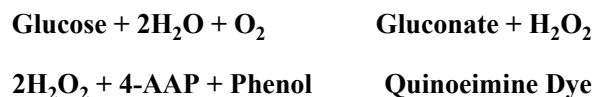
Absorbance of the colored solution at 505nm is directly proportional to the glucose concentration. **(Reference Range: 70 – 110 mg/dl)**

s Vitamin D Level Estimation: Serum vitamin D levels estimated by immunoenzymetric assay (ELISA).

Principle:

1. First 2 hours incubation step -vit.D present in calibrators,controls,samples is dissociated from binding serum proteins to fix on binding sites of a specific monoclonal antibody.

2. After 1 washing step a fixed amount of 25Oh vitamin D labelled with biotin in presence of horsedish peroxidase (HRP),compete with unlabelled 25OH vitamin d2 and 25OH vitamin D3 present on binding sites of the specific monoclonal antibody.

3. After 30 min incubation at room temperature the microtiter plate is washed to stop the competition reaction.

   The DIA source 25OH vitamin D total ELISA is a solid phase enzyme linked immunosorbent assay performed on microtiter plates.

4. The chromogenic solution is added and incubated for 15 mins.

   The reaction is then stopped with the addition of stop solution and the microtiter plate is then read at the appropriate wavelength.

5. The amount of substrate turnover is determined colourimetricaly by measuring the absorbance,which is inversely proportionaly to the total 25OH vitamin D(D2 and D3) concentration.

(Reference range: 30 – 150 ng/ml)

HbA1c Level Estimation

HbA1c Level estimated by Immunoturbidimetry

Principle - Competitive binding

Reference Values:

Good control (5.6% - 7.0%)

Fair control (7.01% - 8.0%)

Unsatisfactory control (8.01% - 10.0%)

Poor control (>10.01%)

Estimation of Malonylaldehyde

2 mL of blood was collected .Itwas centrifuged at 3000 rpm for 5 min. Then the serum MDA was measured using the method of Buege (1978).

The samples are kept in boiling water bath for 15 min.

To the diluted sample 1 mL of Trichloroacetic acid TCA-2-thiobarbituric acid (TBA)–HCl reagent is added. The reaction mixture is cooled and centrifuged. The supernatant is taken and the optical density of the pink colour formed is read at 535 nm.

The concentration of MDA in the sample is got by plotting the obtained absorbance against the standard graph. (normal range- 2.02-4.65 μM/L.)

Assessment of Cognition

Overview of the MoCA

Takes approximately 15 minutes to administer

Requires informed consent

It is a screening tool and not diagnostic

MoCA Scoring

- The total points was added.

- One point was added if the patient had less than 12 years of formal education. •

- Normal score is equal to or greater than 26/30. •

Results

All data was analysed using microsoft excel.

Unpaired student t test was performed. P value <0.05 was considered significant.

Table-1: Demographic and Clinical Charecteristics of Total Type 2 Diabetic Patients with and Without MCI

Number of Patients Male/Female	NO MCI N=43(43%) (28/15)	MCI N=57(57%) (19/38)	P Value
Age (Years)	48.55 ± 7.651	57.80 ± 5.664	<0.05
Duration of Diabetes (Months)	40.11 ± 36.198	156.42 ± 8.950	<0.05
RBS (mg%)	164.34 ± 38.999	210.12 ± 49.079	<0.05
HbA1c (%)	7.93 ± 0.462	9.18 ± 1.137	<0.05
Serum vitamin D level (ng/ml)	41.17 ± 11.544	21.97 ± 7.063	<0.05
MOCA Score	28.16 ±1.252	20.70 ± 2.456	<0.05
MDA Levels	0.93± 0.39	2.65 ± 1.33	<0.05

Table 2: Correlations of MOCA Score with Other Parameters in Total Patients with MCI (n=57)

	MOCA Score	
	R	**P**
Log10[25(OH)d]	+0.512	0.001
Duration of DM in months	-0.103	0.444
RBS (mg%)	-0.062	0.647
HbA1c (%)	+0.003	0.984
MDA	+ 0.40	0.001

Table 3: Comparision of Male and Female Patients with MCI

Number of Patients	Male (n=19)	Female (n=38)	P Value
Age (Years)	58.68 ± 4.308	57.36 ± 6.240	0.412
Duration of DM (months)	167.36 ± 50.354	150.94 ± 62.719	0.326
RBS (mg%)	214.73 ± 49.252	207.81 ± 49.489	0.620
HbA1C (%)	9.24 ± 1.039	9.15 ± 1.195	0.781
Serum Vitamin D Level (NG/ML)	26.57 ± 5.695	19.68 ± 6.592	0.0001
MOCA Score	21.73 ± 2.921	20.18 ± 2.038	0.023
MDA Level	2.67 ± 0.45	2.98 ± 0.53	0.014

