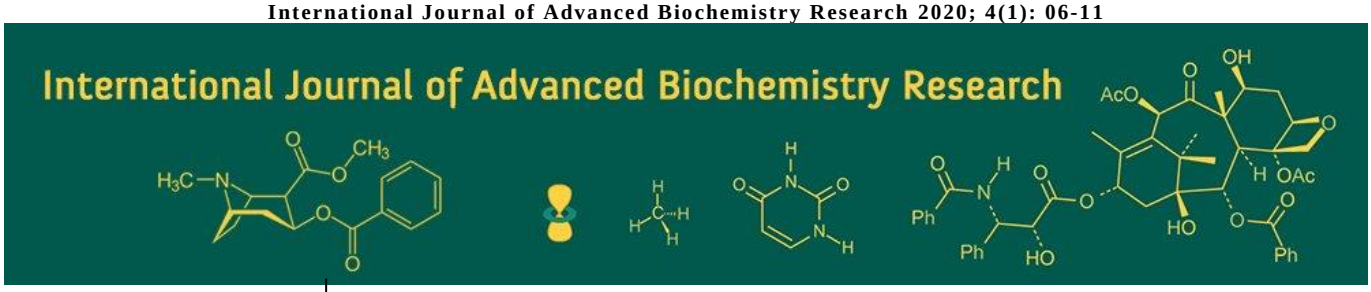

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2020; 4(1): 06-11
www.biochemjournal.com
Received: 05-11-2019
Accepted: 07-12-2019

Dr. Arshiya Masood Osmani
Assistant Professor,
Department of Biochemistry,
Dr. VRK Womens Medical College, Hospital and Research Center, Hyderabad, Telangana, India

Mrs. Shaikh. Haseena
Assistant Professor,
Department of Biochemistry,
Dr. VRK Women Medical College, Hospital and Research Center, Hyderabad, Telangana, India

Corresponding Author:
Mrs. Shaikh Haseena
Assistant Professor,
Department of Biochemistry,
Dr. VRK Womens Medical College, Hospital and Research Center, Hyderabad, Telangana, India

A possible correlation between low serum vitamin-D levels and type 2 diabetes mellitus

Dr. Arshiya Masood Osmani and Shaikh Haseena

DOI: https://doi.org/10.33545/26174693.2020.v4.i1a.40

Abstract
Background: Diabetes Mellitus is a metabolic disease which continues to be a major public health concern. Vitamin D deficiency has been found to be associated with Diabetes Mellitus, decreased Insulin secretion and increased insulin resistance in animals and humans. Recent Research is now beginning to uncover the role of vitamin D in pancreatic beta cell function, Insulin action and inflammation. The beta cells of pancreas possess Vitamin D receptors and the enzyme 1 alpha Hydroxylase is expressed in pancreatic tissue which coincides with the expression of Insulin. Insulin secretion is calcium dependent and it is reported that vitamin D deficiency impairs glucose mediated insulin release.
Aim & Objective: The main of the current study to evaluate serum Vitamin D Levels in newly diagnosed Type 2 Diabetes Mellitus patients.
Methodology: The study was conducted on 50 newly diagnosed Type 2 Diabetes Mellitus patients attending the outpatient department of Dr. VRK Women Medical College, Hospital and Research center, Hyderabad. Glycemic status was estimated by measuring FBS, PLBS and HbA1c levels. 25(OH) Vitamin D estimation, by HPLC method, was done to estimate vitamin D levels.
Results: The mean 25(OH) Vitamin D levels in Diabetic patients were 8.05 ±1.5 whereas the mean serum 25(OH) Vitamin D concentration in the control group was 34.28 ±13.42.Our study showed significantly lower levels of Vitamin D in Type 2 Diabetes Mellitus patients than in the control group. Also there was an inverse relation between HbA1c and Vitamin D levels which also emphasizes that those diabetics with Vitamin D deficiency had higher HbA1c levels.
Conclusion: Vitamin D appears to play a role in blood glucose regulation and low levels of Vitamin D may be a risk factor in development of Type 2 Diabetes Mellitus. There might be a beneficial effect in supplementation of Vitamin D to improve glycemic control in patients with Type 2 Diabetes Mellitus.

