Research Article

25-Hydroxy Vitamin D level in Type 2 Diabetics and Non Diabetics: A Comparative Study

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) affects about 150 million populations worldwide and this figure is expected to be double in next two decades. Although role of vitamin D deficiency in musculoskeletal diseases are well documented, its connection with other diseases including DM pathophysiology has been implicated. Aim: Therefore, an attempt has been made to measure 25(OH) vitamin D levels in T2DM and non-diabetic population and to determine their relation with glycosylated hemoglobin (HbA1c). Material and methods: In the present study, serum 25(OH) vitamin D and HbA1$_C$ levels along with fasting and postprandial plasma glucose levels were measured in 50 diabetic patients and statistically compared with that of age matched 50 non diabetics subjects, served as control by using student's t test and Pearson correlation coefficient analysis. Results: Serum 25(OH) vitamin D levels were found to be decreased significantly ($p<0.001$) in T2DM subjects as compared to the control. HbA1c, fasting and post prandial blood sugar levels were increased significantly ($p<0.001$). In addition, 25(OH) vitamin D level was negatively correlated with HbA1c ($p<0.05$; r = -0.528) in diabetic patients. Conclusion: On the basis of the present study, we conclude that hyperglycemia is linked with poor vitamin D status and the effects of this deficiency during type 2 diabetes seem to have negative consequences on insulin resistance and glucose homeostasis.

Keywords: Insulin resistance, glycosylated hemoglobin, hyperglycemia, vitamin D, oxidative stress.

INTRODUCTION

Contrary to common belief type 2 diabetes mellitus (T2DM) is not a trivial illness but a major medical condition that affects the quality of human life of developed and developing countries as well[1]. As the medical science is advancing, the incidence of diabetes is increasing at alarming pace. This significant increase in number of people with T2DM makes this disease a global threat in 21st century. It is estimated that diabetics affect about 150 million population worldwide and this figure is expected to be doubled in next two decade[2]. T2DM is characterized by two defects; insulin deficiency and insulin resistance. In addition, T2DM is also defined by impaired glucose tolerance, chronic hyperglycemia, altered insulin secretion, and complications that come from induction of oxidative stress.

One of novel strategy toward prevention and control of T2DM is vitamin D supplementation which reflects that, apart from role of vitamin D in calcium homeostasis and bone metabolism, beneficial effects of vitamin D on non-skeletal diseases have been also attracted the attention of researchers[3]. Furthermore, vitamin D is essential for normal insulin secretion in response to glucose and also for maintenance of glucose tolerance[4]. At a molecular level, vitamin D appears to reduce oxidative stress[5]. Although little information is available regarding the association between vitamin D and Type 2 diabetes, the assessment of vitamin D level in North Indian T2DM patients has yet not been carried out.

It is conceivable that there is a close link between altered levels of vitamin D and hyperglycemia in increasing the frequency and complexity of T2DM patients. Inspite of improvement in our knowledge on T2DM from pathologic point of view, the intimate mechanisms involving vitamin D, hyperglycemia and glycosylated hemoglobin (HbA1C) in T2DM patients. Therefore, the present work aims to evaluate the levels of vitamin D, blood glucose and HbA1C in North Indian T2DM patients and to determine their role in etiopathogenesis of disease.

MATERIAL AND METHOD

In the present study 50 patients of either sex with Type 2 diabetes mellitus (31 males and 19 females) belonged to age group 35-65 years were recruited as patient group. 50 age and sex matched healthy subjects with fasting and postprandial plasma glucose less than 100 mg/dl and 140 mg/dl were recruited as controls from volunteers and staff of Shards University and Sharda Hospital, Greater Noida. A general information or pre-experimental questionnaire regarding demographic information, family history and

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Table 1: Statistical analysis of association of major risk factor with Type 2 Diabetes

S. No	Risk factor	Diabetes		Total	x^2	Result (Null Hypothesis)
		Male	Female			
1.	Age (years)					
	35-45	8	4	12		
	46-65	23	15	38	3.2	Rejected
	Total	31	19	50		
2.	Family history					
	Yes	21	14	35		
	No	10	5	15	0.18	Rejected
	Total	31	19	50		
3.	Obesity					
	Obese	20	14	34		
	Non obese	11	5	16	0.44	Rejected
	Total	31	19	50		
4.	Sedentary life style					
	Yes	23	13	36		
	No	8	6	14	0.184	Rejected
	Total	31	19	50		
5.	Drinking habit					
	Alcoholic	17	7	24		
	Non alcoholic	14	12	26	8.26	Accepted
	Total	31	19	50		
6.	Smoking habit					
	Smoker	19	3	22		
	Non-smoker	12	16	28	9.89	Accepted
	Total	31	19	50		

Null Hypothesis: The risk factor is not associated with severity of disease.

x^2 = Calculated value of chi square.

