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Serum Vitamin D in Patients With Type 2 Diabetes Mellitus a Cross-Sectional Study With Controls

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ABSTRACT

Accumulating researches suggest that serum Vitamin D has an effect on insulin synthesis, secretion and insulin resistance revealing an inverse relationship with glucose tolerance. The present study was undertaken with an objective to evaluate the serum Vitamin D status in the patients of Type 2 Diabetes Mellitus with and without complications and its association with the disease process. The serum vitamin D concentrations were found to be significantly reduced in patients of type 2 DM as compared to healthy individuals with significantly lower level observed in complicated cases. A significant negative association was observed between HbA1C level and serum Vitamin D concentration in the study population

KEYWORDS Vitamin D; Diabetes mellitus; HbA1c; VDR; DBP

INTRODUCTION

Diabetes Mellitus, characterized by impaired insulin secretionor variable degrees of insulin resistance includes a heterogeneous group of disorders associated withhyperglycemia and dyslipidemia leading to many micro vascular and macro vascular complications. (1). Although prevalence of both Type 1 and Type 2 DM(Type 2 Diabetes mellitus) is increasing worldwide, the rise in Type 2 DM is much more rapid, presumably because of increasing obesity, reduced level of physical activity as countries are becoming more industrialized, altered environmental factors and increase in aging population. (1).Among the several environmental and dietary causes, Vitamin D deficiency is now being studied extensively as a predisposing factorsupposedly contributing to the development of diabetes mellitus. Accumulating research suggestan inverse relationshipof serum Vitamin Dconcentration with blood glucoselevel and insulin resistance (2-7).Moreover Vitamin D deficiency has been shown to alter insulin synthesis and secretion in the beta cells of pancreas in both human and animal models (8-10).Vitamin D replenishment in establishedhypovitaminosis D caseshas shown improvement in the glycemic status and insulin secretion in patients with Type2 DM(10). The presence of vitamin D receptors (VDR) and vitamin D binding proteins (DBP) in pancreatic tissue and the relationship between certain allelic variations in the VDR and DBP genes with glucose tolerance and insulin secretion have further supported the hypothesis (5,11).

Hence, perceiving the importance of the role of Vitamin D in Diabetes mellitus, this study focuses on identifying any substantial association between serum vitamin D level and glycaemic status in Type2 Diabetes mellitus patients, along with the significance of serum vitamin D concentrations in the occurrence of chronic complications in these patients.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry in collaboration with Department of General Medicine, S.C.B. Medical College and Hospital, Cuttack, Odisha. 68 patients of age group 35-74 years, attending OPD and indoor in the Department of Medicine were included in the study. Patients with Type 2 Diabetes Mellitus were selected and diagnosed on the basis of their history, physical examination, biochemical investigations and according to the ***Criteria for the diagnosis of Diabetes Mellitus given by WHO 2011*** having any one of the following features:

i.Classic symptoms of diabetes and random plasma glucose concentration ≥200mg/dl

ii. Fasting plasma glucose ≥126mg/dl

iii. HbA1c> 6.5%

iv. 2-hour post prandial plasma glucose concentration ≥200mg/dl during the OGTT

The control group consisted of 64 age and sex matched healthy adults with fasting plasma glucose levels ≤ 99mg/dl and post prandial plasma glucose ≤ 139 mg/dl having no family history of diabetes mellitus, previous hypertension, renal diseases, ongoing tuberculosis and chronic metabolic diseases.

Patients with Type 2 diabetes mellitus having acute complications like hypoglycemia, ketoacidosis, hyperosmolar nonketotic coma or any infections, with pregnancy or Gestational Diabetes Mellitus, with known thyroid/parathyroid disorders or any other endocrinopathy, cases of essential hypertension, immunosuppression or history of illicit drug use, taking anticonvulsants, steroids, thyroxin group of drugs, vitamin supplements were excluded from the study group.

All the case records were collected in a specified proforma. Written, informed consent was obtained from all subjects. The study was approved by institutional ethical committee.

3 ml of blood was collected after overnight fasting of 8 hours from all enrolled patients and healthy controls for the assessment of Vitamin D levels and other biochemical parameters. Fingerprick blood sample was taken at the same time for as-

sessment of HbA1c levels from all patients and healthy controls.

