Original Research Article

Assessment of vitamin D status and its association with insulin resistance among type 2 diabetic subjects

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ABSTRACT

Background: The 21st century has seen the rise of diabetes mellitus as one of the major metabolic issues as is vitamin D deficiency which has been found to be pandemic worldwide. The present study is an endeavor to study the status of serum vitamin D levels in relation to the glycemic and insulin resistance status in type 2 diabetes mellitus patients.
Methods: The present is a cross-sectional study with a sample size of 100 type 2 diabetic subjects in the age group of 30-60 years. Serum vitamin D and Insulin levels were estimated using the ELISA technique. HbA1c levels were measured using immunoturbidimetric assays and plasma glucose levels were determined using glucose oxidase-peroxidase method.
Results: The fasting plasma glucose, HbA1c and serum insulin levels were found to be significantly higher in those with vitamin D levels below the normal cut-off value of less than 30ng/ml (p value <0.01). Also, the insulin resistance calculated using HOMA-IR was found to be higher in those subjects having vitamin D deficiency or insufficiency.
Conclusions: Hence, the study suggests that vitamin D deficiency contributes to further insulin resistance and poorer long-term diabetic control in type 2 diabetes mellitus subjects.

Keywords: Insulin, Insulin resistance, Type 2 diabetes, Vitamin D

INTRODUCTION

Diabetes mellitus is a disease that has lived with the mankind since the times ancient to the present and is expected to wreak havoc in the future, according to estimates. The ancient Egyptians knew about it almost 1500 years before Christ was born, numerous Associations and Committees of today are studying this disease and the International Diabetic Federation says that it is here to stay with our future generations. According to the latest estimates provided by the International Diabetic Federation Diabetes Atlas, Seventh Edition, 2015 approximately 415 million adults are afflicted with diabetes mellitus, presently. A figure which is expected to rise to 642 million by 2040. Type 2 DM being the most prevalent type with the proportion of

people suffering from type 2 DM increasing in most countries. Lifestyle changes and changes in the socio-economic structure are deemed to be important causes for this increase in prevalence of type 2 DM, especially, in low and middle income countries where the prevalence continues to rise at an alarming rate. In fact, 75% of adults with diabetes mellitus live in these countries at present.[1]

About 90% of patients of diabetes mellitus belong to the type 2 category. Risk factors include overweight (BMI ≥25kg/m²), family history of diabetes, sedentary lifestyle, advanced age (≥45 years), ethnicity and polycystic ovary disease, as well as history of gestational diabetes, dyslipidemia, hypertension, vascular disease, impaired fasting glucose or impaired glucose tolerance, and other

conditions associated with insulin resistance (i.e., acanthosis nigricans). Most people with type 2 diabetes are obese, insulin resistant and have a relative or absolute deficiency in insulin secretion. Inappropriately high hepatic glucose production occurs, along with impaired glucose utilization peripherally. Decreased glucose transport can be demonstrated in muscle and adipose tissue. If the pancreas is unable to secrete sufficient insulin, impaired glucose tolerance or type 2 diabetes results. Hyperglycemia is toxic to beta cell function and further impairs insulin secretion. Over time, beta cell failure is usually progressive, and the beta cells produce lesser amounts of insulin, contributing to increasing insulin deficiency. Although many people with type 2 diabetes can be effectively treated with diet, exercise, and oral glycemic control agents, others require insulin therapy.[2]

The principle hormone regulating blood glucose is Insulin. Insulin is released from the beta cells in the islets of Langerhans found in the pancreas. Glucose enters the cell and ATP is produced in the mitochondria through the Krebs cycle and electron transport chain. This increase in ATP causes channels to close. These channels allow potassium cations to flow into the cell.[3,4] With these channels closed, the inside of the cell becomes more negative causing calcium channels to open allowing calcium cations to flow into the cell. Calcium cations flow into the cell due to a concentration and elector chemical gradient that favors the influx of calcium cations. Calcium ions are key in the vesicle excretion processes. A protein on the vesicle called the v-SNARE protein becomes entangled with a t-SNARE protein on the beta cell surface. With calcium facilitating the interaction amongst the SNARE proteins the vesicle is forced to merge with the cell membrane and insulin is excreted into the blood stream.[3-5]