Table value of chi square for 1 Degree of freedom & at 5% level of significance = 3.84.

limited physical examination including blood pressure measurement was completed from all the subjects after taking their informed consent and approval of protocol by ethics committee of college. All patients had T2DM, defined as per revised American Diabetic Association criteria (ADA 2013).

Inclusion criteria

Subjects, who gave informed consent for study, don't under any medical treatment (anti-inflammatory drug) or taking antioxidant supplement and vitamin D analogues for at least 1 month prior to blood collection were included.

Exclusion criteria

Patients with acute and chronic infections, fever, malignancy, renal disease, hepatic disease, hypertension, those taking antioxidant vitamin supplements or non-steroidal anti–inflammatory drugs and with other connective tissue disease like systemic sclerosis and osteoarthritis were excluded.

Fasting blood sample (8 ml) was collected from the anticubital vein of the study group subjects and divided into three parts. First part was collected in fluoride vial for glucose estimation, second part in EDTA vial for HbA1c estimation and third part was kept in a syringe for half an hour for proper coagulation followed by serum separation at 2000 rpm to estimate 25 (OH) vitamin D levels. Fasting and postprandial blood glucose levels were measured by using enzymatic kit based on glucose oxidase method. Glucose, in presence of glucose oxidase,

converted into gluconic acid along with production of Hydrogen peroxide, which later oxidatively coupled with 4-aminoantipyrine /phenol (in presence of peroxidase) and red quinoneimine dye was produced. The intensity of the color complex was directly proportional to the glucose in specimen and showed absorption maxima at 505 nm.

Glycosylated hemoglobin (HbA1c) was estimated by using the VITROS chemistry products HbA1c reagent kit in conjunction with the VITROS chemistry products Calibrator Kit 18 and VITROS chemistry products FS Calibrator 1 on the VITROS 5.1 FS. Whole blood samples were hemolyzed on the VITROS 5.1FS and the concentration of HbA1c was measured in the hemolyzed samples, controls and calibrators. After performing a calibration for each reagent lot, HbA1c concentration in the unknown samples was determined using the stored calibration curve and absorbance was measured at 340 nm after a fixed incubation time.

Estimation of serum 25 OH vitamin D level was done in VITROS EciQ immunodiagnostic system by chemiluminescence method which involve the release of the 25 (OH) vitamin D in the sample from the binding protein using a low pH denaturant and the subsequent competition of the free 25 (OH) vitamin D with horse radish peroxidase (HRP) labeled 25 (OH) vitamin D reagent for monoclonal anti- Vitamin D bound to the wells. Unbound materials were removed by washing. The bound HRP conjugate was measured by a

Table 2: Glycemic profile and serum 25(OH) vitamin D levels in study group subjects.   (Mean ± S.D.)

S. No.	Particulars	Control Group (n=50)	Patient Group (n=50)	P- value
1.	Fasting Blood Glucose (mg/dl)	84.14 ± 7.63	156.40 ± 44.64	p < 0.001
2.	Post Prandial Blood Glucose (mg/dl)	125.5 ± 7.76	270.28 ± 84.65	p < 0.001
3.	HbA1C (%)	4.49 ± 0.61	8.91 ± 2.16	p < 0.001
4.	25(OH) vitamin D (ng/ml)	31.78 ± 2.00	19.65 ± 6.75	p < 0.001