Keywords: Vitamin D, diabetes mellitus, vitamin D receptor (VDR), insulin sensitivity

Introduction
Diabetes Mellitus is a syndrome of chronic hyperglycemia due to relative Insulin deficiency, Insulin resistance or both. Diabetes Mellitus and its late complications result in reduced life expectancy and major health costs. It is estimated that 109 million individuals will be affected with diabetes mellitus in India by the year 2035 [1, 2]. The prevalence of Diabetes is increasing worldwide because of increasing obesity, decreased level of physical activity, altered environmental factors and increase in ageing population [3]. In India, the number of Diabetics is increasing from 51 million people in 2010 t0 109 million in by 2035(2). Globally, an estimated 422 million adults are living with diabetes, according to the latest 2016 data from the World Health Organization (WHO).The prevalence of Diabetes in India has been 11.8% in the last 4 years, according to the National Diabetes survey report released by the Health and Family Welfare Ministry. There are estimated 72.96 Million cases of Diabetes in adult population of India. According to Indian Heart Association, India is projected to be home to 109 Million individuals with Diabetes by 2035(2).In previous research works, India now has more than 50 million diabetic patients than any other nation therefore calling it as the diabetic capital of the world [4].
Recently Vitamin D has evoked widespread interest in the pathogenesis of Diabetes Mellitus. Vitamin D deficiency is being studied extensively as a predisposing factor contributing to the development of Diabetes Mellitus. Approximately 1 billion people suffer from vitamin D deficiency [5], which may be due to reduced sunlight exposure, long term wearing of covered clothes, use of sunscreens excessively and malabsorption syndrome [6]. Vitamin D is a fat soluble hormone. The active form of Vitamin D is 1,25

Dihydroxycholecalciferol. It is a major steroid hormone involved in mineral ion homeostasis and regulation. Vitamin D and its metabolites are hormone and hormone precursors rather than Vitamins. They can be synthesized endogenously. In response to UV radiation from sunlight, a photochemical cleavage results in formation of Vitamin D from 7 dehydrocholesterol. Vitamin D enters circulation and is absorbed from the intestine or synthesized cutaneously bound to Vitamin D binding protein, and alpha globulin which is synthesized in the liver. Vitamin D is subsequently 25 hydroxylated by the liver by cytochrome P 450like enzyme in the mitochondria and microsomes.25 dihydroxy Vitamin D is the major circulating form of Vitamin D. The final hydroxylation takes place in the kidneys which leads to formation of 1,25 dihydroxyvitamin D.

The biosynthesis of calcitriol (The Active Form of Vitamin D)