All the routine biochemical parameters like FPG (Fasting Plasma Glucose), PPPG (Post prandial Plasma Glucose), serum Urea, Creatinine, Cholesterol, Triacylglycerol and HDL (High Density Lipoprotein)were done in an Automated Clinical Analyzer using commercially available kits. Serum LDL (Low Density Lipoprotein) and VLDL (Very Low Density Lipoprotein) were calculated using Friedewald's formula. Special parameter like serum ionized calcium was estimated by ISE method, Capillary blood HbA1c level measured by immunoturbidimetricmethod and serum 25-OH Vitamin D concentration assay was done by ELISA.

The results obtained were statistically analyzed using SPSS version 16.0 software.All data are expressed as mean ± SD. Student's t-test and ANOVA are used to compare mean values between different study groups and calculate significance. A p-value <0.05 is considered to be statistically significant.Pearson's coefficient of correlation was used to assess the association between Vitamin D and other variables.

OBSERVATIONS

In our study, the mean FPG in mg/dl was 84.09 ± 8.67 in the control group, 154 ± 16.35 in the uncomplicated DM cases and 207.26 ± 27.96 in the complicated DM cases and the mean PPPG in mg/dl was 103.71± 11.17, 192.5± 19.35 and 263.5± 38.53 in the controls, uncomplicated and complicated DM cases respectively. The FPG and PPPG values were significantly higher in the cases compared to controls with a p<0.001(Table 1)

The mean serum urea in mg/dl was 28± 3.88 in the controls, 32.05± 2.86 in uncomplicated DM cases and 56.3 ± 12.01 in complicated DM cases. The mean serum creatinine in mg/dl was 0.88± 0.1 in the controls, 1.01± 0.16 in uncomplicated DM cases and 1.94 ± 0.45 in complicated DM cases. The serum urea and serum creatinine were found to be higher in complicated cases of Type 2 DMwhich was statistically significant when compared to controls and uncomplicated cases. (Table 1)

Dyslipidemia was very clearly evident in the cases compared to controls with the derangement in lipid profile being more marked in the complicated cases of DM (Table 1).The mean total cholesterol level was161.5±20.57 in controls, 186±21 in uncomplicated DM cases and 222.4 ± 37.33 in complicated DM cases. The mean serum triglycerides level was estimated to be 126.5±23.95,138.9±20.33 and 174.1 ± 33.13in the controls, uncomplicatedand complicated DM cases respectively. The serum LDLc level in controls, uncomplicated cases and complicated cases was 93.08 ± 17.8, 119.8±19.33 and 150.3±33.75 while the serumVLDLc level in controls, uncomplicated cases and complicated cases was 25.31 ± 4.79, 27.91±3.98 and 34.49±6.64 respectively.SerumHDLc level was 43.92±2.42 in controls, 37.61±2.70 in uncomplicated cases and 40.35±4.58 in complicated cases.

The mean serum Vitamin D concentration in the control group was found to be 33±3.33 with a 95% confidence interval of 32.41–33.59. Patients with uncomplicated DM had a mean serum Vitamin D concentration of 19.94±2.41ng/ml and 95% confidence interval of 19.38 -20.51.In patients with complicated DM the mean serum Vitamin D level was found to be 13.4±1.45 and 95%confidence interval of mean was 12.98 – 13.73.The serum Vitamin D concentration was significantly lower in cases compared to controls which was more discernible inpatients diagnosed with complications of DM (Table 2).

The assessment of the mean HbA1c (%) level showed a value of 4.67±0.39 % in the control group .HbA1c level in the uncomplicated DM cases was 7.36±0.42% and in patients with complicated DM the HbA1c (%) level was found to be 8.66±0.33%.Long term hyperglycemia was prominent in the cases compared to controls, it being more marked in the complicated DM cases (Table 3).

Table – 4 depicts the serum ionized calcium levels in mmol/L. The serum ionized calcium levels were markedly reduced in patients with Type 2 diabetes mellitus as compared to the healthy individuals in the control group (1.21±0.09, 0.93±0.10 and 0.81±0.06 in the controls, uncomplicated and complicated cases respectively.

