Study of Effect of Vitamin D Supplementation on Selected Hepatic and Renal Parameters in T2DM with Vitamin D Deficiency

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Vitamin D has been studied as modifiable risk factor in DM. Apart from its role in glucose homeostasis, the anti-inflammatory effect of vitamin D is claimed to have important effect on beta cell survival and on hepatic cells. Vitamin D is said to have anti-inflammatory, anti-proliferative and anti-fibrotic actions in liver. VDD is more prevalent in T2DM, obese and NAFLD even when these conditions occur separately. Literature states the protective effective of vitamin D on kidney. Association of VDD with albuminuria and chronic kidney disease in diabetics has also been reported. This is a type of comparative and interventional study. 63 T2DM patients aged 30 – 60 years with VDD were included. Baseline investigations determined blood levels of vitamin D, calcium, phosphate, liver enzymes (AST, ALT, ALP) and serum creatitine. Patients received vitamin D intervention orally in the dose of 2000 IU daily for 12 weeks. After 12 weeks blood levels of vitamin D, calcium, phosphate, liver enzymes (AST, ALT, ALP) and serum creatitine were determined. There was no correlation of vitamin D with urea, creatinine, calcium, phosphate, AST, ALT and ALP. There was extremely significant rise in vitamin D, significant fall in phosphate level, non-significant fall in creatinine, AST, ALT, ALP and non-significant rise in calcium, urea after 12 weeks of vitamin D supplementation. There was no correlation of vitamin D with hepatic and renal parameters. Also 12 weeks of vitamin D supplementation had no significant improvement in these parameters in T2DM.

Keywords: Endocrine; Hepatic; Kidney; Metabolism; Nutrition.

Diabetes mellitus (DM) per se is caused by decreased insulin secretion and/or increased insulin resistance. However, genetic and environmental factors play important role in its progression.¹ Nutrient deficiency, stress, physical inactivity, obesity are some modifiable risk factors associated with DM.² Though medical therapy of DM achieves glycemic control over short term to medium term, eventually it progresses to beta cell failure and loss of glycemic control.³ Also DM in the long run leads to several microvascular and macrovascular complications.¹ Type II diabetes mellitus (T2DM) prevalence in 2019 was 463 million adults worldwide and 77 million adults in
India. The prevalence in India is expected to rise to 101 million by 2030. This puts tremendous burden on the family, society and economy.

Vitamin D has been studied as a modifiable risk factor in DM. This is because several researchers have mentioned the association of vitamin D deficiency (VDD) with decreased insulin secretion, increased insulin resistance and blood glucose level. Apart from its role in glucose homeostasis, the anti-inflammatory effect of vitamin D is claimed to have important effect on beta cell survival and on hepatic cells.

The prevalence of non-alcoholic fatty liver disease (NAFLD) is reported to be 90% in obese T2DM and 20% in general population. NAFLD in T2DM is said to be associated with increased Insulin resistance (IR), deteriorated metabolism and increased micro and macroangiopathies. Like NAFLD, VDD is also more prevalent in obese T2DM. In fact, it is said that NAFLD increases the risk of VDD by 26%. Diabetes can accelerate NAFLD resulting in liver damage and its complications.

VDR are expressed in hepatic cells. VDD and vitamin D receptor (VDR) expression is associated with chronic inflammatory injury to liver. Vitamin D is said to have anti-inflammatory, anti-proliferative and anti-fibrotic actions in liver. VDD is more prevalent in T2DM, obese and NAFLD even when these conditions occur separately.

Though there are separate studies on occurrence of Vitamin D Deficiency in NAFL or VDD in T2DM or NAFLD in T2DM, the number of studies exploring the relation of vitamin D in liver damage in T2DM is quite limited. Therefore we proposed this study to determine the relation of vitamin D with parameters of liver damage in T2DM and the effects of vitamin D supplementation on these parameters.

**Aim and Objectives**

To study the effects of vitamin D supplementation on selected liver and renal parameters in type II diabetic patients with VDD

**Objectives**

1. To study the selected liver and renal parameters in T2DM patients with VDD
2. To study the selected liver and renal parameters after 12 weeks of vitamin D supplementation.
3. To find the association of vitamin D with selected liver and renal parameters.