Post binding defects in insulin action primarily are responsible for the insulin resistance in type 2 diabetes. Insulin travels through the blood stream to muscle, brain or adipose tissue. Once there, the insulin binds to a dimeric transmembrane receptor. This receptor auto phosphorylates and causes many downstream pathways relating to glucose regulation, energy storage and DNA transcription. For the glucose regulation path way the receptor auto phosphorylates then causes insulin receptor substrate 1 (IRS-1) to be phosphorylated.[3,4] IRS-1 then phosphorylates Phosphatidylinositol 3-kinase (PI3K). PI3K cleaves Phosphatidylinositol 4,5-bisphosphate (PIP2) leaving diacyl glycerol (DAG) in the cell membrane and inositol 1,4,5-trisphosphate (IP3) in the cytosol. IP3 travels to the smooth endoplasmic reticulum (SER) and cause calcium channels to open in the SER, releasing cations into the cytosol. DAG activates a kinase named protein kinase C which also opens calcium channels in the SER. This ambient increase in calcium facilitates the binding of vesicles to the membrane. These vesicles have Glut4 proteins imbedded in the membrane. Glut4 proteins are channel proteins that allow glucose

into the cell. Once these vesicles bind with the cell membrane glucose flows through the Glut4 protein and into the cell, reducing blood glucose levels.[3] The process by which insulin is degraded and metabolized is poorly understood. However, it is known that the liver is responsible for the majority of insulin break down. Since the liver is so important to proper insulin levels when the liver is damaged perhaps by alcohol the regulatory system can be interfered with. The kidney is also key in the breakdown of insulin. Insulin clearance rates are shown to decrease in those who are obese or have diabetes. This may create insensitivity to insulin.[6]

Vitamin D has been known to man since ages. It has been reported that the earliest phytoplankton life forms that have existed unchanged in the Atlantic Ocean for more than 750 years can make vitamin D when exposed to sunlight.[7,8] Most vertebrates, including amphibians, reptiles, birds, and lower primates, depend on sun exposure for their vitamin D requirement.[8] The lack of sunlight and its association with the devastating bone deforming disease rickets in children was first recognized by Sniadecki.[9] It was classified as a vitamin in the early 20th century and as a prohormone (conditional vitamin) in the second half of the 20th century.[10]

It was thought that in tropical countries like India vitamin D deficiency will not occur due to abundant sunlight but studies have shown that high incidences of vitamin D deficiency is occurring among population of all ages.[11,12] In a study from north India it was found that out of the total study population more than half of population was vitamin D deficient.[13] Many other studies also have reported the evidence of vitamin D deficiency in majority of population. Vitamin D deficiency is pandemic yet most under diagnosed condition and also under treated in most part of the world.[14,15]

It has been suggested that vitamin D has a protective role in a number of diseases including diabetes mellitus. The present study is an attempt to study the vitamin D status in type 2 diabetes mellitus patients in relation to glycemic and insulin resistance status.

METHODS

The present study is a cross-sectional observational study done in 100 type 2 diabetes mellitus patients attending the OPD Clinical Biochemistry Laboratory of Medical College, Kolkata between January 2016 to February 2017. The study was conducted after obtaining permission from ethical committee and written consent from subjects before commencing the same.

Sample size calculation and subject selection

Sample size was determined by using the formula z^2pq/L^2 taking the prevalence rate of diabetes mellitus as 8.8%. The patients were randomly chosen from among those who were diagnosed to be suffering from type 2 Diabetes

mellitus, i.e. having fasting plasma glucose levels ≥126mg/dl (WHO 2006 recommendation) and were on oral hypoglycaemics or diet. Patients in the age group of 30-60 years were included. Patients treated with insulin, critically ill patients, patients with compromised liver function, compromised renal function, patients with underexposure to sunlight were excluded. Also, those on vitamin D supplementation or suffering from other endocrinopathies like thyroid, pituitary or adrenal dysfunction were excluded.

Procedure

All subjects after screening for inclusion and exclusion criteria were asked for detailed history followed by meticulous examination after obtaining proper consent in prescribed consent form and were subjected to appropriate biochemical investigations.

The parameters from the biological samples of the study subjects were estimated to fulfill the objectives of the study by standard procedures in the clinical biochemistry laboratory of the Medical College Hospital, Kolkata with optimum parameters for maintaining the quality control of the tests performed.