Table 4: Average MOCA Score in All the Patients According to Different Serum Vitamin D Levels

Range of serum vitamin D (ng/ml)	Average serum vitamin D level (ng/ml)	Average MOCA score	P value
<20(n=28)	15.9 ± 2.916	19.71 ± 4.243	0.0001
20-30(n=28)	25.79 ± 5.187	22.64 ± 3.234	0.009
>30(n=44)	42.18 ± 10.09	27.382 ± 2.470	0.0001

Table 5: Association of Serum Vitamin D Level & Moca Score in Patients with MCI in Two Age Groups

Age Group	Serum vit.D (ng/ml)	MOCA score	P Value
<50 yrs	23.51 ± 13.454	21.28 ±3.400	0.43
>50 yrs	21.76 ± 11.766	20.62 ± 3.866	0.04

Discussion

In our study, HbA1 cpositively correlated with MOCA scores. In a study by Roy et al., cognitive impairment was observed in 11.6% of the patients who had optimal glycemic control (HbA1c under 7%) and 30.2% with HbA1c 7% or above.[5] Khullar et al. showed that subjects having glucose levels >125 mg/dl had 1.73 times higher risk of developing neurocognitive impairment.[6,7] ACCORD-MIND trial done on 2977 type 2 diabetes subjects found a statistically significant age-adjusted association between HbA1c level and score on four cognitive tests.[10] Both clock in a box and clock-drawing test have been shown to inversely correlate with HbA1c.[11] Hence, our results are consistent with existing literature that poor glycemic control in type 2 diabetes is associated with cognitive decline.

The diabetes control and complications trial in type 1 diabetes demonstrated that improved HbA1c was related to improved cognition in nonamnestic domains.[12] Luchsinger et al. showed that improving HbA1c levels in an elderly population over a period of 5 years was associated with slowing down of global cognitive decline.[13] Being a woman and longer duration of diabetes have been shown to be independent risk factors in previous studies.[6] Our study did not find any difference between sex or any relation to duration of diabetes perhaps because of inadequate sample size. The MoCA is now accepted as an excellent tool for brief cognitive screening measure and is freely available with multiple editions in various languages. The original MoCA reported a sensitivity of 100% and specificity of 87% in detecting mild AD using a cutoff score of 26.[9] Amnestic MCI (a MCI) is said to have a high likelihood of progressing to AD.[10] Hence, the differences noted in the MOCA scoring in our study could be suggestive of risk for development of AD in the future.[15] A study on the effectiveness of cognitive training program in people with MCI underlines the importance of early detection of MCI.[16]. In summary, our study shows a high prevalence of undetected MCI in type 2 diabetes mellitus patients attending an outpatient clinic setting. A strong negative correlation was noticed between all parameters of glycemic control and MOCA scores representative of cognitive function. These observations make a strong case for routine screening of type 2

diabetes mellitus patients to detect MCI with a sensitive tool such as MoCA. Studies on the benefits of improved glycemic control on cognitive function would need to be performed in the future to help us understand the significance of our finding in the long-term management of these patients.

Vitamin D and Oxidative Stress: When vitamin D status is adequate, many of the intracellular oxidative stress-related activities are downregulated. Having suboptimal concentrations of serum 25(OH) D fails to subdue oxidative stress conditions, augment intracellular oxidative damage and the rate of apoptosis. The intracellular Nrf2 level is inversely correlated with the accumulation of mitochondrial ROS [17] and the consequent escalation of oxidative stress. Thus, Nrf2 plays a key role in protecting cells against oxidative stress; this is modulated by vitamin D. In addition, vitamin D supports cellular oxidation and reduction (redox) control by maintaining normal mitochondrial functions[29-31]. Loss in the redox control of the cell cycle may lead to aberrant cell proliferation, cell death, the development of neurodegenerative diseases, and accelerated aging[31-35]. Peroxisome proliferator-activated receptor-coactivator 1α (PGC-1α) is bound to mitochondrial deacetylase (SIRT3). PGC-1α directly couples to the oxidative stress cycle [31] and interacts with Nrf2. This complex regulates the expression of SIRT3; this process is influenced by vitamin D metabolites[32]. Calcitriol has overarching beneficial effects in upregulating the expression of certain antioxidants and anti-inflammatory cytokines[33], thereby protecting the tissues from toxins, micronutrient deficiency-related abnormalities, and parasitic and intracellular microbe-induced harm [34]. It regulates ROS levels through its anti-inflammatory effects and mitochondrial-based expression of antioxidants through cell-signaling pathways [34].In our study there was a positive correlation between vitamin D levels and MOCA scores. A positive correlation was also seen between HbA1c, MDA levels and MOCA scores.

Supplementation of vitamin D in Diabetic patients can prove to be beneficial in reducing oxidative stress, improving cognition as well as help control blood sugar levels.

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