The Pearson's correlation analysis between Vitamin D and HbA1c % in the DM cases revealed a significant negative association with a r value of -0.883 and p value of <0.001(Graph 1).Serum Vitamin D concentrationalso demonstrated a negative correlation with the fasting plasma glucose level in the uncomplicated and complicated cases of the study group with a r value of -0.156 (Graph 2) and r value of -0.334 (Graph 3) which was statistically non-significant with a p value of 0.356 and 0.066 respectively.The observations markedly points towards a negative association of Vitamin D level with the diseases process implicating its importance in relation to long term hyperglycemia denoted by HbA1c% in comparison to immediate plasma sugar level revealed by FPS and PPPS levels.

DISCUSSION

The present study was undertaken with an objective to evaluate the serum Vitamin D status in the patients of Type 2 Diabetes Mellitus with and without complications and its association with the disease process.

The study population comprised of 132 subjects out of which 68 were patients with type 2 diabetes mellitus and 64 served as age and sex matched healthy controls. Out of the 68 cases 37 consisted of uncomplicated cases and 31 cases were with complications related to long term DM.

Hyperglycemia, revealed by a significantly raised FPG and PPPG level in the cases compared to controls along with increased HbA1clevel was observed in our study population which is a quintessential feature of Type 2 DM. An obvious dyslipidemia exists in type 2 diabetes patients with higher levels in complicated cases in the present study. This observation accorded with the observation of Gordon L et al (12) and Mahato RV et al (13) who also reported an increased occurrence of dyslipidemia in patients with uncontrolled diabetes. Theabove metabolic derangements like hyperglycemia and dyslipidemia can be attributed toimpaired insulin secretion with increased hepatic glucose production along with insulin resistance in adipose tissue as a result of which free fatty acid (FFA) flux from adipocytes is increased, leading to increased lipid synthesis in hepatocytes.(1).

The HbA1c is a better index of glycaemia as it is a long term marker reflecting the plasma sugar level over a period of 12 weeks, is not affected by food intake, emotional stress, exercise or time of day and it correlates better with complications. In our study, the HbA1c% levels were significantly raised in patients of diabetes and were found to be still higher in complicated cases as compared to uncomplicated cases (p<0.001) which agrees with the observations made by many authors supporting that HbA1cis an indicator of long term blood glucose concentrations and is an apt gage for the development of complications in patients with diabetes mellitus (14-16).

A raised serum urea and creatinine level was observed in the complicated cases of DM in the study population compared to uncomplicated cases and healthy controls .This is in covenant with the view of Powers Alvin C (1) who documenteda rise in blood urea nitrogen and creatinine due to micro vascular complications of diabetes mellitus like diabetic nephropathy.

In our study the non-diabetic healthy individuals had Vitamin D levels ranging from 29.7 to 36.3ng/ml and the lowest values were observed in complicated type 2 diabetes patients with a range of 11.9 to 14.9 ng/ml. In patients with uncomplicated diabetes the Vitamin D levels ranged from 17.5 to 22.3

ng/ml which was intermediate between the levels found in controls and complicated cases. Our finding agrees with the observations made by Pittas et al (17) who reported a serum Vitamin D concentration in the range of 27to34.5 ng/ml in their highest tertile and 10.4to14.9 ng/ml in the lowest tertile.

Our study documented an inverse relationship of serum Vitamin D concentration with FPG in the cases of DM which was however statistically non-significant. Need et al (18) previously reported that lower serum VitD levels was associated with higher fasting plasma glucose throughout the measured array and is most marked when serum VitD levels were less than 40nmol/L (16.0 ng/ml). The association of increased FPG and decreased vitamin D levels may be explained by the regulatory role of vitamin D on insulin sensitivity and increasing insulin secretion from the pancreatic β cells (8, 9).

On analyzing the correlation between serum Vitamin D concentrations and the HbA1c% levels in the study population by Pearson's correlation analysis,a negative association between them was revealed which was statistically significant($p<0.001$). This observation was in agreement with that of Kositsawat J et al (19) who established a mechanistic link between serum vitamin D concentration and glucose homeostasis manifested by HbA1c% levels.