About 8ml of fasting blood sample were collected by venepuncture from each case- 4ml of blood in clot-activator vial, 2ml in glucose vial (containing sodium fluoride and potassium oxalate), 2ml in EDTA vial. The tubes were placed in a centrifuge and spun at 3000rpm for 10 minutes to obtain the plasma and serum. Plasma glucose was measured immediately and the serum and plasma for the measurement of other biochemical variables were stored at -20°C until analysis. Serum 25-hydroxyvitamin-D and serum insulin were estimated by Enzyme Linked Immunosorbent Assay (ELISA). Plasma Glucose was estimated by Glucose Oxidase-Peroxidase (GOD-POD) method. HbA$_{1c}$ was estimated using ion-exchange resin method.

The Homeostasis Model of Assessment-Insulin Resistance Index (HOMA-IR) was used as an index of insulin resistance and was calculated from the formula:

HOMA-IR = {Fasting insulin (µIU/ml) x Fasting Glucose(mg/dl)}/405

Statistical analysis

For statistical analysis, the data obtained were placed into a Microsoft excel sheet and then analyzed by SPSS 20.0.1 version and Graph Pad Prism version 7.03. Data have been summarized as mean and standard error of Mean for numerical variables and count and percentages for categorical variables. The median and the interquartile range have been stated for numerical variables that are not normally distributed. Chi-square test was done to compare the sex ratio in the two groups. Student's unpaired t-test with Welch correction was applied to

compare between the normally distributed numerical variables. Correlation was calculated by Spearman correlation analysis. In this study, p-value <0.05 has been considered to be statistically significant.

RESULTS

The present cross-sectional study of sample size of 100 type 2 diabetic patients intended to see their underlying vitamin D as well as lipid profile status. The study population was subdivided into two groups considering their vitamin D levels: Normal (>30ng/ml) and below normal groups (<30ng/ml). About 66% of the study population were either vitamin D levels deficient or insufficient while the rest 34% had normal vitamin D levels (Figure 1).
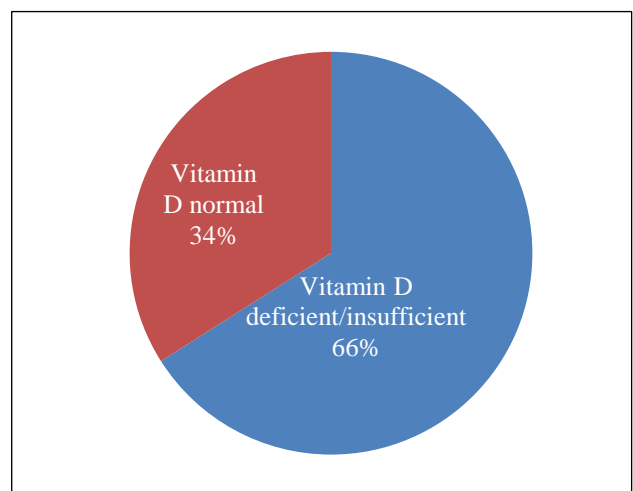

Figure 1: Vitamin D levels in the study population.

The first group (25 OHD levels >30ng/ml) included 18 females and 16 males while the second group had 39 females and 27 males. No significant difference was found between the age and sex ratios of the 2 groups. The fasting plasma glucose, HbA1c and fasting insulin levels were found to be significantly higher in the group having vitamin D levels below normal. Also, the insulin resistance as calculated by using HOMA-IR was found to be higher in the second group (Table 1).

Table 1: Comparison between the two groups of the study population.

Variable	25-OHD levels >30ng/ml	25-OHD levels <30ng/ml	p-value
Age	46.94	48.34	0.329
Sex (Female:Male)	18:16	39:27	0.65
Fasting plasma glucose	152.1±2.4	164.7±2.5	<0.01
Hb A1c	7.3±0.11	7.8±0.09	<0.01
Insulin	12.9±3.09	14.7±3.81	0.019
HOMA-IR	4.54±1.11	5.34±1.38	0.0038

Inverse correlation was found between 25 OHD levels and HbA1c, insulin and HOMA-IR for both the groups as is suggested by the spearman correlation coefficient values (Table 2).

Table 2: Spearman correlation coefficient(r) of 25-OHD, serum insulin, HBA1c and HOMA-IR in the two groups.