**MATERIAL AND METHODS**

The present study was approved by the Institutional Ethics Committee. This is a type of comparative and interventional study (before and after). 63 T2DM patients aged 30 – 60 years with VDD and on oral hypoglycemic willing to participate in the study were included. Patients with T1DM, T2DM on insulin or having cardiovascular, neural, thyroid, renal or liver complications and metabolic diseases (Paget’s disease or osteomalacia), hyperparathyroidism, renal stone disease were excluded. Informed written consent was obtained after explaining the study procedure. Research fund allotment committee approved the funding for the project.

Baseline investigations were done to determine blood levels of vitamin D, Calcium and Phosphate, liver enzymes (AST, ALT, ALP) and Serum Creatitine. Patients received vitamin D intervention orally in the dose of 2000 IU daily for 12 weeks. Compliance to the supplementation was also supervised by research worker once a week by telephonic conversation with the patient. During study period, antidiabetic medication of patients was continued as usual & patients were advised to maintain their normal diet & continue their habitual physical activity. After 12 weeks blood levels of vitamin D, Calcium and Phosphate, liver enzymes (AST, ALT, ALP) and Serum Creatitine were determined.

Venous blood sample was collected after an overnight fast by using disposable needles and syringes. After two hours, the samples were
centrifuged at 3000 RPM for 5 minutes, serum from plain blood and plasma from anticoagulated blood were separated.

Estimation of vitamin D was done by Enzyme Immunoassay.\(^8\) 25- OH vitamin D is a better indicator of status of vitamin D in the body though biologically active form is 1, 25- OH vitamin D. The ST AIA- PACK 25- OH vitamin D is a one step delayed competitive enzyme immunoassay done on TOSOH autoanalyzer.

Test cups contain lyophilized twelve magnetic beads coated with anti-25-OH vitamin D sheep monoclonal antibody with sodium azide as a preservative. Vitamin D Conjugate contains vials with liquid 25- OH vitamin D conjugated to bovine alkaline phosphatase.

Before actual determination of vitamin D, first serum was pretreated with the pretreatment reagent containing sodium hydroxide which dissociates 25-OH vitamin D from its binding proteins. During first incubation, 25- OH vitamin D from the pretreated sample is bound to 25- OH vitamin D specific monoclonal antibody immobilized on magnetic beads. In this test enzyme labeled 25-OH vitamin D was added to the reaction mixture which competes with the 25- OH vitamin D for binding to antibody on magnetic beads in the reaction mixture. The magnetic beads are washed to remove unbound enzyme labeled 25- OH vitamin D during second incubation and then incubated with 4MUP which is a fluorogenic substrate.

25- OH vitamin D in the test sample is inversely related to the amount of enzyme labeled 25- OH vitamin D that binds to the beads. The machine constructs a standard curve and unknown vitamin D concentration was calculated.

The values obtained have units in ng/ml.

Estimation of Calcium and Phosphate were done by standard method on autoanalyzer.\(^9\)

**Determination of Calcium**

A colored chromophore is formed at pH 6.5 when calcium and Arsenazo II are combined. The absorbance is measured at 650 nm which is proportional to calcium concentration.

**Determination of Phosphate**

Inorganic phosphorus and ammonium molybdate combines to form phosphomolybdate in the presence of strong acids. This phosphomolybdate is directly proportional to the inorganic phosphorus when measured at 340 nm.

Estimation of liver enzymes (AST, ALT, ALP) was done by standard method.\(^10\)

**Determination of AST**

AST in the sample transfers amino group from L- aspartate to 2-oxoglutarate forming oxaloacetate and L-glutamate. Malate Dehydrogenase (MDH) reduces oxaloacetate in the presence of NADH to L- malate and NAD. Oxidation of NADH to NAD is monitored by measuring rate of decrease in absorbance at 340nm. Complete and rapid reduction of endogenous pyruvate is necessary to avoid interference in the reaction which is done by LDH (Lactate Dehydrogenase) added to the reagent.