where,
 p<0.1: Non-significant
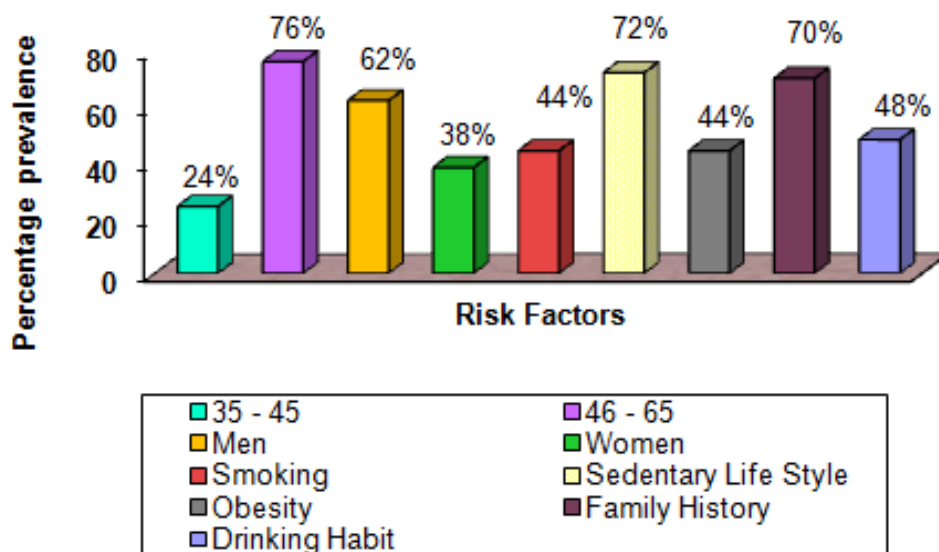 p<0.05: Significant
 p<0.001: Highly significant

Figure 1: Percentage prevalence of risk factors of type 2 diabetes

luminescent reaction. A reagent containing luminogenic substrates (a luminal derivative and a peracid salt) and an electron transfer agent was added to the wells. The oxidation of luminol derivative was catalyzed by HRP bound conjugate and the produced light signals were read by the system. The amount of HRP conjugate bound was indirectly proportional to the 25 (OH) vitamin D concentrations present in the sample.

Statistical analysis
The data collected from study group subjects were entered separately in Microsoft Excel sheet of windows 2007 and values were expressed as Mean ± SD. The significance of mean difference between study group subjects was compared by using Student's t test. The distribution of 't'- probability was calculated depending on 'n' and significance of test was obtained. P value <0.05 and <0.001 were considered as significant and highly significant respectively. In addition, relation of various risk factors with T2DM was determined by chi square test and correlation analysis between 25 (OH) vitamin D and HbA1c level was performed by using Pearson correlation test.

RESULT
In the present study, 50 patients of Type 2 Diabetes and 50 healthy individuals, served as controls were included.

Percentage prevalence of major risk factors in the study groups are represented in Fig 1. Out of the selected patients of each group, more patients of Diabetes belonged to age group 46 - 65 years i.e. 76% followed by young patients (24%). In addition, the prevalence of diabetes was higher in men than women i.e. 62% men and 38% women were diabetic subjects. There was a high prevalence of family history of diabetes in study group subjects i.e. 70%. In the patient group, 68% diabetic subjects were obese [(BMI > 25 kg/m^2) and 72% adopted sedentary life style. However, the prevalence of drinking habit and smoking habit was lower in study group subjects i.e. 48% & 44% (Fig. 1). On applying Chi square test, it was observed that ageing, family history, obesity and sedentary life style were significantly associated with T2DM diabetes whereas drinking and smoking habit were not associated with Type 2 diabetes, as represented in Table 1.

Abnormalities in biochemical markers such as glycemic profile and serum 25(OH) vitamin D levels in study group subjects are represented in Table 2. Significantly high levels of fasting blood glucose (P< 0.001; 85.88 % high), postprandial blood glucose level (P< 0.001; 115.36% high) and glycosylated hemoglobin (HbA1c) level (P< 0.001; 98.44% high) were observed in patient group as compared to healthy controls. Serum 25(OH)

vitamin D level was significantly lower (P< 0.001, 38.15% low) in patient group as compared to healthy control which authenticated the fact that low vitamin D itself may be an important risk factors for Diabetes mellitus and its related complications. Moreover, 25(OH) vitamin D level was negatively correlated with HbA1c (p<0.05; r = -0.528) in diabetic patients.

DISCUSSION

The results of the present study revealed that family history, age and sex are non-modifiable risk factors and were directly related to the incidence of Type 2 diabetes mellitus. These diseases can develop in either of the sex but are more prominently present in males. Besides this, modifiable risk factor includes obesity, alcohol consumption, sedentary life style and smoking habit. In the present study, aging, obesity, family history and sedentary life style were found to be significantly associated with Type 2 diabetes mellitus (Table 1). These findings are in concordance with the findings of Meisinger et al. and Field et al; who have also reported that rate of increasing burden of Type 2 diabetes mellitus can be reduced by modifying these risk factors (obesity, sedentary life style and dietary pattern) which precipitate the early onset of T2DM[6,7].