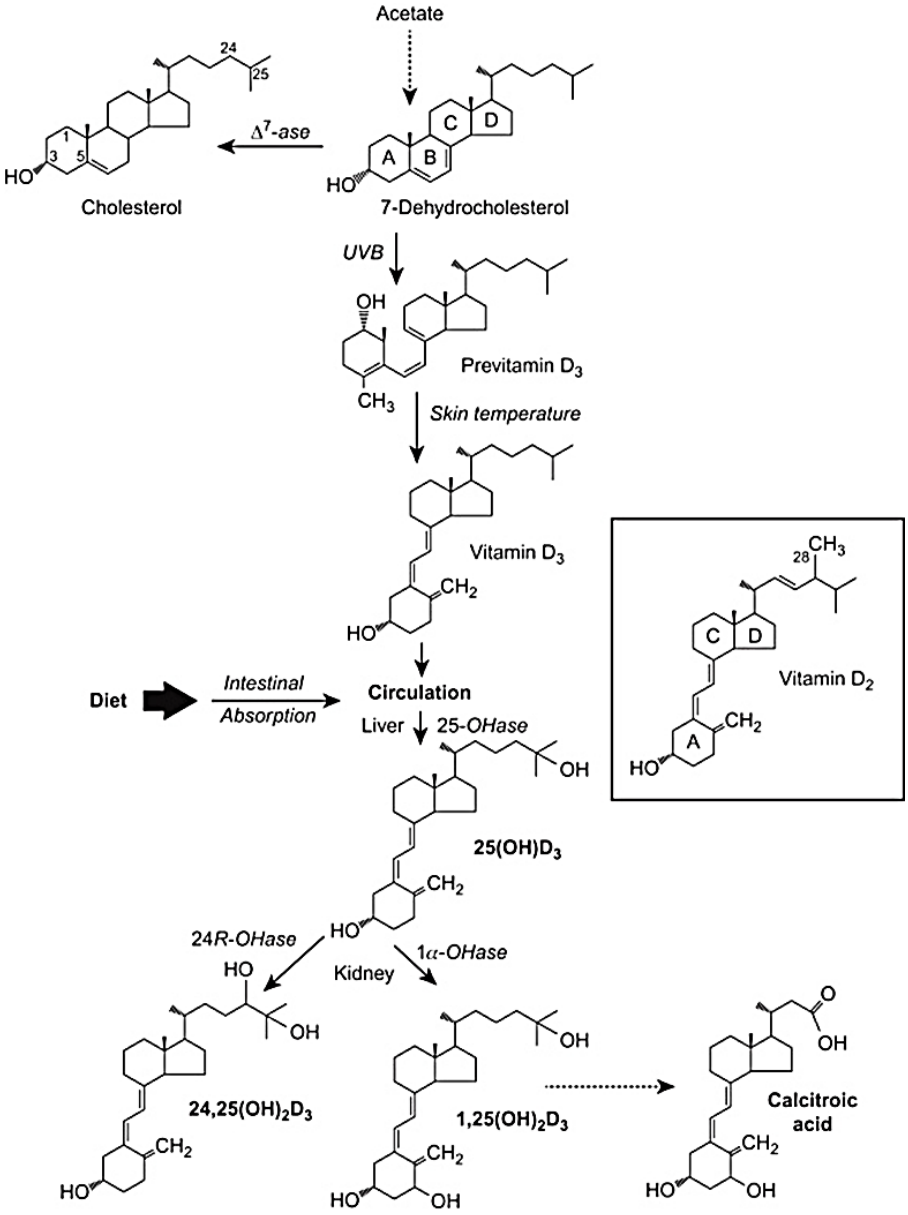

Fig 1: Biosynthesis of the active form of vitamin D in the body. The precursor in the skin is activated by UVB rays in sunlight. The active form that is the ligand for the vitamin D receptor is calcitroic acid (or 1,25-dihydroxycholecalciferol or calcitriol). Δ^7-ase, 7-dehydrocholesterol reductase; *25-OHase*, vitamin D 25-hydroxylase; *1α-OHase*, 25-hydroxyl-D-1α-hydroxylase; *24R-OHase*, 25-hydroxyl D-24R-hydroxylase. Inset, the structure of vitamin D2. Taken from M.F. Holick, 1996.

1,25 Dihydroxy vitamin D mediates the biological effects by binding to a number of nuclear receptors, super family, the vitamin D receptor(VDR).This receptor belongs to the sub family which includes the thyroid hormone receptor, the retinoid receptor and the peroxisome proliferator activated receptor. The tissues with highest number of VDR content are intestine, kidney, parathyroid gland and bone all of which are associated with maintenance of calcium homeostasis. VDR plays a central role in the biological actions of vitamin D [7]. The VDR binds to target DNA sequences as a hetero dimer with the retinoid x receptor recruiting a series of co activators that result in the induction of target gene expression. When the VDR causes repression of target gene expression, it either interferes with the action of activating transcription factor or recruits normal protein to the VDR complex that causes transcriptional regression[7] Vitamin D reduces insulin resistance probably through its effects on calcium and phosphorus metabolism through the up regulation of insulin receptor gene [8].