**Determination of ALT**

The amino group from alanine is transferred enzymatically to the carbon atom of 2-oxoglutarate forming pyruvate and L- glutamate. Pyruvate is then reduced by LDH present in the reagent to lactate causing simultaneous oxidation of NADH to NAD. The rate of decrease in absorbance due to NADH oxidation is measured at 340 nm. To avoid interference during the assay endogenous sample pyruvate is rapidly and completely reduced by LDH during initial incubation.

**Determination of Alkaline Phosphatase**

4-nitrophenol is used as substrate. At alkaline pH, 4-nitrophenol has an intense yellow color. In the reagent, to maintain the optimal concentration of zinc and magnesium a metal ion buffer system is present. This buffer system can chelate other potentially inhibitor ions. ALP concentration in the serum is directly proportional to the rate of increase in the absorbance at 415 nm.

Estimation of Serum Creatitine was done by using Creatinine Reagent (Modified Jaffe’s reaction) by a standard method.\(^11\)

**Principle-** Creatinine produces red colored reaction (Jaffe reaction) when combines with alkaline picrate. Kinetic method has improved the specificity of the tests as many substances can give this non specific reaction.

Estimation of Serum Urea was done by using Urea Reagent by a standard method.

**Principle-** Urease hydrolyses urea in the presence of water and forms ammonia and carbon dioxide. Ammonia and NADH combines in the presence of GLDH (Glutamate Dehydrogenase) and á- keto glutarate to form L- glutamate. As NADH is converted to NAD, the reaction is
monitored at 340 nm by measuring rate of decrease in absorbance.

**Statistical Analysis**

Instat 3 software was used for statistical analysis. Correlation of the parameters at baseline with vitamin D intake was done by Pearson correlation. Parameters before and after intervention in were compared by paired t test.

**RESULTS**

1) Study group (n=63)
2) Age group: -50.4 ± 6.20
3) Sex: - Male: Female 34:29

Table 1 shows extremely significant rise in vitamin D, significant fall in phosphate level, non-significant fall in creatinine, AST, ALT, ALP and non-significant rise in calcium, urea after 12 weeks of vitamin D supplementation.

Table 2 shows no correlation of vitamin D with urea, creatinine, calcium, phosphate, AST, ALT and ALP.

**DISCUSSION**

We found a significant increase in the blood vitamin D level after 12 weeks of vitamin D supplement but failed to achieve normal level of 20 ng/ml. Similar findings have been reported by Ivan Al-Shahwan MA, et al,2 A Sadiya et al,5 Parini Patel, et al12 and Al-Daghri NM, et al.13

The probable causes are – inadequate dose and/or duration of vitamin D, ethnic differences and less bioavailability of vitamin D.5

**Vitamin D and Liver Enzymes**

There was no correlation of vitamin D with AST, ALT and ALP at baseline. On 12 weeks of vitamin D supplementation there was non-significant fall AST, ALT and ALP.

Ravindra Shukla et al14 reported significant rise in ALP in diabetics. Ýlker Boyraz et al15 reported normal AST and ALT levels in T2DM patients.