Tobacco smoking is considered as one of the many possible risk factors that might be associated with type 2 diabetes mellitus. Accumulating lines of epidemic evidence have suggested that chronic smokers have a higher risk to develop insulin resistance, exhibit several aspects of the insulin resistance syndrome, and develop type 2 diabetes mellitus[8,9]. However, the prevalence of smoking habit in our study was found to be low and in concordance with the findings of Wilson et al.[10] According to them, smoking was not identified as a risk factor of Type 2 diabetes mellitus. Moreover, the present study did not reveal any significant association of drinking habit with Type 2 diabetes mellitus. Interestingly, Swade and Emmanuele in their report also mentioned that drinking of large amount of alcohol should be avoided as such behavior can cause ketoacidosis, hypertriglyceridemia, and if taken outside the context of meal can cause hypoglycemia[11].

In addition to above mentioned risk factors, the extra skeletal effects of vitamin D have raised considerable interest[12,13]. In previous studies, vitamin D has been found to be effective in maintaining calcium homeostasis, in improving immunity, endothelial dysfunction and nitric oxide availability, and reducing atherosclerotic parameters. Vitamin D modulates contraction, inflammation and remodeling tissue. Deficiency of vitamin D have also been found to be associated with age related diseases, hypertension and in pregnancy related complications[14]. However, the relationship between altered vitamin D status and incidence of T2DM in developing countries has yet not been fully elucidated. However, previous studies have also shown that low vitamin D ingestion may be related with a higher risk for the development of diabetes mellitus type 2 and the metabolic syndrome[15,16].

In the present study, vitamin D levels were found to be significantly low in (p<0.001; Table 2) in Type 2 diabetes patients as compared to healthy control whereas glycosylated hemoglobin levels along with fasting blood glucose and postprandial blood glucose levels were significantly high. This shows that vitamin D deficiency is inversely related with hyperglycemia, which in turn is associated with the development of Type 2 diabetes. It could be explained on the basis of impaired ultra violet rays induced cutaneous synthesis of vitamin D which may be caused by dark skin pigmentation, use of sunscreen, limited time outdoors, latitude, season, lack of dietary sources or excess adiposity. In addition, we also observed negative correlation of vitamin D deficiency with glycosylated hemoglobin in T2DM patients which were in consistent with the findings of Athanssiou et al.[17]. Another possible reason of vitamin D deficiency mediated hyperglycemia in T2dM patients is explained on the basis of crucial role of vitamin D in maintaining extracellular calcium concentrations and calcium influx into β-cells, which is necessary for insulin secretion and thereby glucose uptake in insulin- sensitive tissue[18]. Furthermore, vitamin D has been shown to directly activate the transcription of the human insulin receptor gene and increase expression of the insulin receptor in skeletal muscle, adipose tissue and in the liver[19,20].

CONCLUSION

On the basis of prevalence of major risk factors and clinical findings of Type 2 diabetes mellitus in the population of Greater Noida city, and the consistent findings of previous studies, it is obvious that a combination of increased physical activity, moderation of alcohol intake, life style modification, adoption or consumption of diet rich in fruits, vegetables and low fat dairy products and proper exposure to sunlight to fulfill the vitamin D requirement of the body, can prevent the development of Type 2 diabetes mellitus. Furthermore, apart from routine fasting and postprandial blood glucose estimation, regular assessment of vitamin D levels along with HbA1c is efficient markers in determination of T2DM complexity.

Moreover, the observation made in the current study cannot draw a definitive conclusion, due to inadequacy in sample size which is just 50 and inability to measure insulin levels in T2DM. To validate the findings of the current study, multicenter study with large sample size should be carried out to support the findings of the current observation from this study.

ABBREVIATION
25(OH) vitamin D: 25 Hydroxy vitamin D
BMI: Body Mass Index
HbA1c: Glycosylated hemoglobin
T2DM: Type 2 diabetes mellitus

Conflict of interest: There is no conflict of interests. All authors are equally contributed.

Ethical approval: Approved

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