Diabetes mellitus (DM) refers to a group of common metabolic disorders with hyperglycaemia being the archetypal feature. Type 1 Diabetes is the result of complete or near-total insulin deficiency while Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Based on current trends, it is projected that >360 million individuals will have diabetes by the year 2030. (1, 20)Studies from various urban areas of India have shown a several fold increase in the prevalence of Type 2 diabetes in the last two decades. (21, 22)The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease.The risk of chronic complications increases as a function of the duration of hyperglycemia; they usually become apparent in the second decade of hyperglycemia. Since type 2 DM often has a long asymptomatic period of hyperglycemia, many individuals with type 2 DM have complications at the time of diagnosis. (1)

The ubiquitous expression of the vitamin D receptor (VDR) in all nucleated cells, the presence of functional 1β-hydroxylase in several other tissues apart from the kidney and the extensive number of genes that are under control of VitD depicts a more universal role of the vitamin D endocrine system than just regulation of calcium, phosphate and bone metabolism. The suggested relationship between type 1 diabetes mellitus and vitamin D deficiency has been extensively reported (23,24). Vitamin D treatment has been shown to improve, and even prevent, type 1 diabetes mellitus in both human and animal models (25- 27). These effects have been mainly ascribed to the immunomodulatory actions of vitamin D. (24).The association between Vitamin D and Diabetes mellitus is still in experimental stage and absolute conclusive evidence of interaction is yet to be determined. However studies shows that Vitamin D deficiency causes reduced insulin secretion in rats and humans, and its replenishment improves β-cell function and glucose tolerance.(8-11) Moreover, certain allelic variations in the vitamin D receptor (VDR) and vitamin D–binding protein (DBP) might influence glucose tolerance and insulin secretion,(5,11,28)thus contributing to the genetic risk for type 2 diabetes. Accumulating evidences suggests a role for vitamin D in insulin secretion, which includes the presence of the VDR in β cells and the vitamin D–dependent calcium-binding proteins (DBP) in pancreatic tissue(29-31). Therefore the effects of vitamin D on insulin secretion may follow several pathways. Evidence furthermore points towards influence of Vitamin D on β-cell insulin secretion through a rise in intracellular calcium concentration via non-selective voltage-dependent calcium channels (32, 33).The present study registered a low serum ionized Calcium in cases compared to controls (Table 4) which explains the above mentioned role of Vitamin D on β-cell insulin secretion .It has also been advocated that Vitamin D improves insulin secretion by activation of protein biosynthesis in pancreatic islets cells(34).

As vitamin D modulates insulin receptor gene expression and insulin secretion, it is an interesting environmental candidate for type 2 diabetes mellitus pathogenesis and development. (5). Studies have documented that prolonged treatment of osteomalacia with vitamin D increases insulin secretion and improves glucose tolerance (35, 36).Chiu et al found that healthy normoglycaemic subjects with hypovitaminosis D had a greater prevalence of developing metabolic syndrome later compared to subjects without hypovitaminosis D (6). They also found a positive correlation between Vit Dconcentration and insulin sensitivity and an alteration in β-cell function associated with hypovitaminosis D, suggesting that hypovitaminosis D might be an independent risk factor for insulin resistance, type 2diabetes and the metabolic syndrome.

CONCLUSION

Vitamin D deficiency is a common problem and the clinical consequences are protean. In addition to its essential role in bone health, vitamin D has multiple extra skeletal beneficial effects. Experimental studies and clinical observations suggest an association between vitamin D deficiencies, abnormal glucose metabolism, type 2 diabetes mellitus and metabolic syndrome.

In this study we observed an inverse association between glycosylated hemoglobin level and serum vitamin D concentration in type 2 diabetes patients. Though it was observed that fasting plasma glucose was higher in patients with lower vitamin D levels, it was not statistically significant. Glycosylated hemoglobin, a better long term marker of glycemic status showed a significant negative correlation with serum Vitamin D concentration in the study population.

Many studies have suggested that supplementation with vitamin D could reduce incidence of diabetes in individuals at risk and also improve glycemic status in diabetics by increasing insulin secretion from beta cells of pancreas and decreasing insulin resistance. Thus, further studies with larger cohorts are essential to establish an association between Vitamin D status and Diabetes mellitus and vitamin D supplementation could serve as an element in the prevention and complex treatment of type 2 diabetes mellitus.