Variable	25-OHD levels >30ng/ml	25-OHD levels <30ng/ml
Hb A1c	-0.42 (0.0108)*	-0.39 (0.001)**
Insulin	-0.34 (0.0425)*	-0.30 (0.0129)*
HOMA-IR	-0.39 (0.0187)*	-0.44 (0.0002)**

*p-value<0.05; **p-value <0.01

The correlation between vitamin D and Hb A1c as found in the study had r-values of -0.42 and -0.39 in the two study groups respectively is significant (Figure 2).
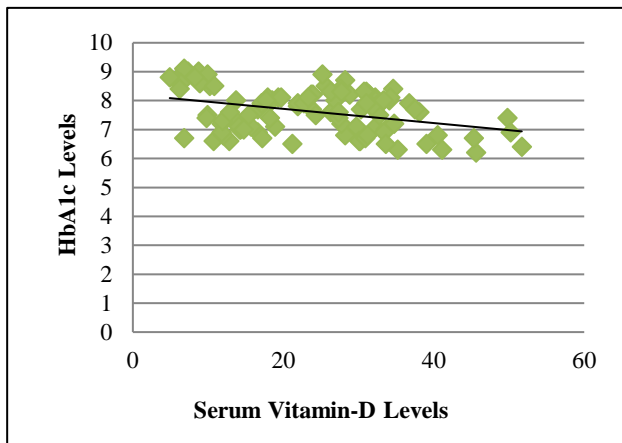

Figure 2: The correlation of vitamin D with Hb A1c levels in the study population.
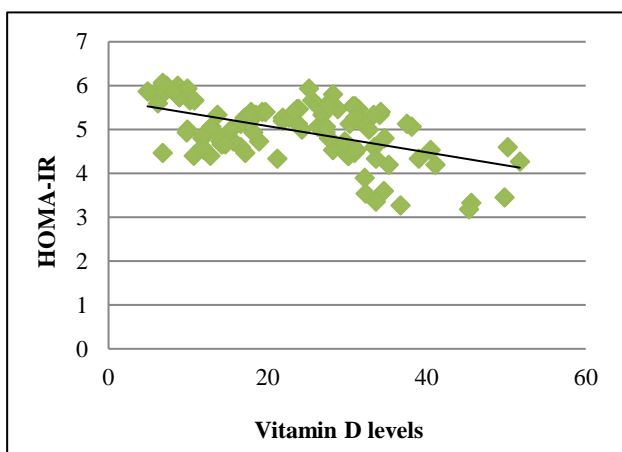

Figure 3: The relation of vitamin D with Hb A1c levels in the study population.

Also, the correlation between vitamin D and HOMA-IR is also negative in the two groups having r- values of -0.39 and -0.44 respectively (Figure 3).

DISCUSSION

Vitamin D deficiency has also been described as a pandemic entity that has affected the population of the whole world, including India. In this present study, it has been found that 66% of the study population were either Vitamin D deficient (<20ng/ml) or vitamin D insufficient (<30ng/ml). In fact, 42 % of the study population had vitamin-D levels <20ng/ml. A number of similar studies, done on the Indian population, found evidence of large-scale vitamin D deficiency, though the exact figures vary.[16]

The present study detected a significant negative correlation between plasma vitamin D and each of insulin levels, HOMA-IR and HbA1c levels in both the studied groups. Modi et al had similar findings in their studies.[17] On the contrary Erdönmez et al, stated that there was no significant association between vitamin D levels and insulin resistance.[18] Also the findings of Sollid et al, suggested that vitamin D supplementation does not improve glycemic status.[19]

Various mechanisms have been suggested for the effects of vitamin D: presence of vitamin D receptors on pancreatic β cells, expression of vitamin D activating 1α hydroxylase in pancreatic β cells, the insulin gene having vitamin D response element and the transcription of insulin receptor genes being increased by1,25(OH)$_2$D.[20-23] Studies on mice by Zeitz et al, where they removed the vitamin D receptors in the pancreatic beta cell showed these animals could not produce as much insulin as they should.[24] Protective effects of vitamin D on diabetes, maybe due to well-known effects of vitamin D, such as its anti-inflammatory properties, its effects on calcium and phosphorus metabolism and regulation of the insulin receptor gene.[23] It has been suggested that vitamin D increases in calcium content of the cells, in turn leading to increased transport of glucose into the muscle.[25] Vitamin D also regulates nuclear PPAR (Peroxisome proliferative activated receptor) that has an important role in the insulin sensitivity.[26] Vitamin D deficiency is associated with increases in inflammation. Vitamin D attenuates the expression of proinflammatory cytokines involved in insulin resistance such as interleukins, IL-1, IL-6, TNF-α, also down regulates NF-Kβ (Nuclear factor) activity.[27]