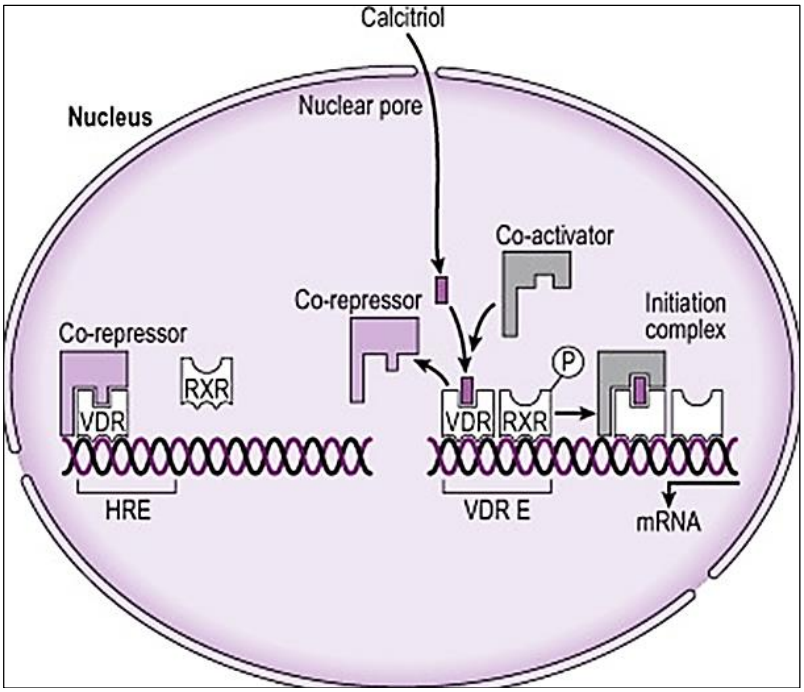

Fig 2: Molecular action of calcitriol: Calcitriol binds to vitamin D receptors (VDR) which are located in the nucleus of target cells. Binding of the calcitriol causes the VDR to become phosphorylated which allows it to recruit the retinoic acid receptor (RXR) to form a dimer which binds to the vitamin D response element (VDRE) in a gene promoter. The dimer attracts co-activators to form an initiation complex and permit gene transcription to proceed.

Deficiency of vitamin D has been associated with type 2 Diabetes Mellitus, decreased insulin secretion and increased insulin resistance in animals and humans [9].

Type 2 Diabetes and Vitamin D deficiency have risk factors in common such as African, Asian, American and Hispanic ethnicity, obesity, ageing and low physical activity [10].

Vitamin D receptors are present in both pancreatic beta cells and immune cells. Vitamin D receptors and the enzyme 1 alpha hydroxylase which catalyst the conversion of 25 (OH) Vitamin D to 1, 25 OH Vitamin D were found in more than 40 types of human cells [11].Therefore Vitamin D levels can effect tissues that are not only involved in bone metabolism and calcium homoeostasis. It has been suggested that altered Vitamin D levels play a role in the development of Diabetes Mellitus [6, 12, 13], metabolic syndrome [13] and cardiovascular diseases.

Research has also suggested that Vitamin D receptors are present on pancreatic beta cells and sufficient levels of Vitamin D helps in improving insulin sensitivity and increase insulin secretion, many studies have reported a link between low Vitamin D levels and decreased insulin sensitivity [14, 15].

Further Vitamin D plays an important part in the regulation of calcium. Calcium helps to control the release of Insulin so alterations in calcium levels can have a negative effect on beta cell function which hinders normal Insulin release. Therefore it is believed that Vitamin D helps in proper Insulin function and its effects maybe because of calcium.

Materials and Methods

The present study was done to evaluate serum 25(OH) Vitamin D levels in newly detected in Type 2 Diabetes Mellitus.

A group of 50 newly detected Type 2 Diabetic patients in the age group of 35 to 50 years with no known history of Osteoporosis, no complications of Diabetes, no history of any renal disease or renal failure, and no history of cancer were selected for this study. Post-Menopausal women and patients taking Vitamin D supplements were excluded from this study. Also patients who take medications which affect Vitamin D metabolism like Isoniazid, Rifampicin, Phenytoin, and Ketoconazole were not included in this study.

A control group of 50 people of the same age group with no history of Diabetes Mellitus or any other disease were included in this study as controls.