TABLE – 1

BIOCHEMICAL PARAMETERS IN THE STUDY GROUPS

Sl No.	Parameter	Controls (n = 64)	Cases (n = 58)	
			Uncomplicated cases	Complicated Cases
		Mean ± SD	Mean ± SD	Mean ± SD
1.	FPG (mg/dl)	84.09±8.67	154±16.35*	207.3±27.96*
2.	PPPG (mg/dl)	103.71±11.17	192.5±19.35*	263.5±38.53*
3.	Serum Urea (mg/dl)	28.0±3.88	32.05±2.86	56.3±12.01*
4.	Serum Creatinine (mg/dl)	0.88±0.10	1.01±0.16	1.94±0.45*
5.	Total Cholesterol	161.5±20.57	186.3±21	222.4±37.33*
6	Triglycerides	126.5±23.95	138.9±20.33	174.1±33.13*
7.	HDLc	43.92±2.42	40.35±4.58	37.61±2.70
8.	LDLc	93.08±17.8	119.8±19.33	150.3±33.75*
9.	VLDLc	25.31±4.79	27.91±3.98	34.49±6.64*

*Statistically significant, p<0.001, as compared to control group.

TABLE 2

SERUM VITAMIN D (ng/ml) CONCENTRATIONS IN THE STUDY GROUPS

CATEGORY		MEAN±SD	CI (MEAN±2SE)
CONTROLS (n=64)		33±3.33	32.41-33.59
CASES (n=68)	UNCOMPLICATED CASES (n=37)	19.94±2.41*	19.38-20.51
	COMPLICATED CASES (n=31)	13.46±1.45*	12.98-13.73

*Statistically significant (p<0.001) as compared to control group.

TABLE – 3

HbA1c (%) LEVELS IN THE STUDY GROUPS

CATEGORY		MEAN±SD	CI (MEAN±2SE)
CONTROLS (n=64)		4.67±0.39	4.09-5.26
CASES (n=68)	UNCOMPLICATED CASES (n=37)	7.36±0.42	6.79-7.93
	COMPLICATED CASES (n=31)	8.66±0.33	8.28-9.04

TABLE-4

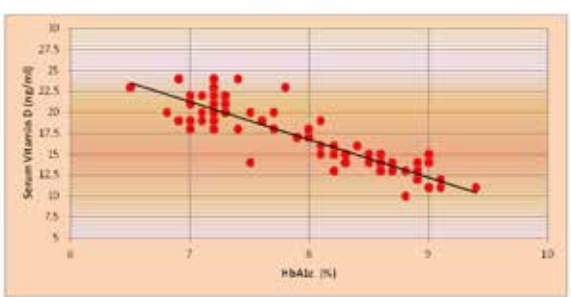
SERUM IONISED Ca^{+2} (mmol/L) CONCENTRATIONS IN THE STUDY GROUPS

CATEGORY		MEAN±SD	CI (MEAN±2SE)
CONTROLS (n=64)		1.21±0.09	1.185-1.230
CASES (n=68)	UNCOMPLICATED CASES (n=37)	0.93±0.1	0.897-0.963
	COMPLICATED CASES (n=31)	0.81±0.06*	0.79-0.832

* Statistically significant (p<0.001) as compared to control group.

GRAPH 1

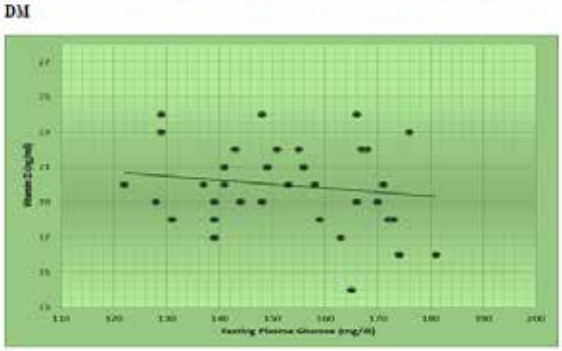
CORRELATION OF SERUM VITAMIN D CONCENTRATION WITH HbA1c (%) LEVEL IN TYPE 2 DM PATIENTS