A review by Mitri et al, found that people with higher vitamin D blood levels (>25ng/ml) had a decreased chance of getting T2D later in life compared to those with the lowest levels (<14ng/ml).[28] A review by Song et al, studies that measured serum vitamin D blood level and then followed participants to see if they got T2D later in life were examined. It was found that Participants with the highest vitamin D levels had a decreased risk of diabetes compared to those with the lowest vitamin D levels. Every 4ng/ml increase in vitamin D was associated with a 4% lower risk of getting T2D later in life.[29]

A recent trial in 2011 gave 2000 adults with pre-diabetes either 2000 IU of vitamin D per day or 400mg calcium. It was found that vitamin D supplementation improved pancreatic B cell function and helped control the rise of plasma glucose level.[30] However, on the other hand, another very recent trial found that vitamin D supplementation in people with pre-diabetes had no effect in reducing people's chances of getting diabetes later. Furthermore, people who supplemented with vitamin D didn't produce more insulin and they weren't any more sensitive to insulin than those taking a dummy pill. They were also just as likely to get diabetes later in life as people taking the dummy pill.[31]

After conducting a meta-analysis and review of the impact of vitamin D and calcium on glycemic control in patients with type 2 diabetes, Pittas et al concluded that insufficient vitamin D and calcium appears to hinder glycemic control and that supplementing both nutrients may be necessary to optimize glucose metabolism.[32] An observational study from the nurses health study that included 83,779 women >20 years of age found an increased risk of type 2 diabetes in those with low vitamin D status. A combined daily intake of >800 IU of vitamin D and 1,000 mg of calcium reduced the risk of type 2 diabetes by 33%.[33] The National Health and Nutrition Examination Survey (NHANES) III study between 1988 and 1992 demonstrated that there is a strong inverse association between low levels of 25(OH)D and diabetes prevalence.[34] Low vitamin D levels have also been shown to be predictive of the future development of type 2 diabetes.[35] Parker J et al, showed that increasing vitamin D serum levels to normal led to a 55% relative reduction in the risk of developing type 2 diabetes.[36]

As with most disease states and vitamin D, prospective studies related to vitamin D supplementation and diabetes are rare and limited. Kayaniyil et al, performed a linear regression analysis of 712 subjects after evaluating serum 25(OH)D levels and assessing insulin sensitivity by means of the homeostasis model of insulin resistance. Their results indicated that vitamin D was significantly correlated to insulin resistance and β-cell function in their multiethnic sample. The researchers concluded that low vitamin D levels may play a significant role in the pathogenesis of type 2 diabetes.[37] The NHANES group (2003-2006) evaluated 9,773 U.S. adults >18 years of age and showed a mechanistic link between serum vitamin D levels, glucose homeostasis, and the evolution of diabetes.[38] On the contrary to the above findings, McGill, AT et.al found no significant relationships between hypovitaminosis D with status of blood glucose and glycaemic control.[39]

The present study has certain limitations. Some of them due to time and resource constraints and some others which could only be realized after the study was concluded. Firstly, the number of study subjects, if higher, would have made the findings more significant.

Secondly, the patients are from OPD of clinical biochemistry of our hospital which is not a true representative of the normal population. Thirdly, presence of confounding effects of unforeseen variables (heterogeneicity of ethnicity and religious distribution, dietary habit, solar exposure, physical activity etc) that influence the variable parameters were not included in the present study and finally, variation in the standard of treatment, reflecting control of the diabetic state is also another confounding factor.

CONCLUSION

These findings seem to suggest that vitamin D deficiency does really associate with insulin resistance and altered glycemic control in type 2 diabetes mellitus subjects. However, prospective studies with vitamin D supplementation on diabetic individuals in a larger study population may help establish the protective role that vitamin D may play in that situation.

ACKNOWLEDGEMENTS

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