Amena Sadiya et al16 reported negative relationship of vitamin D with ALP. Ahern T et al17 reported no associations of vitamin D with ALP.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD Before</th>
<th>Mean ± SD After</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D</td>
<td>14.79 ± 3.64</td>
<td>17.89 ± 4.42</td>
<td>&lt; 0.0001</td>
<td>Extremely significant</td>
</tr>
<tr>
<td>Calcium</td>
<td>8.75 ± 1.17</td>
<td>9.02 ± 1.06</td>
<td>0.2049</td>
<td>Not significant</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>3.44 ± 1.05</td>
<td>3.02 ± 0.66</td>
<td>0.0103</td>
<td>Significant</td>
</tr>
<tr>
<td>Urea</td>
<td>23.53 ± 8.72</td>
<td>24.01 ± 6.59</td>
<td>0.7213</td>
<td>Not significant</td>
</tr>
<tr>
<td>Creat</td>
<td>1.13 ± 0.18</td>
<td>1.11 ± 0.19</td>
<td>0.6921</td>
<td>Not significant</td>
</tr>
<tr>
<td>SGOT</td>
<td>24.61 ± 12.07</td>
<td>24.52 ± 8.63</td>
<td>0.9614</td>
<td>Not significant</td>
</tr>
<tr>
<td>SGPT</td>
<td>24.84 ± 12.33</td>
<td>24.82 ± 10.74</td>
<td>0.9932</td>
<td>Not significant</td>
</tr>
<tr>
<td>Alk Phosphatse</td>
<td>85.39 ± 31.45</td>
<td>84.53 ± 19.75</td>
<td>0.8635</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>r</th>
<th>r squared</th>
<th>P value</th>
<th>Significance</th>
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<tr>
<td>Calcium</td>
<td>-0.09327</td>
<td>0.008699</td>
<td>0.4672</td>
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<tr>
<td>Phosphorus</td>
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<td>0.01699</td>
<td>0.3085</td>
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<tr>
<td>Urea</td>
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<td>0.05948</td>
<td>0.0541</td>
<td>Not significant</td>
</tr>
<tr>
<td>Creat</td>
<td>-0.01884</td>
<td>0.0003549</td>
<td>0.8835</td>
<td>Not significant</td>
</tr>
<tr>
<td>SGOT</td>
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<td>0.006001</td>
<td>0.5462</td>
<td>Not significant</td>
</tr>
<tr>
<td>SGPT</td>
<td>0.0801</td>
<td>0.006416</td>
<td>0.5326</td>
<td>Not significant</td>
</tr>
<tr>
<td>Alk Phosphatse</td>
<td>0.113</td>
<td>0.01277</td>
<td>0.3779</td>
<td>Not significant</td>
</tr>
</tbody>
</table>
Luo C et al\textsuperscript{18} reported no association of vitamin D with AST.

Conghua Ning et al\textsuperscript{19} reported extremely elevated AST and ALT in diabetic rats at baseline and reported significant reduction in AST and ALT following vitamin D supplementation. Luo C et al\textsuperscript{18} reported no significant change in liver enzymes after vitamin D supplementation. Nwosu BU et al\textsuperscript{20} reported a significant decrease in ALT following vitamin D supplementation. Barchetta I et al\textsuperscript{6} and Ryu OH et al\textsuperscript{21} reported no significant changes in AST, ALT after vitamin D supplementation. Leonardo M. Bella et al\textsuperscript{22} reported no significant difference in AST, ALT and ALP levels in diabetic mice even after vitamin D supplementation.

AST (SGOT) and ALT (SGPT) are commonly used and most sensitive markers of liver injury. Normal serum levels of AST and ALT are 5-35 IU/lit and 0-40 IU/lit respectively.\textsuperscript{10} One disadvantage of using aminotransferases as markers of liver injury is that their levels do not correspond with the extent of liver damage. Also they cannot be used for prognostic purpose.\textsuperscript{23} Normal serum ALP levels are 37-147 Iu/lit.\textsuperscript{10} ALP increases in liver injury. But ALP also increases with increased new bone formation.\textsuperscript{14} Thus, expertise is required to interpret alterations in ALP together with clinical correlation.

VDR are expressed on all hepatocytes. Vitamin D exerts its anti-inflammatory, antiproliferative and antifibrotic effect by direct action. Also it increases free fatty acid uptake by its insulin sensitizing effect on hepatocytes.\textsuperscript{6} Raised serum transaminases levels indicate hepatic dysfunction. Thus decreased transaminases levels towards normal signify improvement in hepatic function.\textsuperscript{6,20}

The aminotranseferse and ALP levels in our participants were fairly within normal range. It may be because of the fact that we included T2DM without any complications. Inclusion of T2DM with clinical findings of liver injury may reflect alterations in aminotransferases and ALP. Also correction of vitamin D level in such patients may favorably change liver function and hence levels of aminotransferases and ALP. Nevertheless, vitamin D is said to have anti-inflammatory, anti-proliferative and anti-fibrotic actions in liver. So a study with inclusion of DM with liver damage is warranted before arriving at particular conclusion.