r Value = -0.883; p Value = <0.001

GRAPH 2

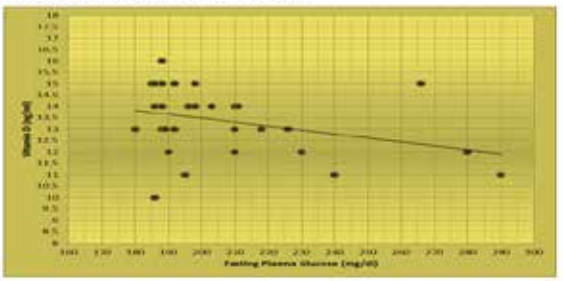
CORRELATION BETWEEN SERUM VITAMIN D CONCENTRATION ANDFASTING PLASMA GLUCOSE IN UNCOMPLICATED CASES OF TYPE 2 DM



r Value = - 0.156;p Value = 0.356

GRAPH 3

CORRELATION BETWEEN SERUM VITAMIN D CONCENTRATION AND FASTING PLASMA GLUCOSE IN COMPLICATED CASES OF TYPE 2 DM



REFERENCES

1. Powers Alvin C. Diabetes Mellitus. Harrison's Principles of Internal Medicine 18th Edition; 2968-2969 | 2. Scragg, R., Sowers, M.,& Bell, C.(2004) Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care. 27, 2813–2818. | 3. Boucher, B.J., Mannan, N., Noonan, K., Hales, C.N., &Evans, SJ.(1995) Glucose intolerance and impairment of insulin secretion in relation to vitamin D deficiency in east London Asians. Diabetologia.38, 1239–1245. | 4. Baynes, K.C., Boucher, B.J., Feskens, E.J.,&Kromhout, D.(1997) Vitamin D, glucose tolerance and insulinaemia in elderly men. Diabetologia. 40.344–347. | 5. Ortlepp, J.R., Metrikat, J., Albrecht, M., von Korff, A., Hanrath, P.,& Hoffmann, R.(2003) The vitamin D receptor gene variant and physical activity predicts fasting glucose levels in healthy young men. Diabet Med. 20.451–454. | 6. Chiu, K.C., Chu, A., Go, V.L., &Saad, M.F.(2004)Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. (2004) Am J ClinNutr. 79.820–825. | 7. Holick, M.F. Diabetes and Vitamin D connection. (2008)CurrDiab Rep.8.393-398. | 8. Norman, A.W., Frankel, B.J., Heldt, A.M., et al. (1980) Vitamin D deficiency inhibits pancreatic secretions of insulin. Science. 209. 823–825. | 9. Tanaka, Y., Seino, Y., &Ishida, M., et al. (1984) Effect of vitamin D3 on the pancreatic secretion of insulin and somatostatin. ActaEndocrinol. 105.528–533. | 10. Kumar, S., Davies, M., &Zakaria, Y., et al. (1994)Improvement in glucose tolerance and beta cell function in a patient with vitamin D deficiency during treatment with vitamin D. Postgrad Med J. 70. 440–443. | 11. Iyengar, S., Hamman, R.F., &Marshall, J.A., et al.(1989) On the role of vitamin D binding globulin in glucose homeostasis: results from the San Luis Valley Diabetes Study. Genet Epidemiol. 6. 691–698. | 12. Gordon, L., Dalip, R., Morrison, Y.A.,&Martorell, E.(2010) Lipid profile of Type 2 Diabetic and Hypertensive patients in a Jamaican population. J Lab Phys. 2(1).25-3. | 13. Mahato, R.V., Gyawali, P., &Raut, P.P., et al. (2011) Association between glycemic control and serum lipid profile in type 2 diabetes patients: Glycated hemoglobin as a dual marker. Biomed Res. 22(3).375-380. | 14. DCCT. (1993)The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin dependent diabetes mellitus.NEJM. 329.977-86 | 15. Goldstein, D.E., Little, R.R., Lorenz, R.A., Malone, J.I., Nathan, D., &Peterson C.M., et al. (2004) Tests of Glycaemia in diabetes. Diabetes care. 27.1761-1773 | 16. Sacks, D.B., Bruns, D.E., Goldstein, D.E., Maclaren, N.K., McDonald, J.M., &Parrott, M. (1992) Guidelines and recommendations for laboratory analysis in diagnosis and management of diabetes mellitus. Clin Chem. 48.436-72 | 17. Pittas, A.G., Jason, N., Mitri, J., Garganta, C., &Hillman, W., et al.