The investigations done in this study were FBS,PPBS,HBA1cto evaluate the glycemic status and Blood Urea, Serum Creatinine, fundoscopy and ECG were done to rule out complications of Diabetes Mellitus.

FBS and PPBS were done by GOD-POD Method on an Automated Analyzer.HbA1c levels were measured by HPLC method.

The reference range for HbA1c is:

4% to 5.6%-Normal range.
5.7% to 6.4%-pre diabetes
>6.5%-diabetes

Blood Urea was done by Urease method and Serum Creatinine by Enzymatic method was done in an Automated Clinical Analyzer using commercially available kits.

25 (OH) Vitamin D3 is the major circulating form of Vitamin D, thus the total serum 25(OH) Vitamin D3 level is considered the best indicator Vitamin level in the body.

25 (OH) Vitamin D 3 was measured by High Performance Liquid Chromatography (HPLC) Method.

The reference range total 25 (OH) Vitamin D level is 25-80 nano grams per ml.

Statistical Analysis

Statistical evaluations of the results were performed using the Statistical application SPSS.

Student's 't' test was used to compare the patient group with the control group.

Results

Table 1: Biochemical parameters in both diabetic & non-diabetic patients

Parameters	Non diabetics	Diabetics
Fasting Blood Sugar	86 ± 6.07	137 ±11.25
Post prandial blood sugar	105 ±15.13	198 ±16.54
Blood Urea	24 ±4.6	32 ±3.2
Serum Creatinine	0.7 +/-0.2	1.02 ±0.16
HbA1 c	4.52 ±0.17	7.52 ±0.52

Table 2: 25(Oh) Vitamin D 3 Levels

Group Statistics					
	Group	N	Mean	Std. deviation	Std. error mean
25(OH) Vitamin D3	Diabetic group	50	8.054000	1.5798747	.2234280
	Non diabetic control group	50	34.288000	13.4232001	1.8983272

The above table describes mean and std. deviation of each group along with standard error.

The Mean serum concentration of Vitamin D3 in Diabetics was 8.05± 1.57 and the Mean serum Vitamin D3 concentration in the control group was found to be 34.21± 13.42 with a 95% confidence interval of-30.02 to-22.44.

The serum Vitamin D3 was significantly lower in Diabetics when compared to normal control group.

Table 3: Comparison of Vitamin D3 Levels in Both Diabetic and Non diabetic (t-Test)

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
25(OH)vit D3	Equal variances assumed	70.563	.000	-13.725	98	.000 <0.05	-26.234	1.9114304	-30.0271714	-22.4408286
	Equal variances not assumed			-13.725	50.357	.000 <0.05	-26.234	1.9114304	-30.0725461	-22.3954539

From the above table p value is significant (sig. value is 0.000 < 0.05)therefore reject null hypothesis. It means that we reject the hypothesis that there is no significant mean difference between diabetic and non-diabetic groups with respect to Vitamin D3.

Table 4: Significance of Vitamin D values in both diabetic and non diabetic

Null Hypothesis	Sig. value	Result
H0: There is no significant mean difference between diabetic and non-diabetic groups with respect to Vitamin D.	0.000 < 0.05	Rejected

Therefore there is a significant mean difference between Vitamin D levels of Diabetics and Control group.

Discussion

The aim of this study was to evaluate the levels of Vitamin D in diabetic and non-diabetic patients and after conducting this study we found that the Diabetic group was having significantly lower levels of Vitamin D when compared to the controls. It can be concluded that there is a role of Vitamin D in regulating the blood sugar levels and improving Insulin sensitivity thereby reducing Insulin resistance which is often the precursor to Type 2 Diabetes Mellitus.

This study correlates well with the study conducted by Chiu *et al.* on 126 healthy, glucose tolerant subjects. 47 subjects were detected to have Vitamin D levels less than 20ngm/ml [15].

In another study done by Kalyan Miyil *et al* performed the linear regression analysis of 712 subjects after evaluating serum 25 (OH) 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 Beta cell function in their samples. They concluded that low Vitamin D levels may play s significant role in pathology of type 2 Diabetes Mellitus.