### Vitamin D and Electrolytes

Vitamin D and Electrolytes

There was no correlation of vitamin D with calcium and phosphate group at baseline. On 12 weeks of vitamin D supplementation there was significant fall in phosphate level and non-significant rise in Calcium.

Ýlker Boyraz et al\textsuperscript{24} reported normal calcium and phosphate levels in T2DM patients. Alam U et al\textsuperscript{25} reported marginally high calcium in vitamin D sufficient group than VDD with T2DM. Al-Shoumer et al\textsuperscript{26} reported significantly lower phosphate in the patients. Conghua Ning et al\textsuperscript{19} reported no difference in calcium and phosphate levels in rats with VDD and without VDD.

Positive relationship of vitamin D with calcium was reported by Amena Sadiya et al\textsuperscript{16} and Lim S et al.\textsuperscript{27} While Ahern T et al\textsuperscript{17} reported no associations of vitamin D with calcium.

Al-Daghri NM et al\textsuperscript{13} reported significant increase in calcium and no significant change in phosphate with 18 months vitamin D supplementation. While no significant change in calcium after vitamin D supplementation was reported by Patel P et al,\textsuperscript{12} Ryu OH et al\textsuperscript{21} and Nazarian S et al.\textsuperscript{28}

We must remember that regulation of blood calcium and phosphate levels depend not only on vitamin D but also on parathyroid hormone (PTH) and calcitonin.\textsuperscript{29} And in uncomplicated T2DM the function of these hormones and hence calcium and phosphate levels are expected to be normal.

Both Calcium and PTH can independently influence insulin release as well as peripheral insulin sensitivity. Thus both can be potential confounding factors after vitamin D supplementation.\textsuperscript{28}

### Vitamin D and Renal Parameters

Vitamin D and Renal Parameters

There was no correlation of vitamin D with urea and creatinine at baseline. On 12 weeks of vitamin D supplementation there was non-significant rise in urea and non-significant fall in creatinine.

Cimbek A et al\textsuperscript{30} reported positive correlations of vitamin D with creatinine. Amena Sadiya et al\textsuperscript{16} reported no association of vitamin D with creatinine.

No significant change in creatinine following 24 weeks vitamin D supplementation was reported by Nwosu B et al.\textsuperscript{20}
Chronic hyperglycemia leads to increased production of advanced glycation end products (AGEs). AGEs are important in the pathogenesis of end organ damage like diabetic nephropathy, diabetic retinopathy. Vitamin D by its antioxidant and anti-inflammatory effects can decrease the accumulation of AGEs. Vitamin D exhibits protective action on kidney through inhibition of renin angiotensin system (RAS). Also it protects kidney from inflammatory damage and fibrosis. The strengths of the study include inclusion of T2DM patients with VDD, estimation of vitamin D with standard method, we ensure patient compliance and we did not change patients’ medications or lifestyle.

The limitations of the study include relatively small sample size, relatively small duration of the study, T2DM patients with complications were not included, HbA1c which is a good indicator of glycemic status was not estimated.

**Suggestions for further studies**

- Studies with high doses of Vitamin D for long intervention duration in identified high risk T2DM patients should be taken. The optimum vitamin D level at which glycemic control is maximum and the ultimate vitamin D levels that cause derangement in glucose homeostasis should be identified.
- Bioavailability of Vitamin D and concentration of free or active vitamin D should be given due consideration.

**CONCLUSION**

There was no correlation of vitamin D with hepatic and renal parameters. Also 12 weeks of vitamin D supplementation had no significant improvement in these parameters in T2DM.

**ACKNOWLEDGEMENTS**

We are grateful to KIMS, Karad for funding this project. We are thankful to the supporting staff of blood collection center and the laboratory staff for their help.

**Conflict of Interest**

No conflict of interest.

**Funding Source**

KIMS, Karad, India.

**Statement of Informed Consent**

Informed written consent was obtained after explaining the study procedure.

**REFERENCES**