(2012) Plasma 25-hydroxyvitamin D and progression to diabetes in patients at risk for diabetes. Diabetes Care. 35.565-573. | 18. Need, A.G., O'Loughlin, P.D., Horowitz, M., &Nordin, B.E.(2005) Relationship between fasting serum glucose, age, body mass index and serum 25 hydroxy vitamin D in postmenopausal women. ClinEndocrinol. 62.738 -741. | 19. Kositsawat, J., Gerber, B.S., Freemen, V.L.,&Geraci, S.(2010) Association of HbA1c levels with vitamin D status in US adults. Data from the National Health and Nutrition Examination Survey. Diabetes Care. 33. 6:1236-1238. | 20. King, H., Aubert, R.E.,& Herman, W.H. (1998) Global burden of diabetes 1995-2025; Prevalence, numerical estimates and projection. Diabetes Care. 21.1414-1431. | 21. Ramchandran, A., Snehalatha, C., Latha, E., Vijay, V., &Viswanathan, M. (1997) Rising prevalence of NIDDM in urban population in India. Diabetologia. 40. 232-237, | 22. Ramaiya, K.L., Kodali, V.R.R.,&Alberti, K.G.M.M.(1990) Epidemiology of diabetes in Asians of the Indian Sub-continent. Diabetes Metab Rev. 6. 125-146 | 23. Luong, K., Nguyen, L.T.H., &Nguyen, D.N.P.(2005) The role of vitamin D in protecting type 1 diabetes mellitus. Diabetes Metab Res Rev. 21. 338–346 | 24. Mathieu, C., Gysemans, C., &Guilietti, A., et al.(2005) Vitamin D and diabetes. Diabetologia. 48. 1247–1257. | 25. Stene, L.C., Ulriksen, J.,& Magnus, P., et al. (2000) Use of cod liver oil during pregnancy is associated with lower risk of type 1 diabetes in the offspring. Diabetologia. 43. 1093–1098. | 26. Mathieu, C., Waer, M., &Laureys, J., et al.(1994) Prevention of autoimmune diabetes in NOD mice by 1, 25-Dihydroxyvitamin D3. Diabetologia. 37.552–558. | 27. Gregori, S., Giarratanam N., &Smiroldo, S., et al.(2002) A 1alpha, 25-dihydroxyvitamin D 3 analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. Diabetes. 51. 1367–1374 | 28. Pratley, R.E., Thompson, D.B., &Prochazka, M., et al. (1998)An autosomal genomic scan for loci linked to prediabetic phenotypes in Pima Indians. J Clin Invest. 101. 1757–1764. | 29. Ishida, H.,&Norman, A.W.(1988) Demonstration of a high affinity receptor for 1, 25-dihydroxyvitamin D3 in rat pancreas. Mol Cell Endocrinal. 60.109–117. | 30. Morrissey, R.L., Bucci, T.J.,&Empson, R.N. et al.(1988) Calcium binding protein: its cellular localization in jejunum, kidney and pancreas. ProcSocExpBiol Med. 149.56–60 | 31. Johnson, J.A., Grande, J.P., &Roche, P.C., et al. (1994)Immunohistochemical localization of the 1, 25(OH) 2 D3 receptor and calbindin D28K in human and rat pancreas. Am J Physiol. 267. E356–E360 | 32. Bhattacharyya, M.H.,& DeLuca, H.F. (1974)The regulation of calciferol-25-hydroxylase in the chick, BiochemBiophys Res Commun. 59.734-741. | 33. Lips, P. (2007) Vitamin D status and nutrition in Europe and Asia. J Steroid BiochemMol Biol. 103.620-625. | 34. Cheng, J.B., Levine, M.A.,& Bell, N.H., et al. (2004) Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase, ProcNatlAcad Sci. 101.7711-7715. | 35. Zittermann, A. (2003) Vitamin D in preventive medicine: are we ignoring the evidence? Br J Nutr. 89. 552–572. | 36. Boucher, B.J. (1998) Inadequate vitamin D status: does it contribute to the disorders comprising syndrome "X". Br J Nutr. 79. 315–327. |