A study conducted by Balasubramian S and *et al* on 50 cases of type 2 Diabetes Mellitus, the mean Vitamin D was 18.492 with mean FBS value being 146.26mg/dl.

Vitamin D is most known for its role in Bone health but research is beginning to uncover its role in many other areas of health. There is growing evidence that Vitamin D deficiency could be a contributing factor in developing Type 2 Diabetes Mellitus. The Beta cell in pancreas that secrete Insulin has been shown to contain Vitamin D receptor as well as the Alpha 1 Hydroxylase enzyme[16].

Evidence also indicates that Vitamin D supplementation also improves glucose tolerance and Insulin resistance [17, 18]

Supplementation with Vitamin D has been shown to restore Insulin secretion in animals [19]

Research has found an indirect effect on Insulin secretion potentially by a calcium effect on Insulin secretion. Vitamin D contributes to normalization of extracellular Calcium which ensures normal calcium flux through the cell membranes. Low Vitamin D levels may diminish calcium's ability to effect Insulin secretion.

The other possible mechanism associated with Vitamin D and Diabetes Mellitus include improving the action of

Insulin by upregulation of insulin receptor, increasing the insulin response for glucose transport, an indirect effect on insulin action via a calcium effect on insulin secretion and improving systemic inflammation by a direct effect on cytokines [12] Vitamin D is found to have a protective effect on Beta cell mass. Beta cell apoptosis maybe due to an increase in cytokines (TNF Alpha,IL-6), Production of reactive oxygen species which are often present in individuals with type 2 Diabetes Mellitus.

Vitamin D prevents apoptosis of Beta cells and thereby preserves Beta cell mass [19]

A study by Riachy *et al* demonstrated that 1,25 (OH) D 3 preserves the Insulin content of human islet cells, prevents MHC expression, IL-6 production and Nitric Oxide release.

Vitamin D may act in these possible ways

It may act directly to induce Beta cell Insulin secretion by increasing the intracellular calcium concentration via non selective voltage dependent calcium channels or it may mediate activation of Beta cell calcium dependent endopeptidases to produce the cleavage that facilitates the conversion of proinsulin to Insulin [1]

Therefore this study was undertaken to determine the serum Vitamin D levels in Type 2 Diabetes Mellitus.

Conclusion

Diabetes Mellitus is a metabolic disease and continues to be public health concern. Vitamin D deficiency has become a common problem having many clinical consequences.

The ubiquitous expression of VDR in all nucleated cells, the presence of functional Alpha 1 hydroxylase enzyme in several other tissues apart from the kidneys, depicts a major universal role of Vitamin D than just the regulation of calcium, phosphate and bone metabolism.

Experimental studies and clinical observation suggest an association between Vitamin D deficiency and Type 2 Diabetes Mellitus.

In this study we observed an inverse relation between Glycosylated Hemoglobin levels and serum Vitamin D concentration in newly detected Type 2 Diabetes Mellitus. Many studies have suggested that supplementation with Vitamin D could reduce the incidence of Diabetes Mellitus in individuals who are at high risk of developing Diabetes Mellitus and improve glycemic status in Diabetics by increasing Insulin secretion from the Beta cells of pancreas and decrease Insulin resistance.

Our study can be further taken forward and more research can be done on Vitamin D and Vitamin D receptors using higher technologies, for the better understanding of a link between Vitamin D deficiency and development of Type 2 Diabetes Mellitus. Detailed Studies are also needed on Vitamin D supplementation and long term observation of glycemic control in Type 2 Diabetes Mellitus.

Our findings suggest that Vitamin D supplementation could facilitate glycemic control and optimal levels of Vitamin D may retard the clinical development of Type 2 Diabetes Mellitus in people with a tendency to develop type 2 Diabetes Mellitus. Therefore low levels of Vitamin D is a risk factor for development of Type 2 Diabetes Mellitus and Vitamin D supplementation in a judicious manner could help in prevention and also in achieving good glycemic control in Type 2 Diabetes Mellitus.

Acknowledgements

We are thankful to Dr Sarib Rasool Khan-Managing Director of Dr VRK Women's Medical College, Teaching Hospital and research center for providing all the facilities for the research work.

References

1. World Health Organization, Global Report on Diabetes. Geneva, Published on, 2016.
2. "Overview" Indian Heart Association, retrieved, 2020, 14-01.
3. Powers Alvin C. Diabetes Mellitus. Harrison's Principles of Internal Medicine 8th edition, 2968-2969.
4. Kumar KPS, Bhownik D, Srivastava S, Paswan S, Dutta AS. Diabetes Epidemic in India-A Comprehensive review of clinical features, management and remedies. The pharma Innovation, 2012, 17-33.
5. Adams JS, Hewison M. Update in vitamin D.J Clin Endocrinol, & Metab. 2010; 95:471-478.
6. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The role of Vitamin D and Calcium in Type 2 Diabetes. A systematic Review and Meta-Analysis. J Clin Endocrinol & Metabolism. 2007; 92:2017-2029.
7. Yongji Wang, Jinge Zhu, DeLuca HF. Where is the Vitamin D receptor? Archives of Biochemistry and Biophysics. Xxx, 2012, 1-12.
8. Maestro B, Molero S, Bajo S. Transcriptional activation of the human insulin receptor gene by 1,25-dihydroxyvitamin d 3.Cell bio-Chemfunct. 2002; 20:227-232.
9. Afzal S, Bojesen SE, Nordestgaard BG. Low 25-Hydroxyvitamin D and risk of Type 2 Diabetes :A prospective Cohort Study and Meta-analysis. Clinical Chemistry. 2013; 59(2):1-11.
10. Taleil A, Mohamadi M, Adgi Z. The effect of Vitamin D on Insulin resistance in patients with Type 2 Diabetes. Diabetology & Metabolic Syndrome. 2013; 5(8):1-5.
11. Grineva EN, Kaonova T, Micheeva E, Belyaeva O, Nikitina IL, Vitamin D. Deficiency is a risk factor for obesity and Diabetes type 2 in women at late reproductive age. AGING. 2013; 5(7):575-581.
12. Zippitus CS, Akobeng AK. Vitamin D. Supplementation in early childhood and risk of Type 1 Diabetes:a systematic review and Meta-analysis. Arch Dis Child. 2008; 93(6):512-517.
13. Liu S, Song Y, Ford ES, Manson JE, Buring JE, Ridker PM. Dietary Ca, Vitamin D and the prevalence of metabolic syndrome in middle aged and older US. Women. Diabetes acre. 2005; 28(12):2926-2932.
14. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, diabetes and ethnicity in the Third National Health and Nutrition Examination Survey. Diabetes Care. 2004; 27:2813-2818.
15. Chiu KC, Chuang LM, Yoon C. The Vitamin D receptor polymorphism in the translation initiation codon is a risk factor for Insulin resistance. In glucose tolerant Caucasians." BMC Medical Genetics. Article 2, 2001, 2.
16. Bland R, Markovich D, Hills C, Hughes S, Chan S, Squires P *et al*. Expression of 25-hydroxyvitamin D3-1 alpha-hydroxylase in pancreatic islets. J Steroid Biochem Mol Biol (Pub Med). 2004; 89-90:121-125.

17. Von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces Insulin resistance in South Asian women living in New Zealand who are Insulin resistant and Vitamin deficient-a randomized,placebo-controlled trial.Br J Nutr.2010.
18. Parekh D, Sarathi V, Shivane VK, Bandgar TR, Menon PS, Shah NS. Pilot study to evaluate the effect of short term improvement in Vitamin D status on glucose tolerance in patients with Type 2 diabetes. Endoer Pract.2010; 16:60-608.
19. Bourlon PM, Billaudel B, Faure-Dussert A: Influence of Vitamin D 3 deficiency and 1,25 dihydroxyvitamin D 3 on de novo Insulin biosynthesis in the islets of the rat endocrine pancreas. J Endocrinol. 1999; 160:87-95.