ASSOCIATION OF SERUM CONCENTRATIONS OF 25-HYDROXYVITAMIN D WITH CHRONIC PERIODONTITIS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS (T2DM) 

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ABSTRACT : Chronic periodontitis in patients with Type 2 Diabetes mellitus (T2DM) has been associated with adverse outcomes. Vitamin D deficiency/insufficiency is common in patients with T2DM and may play a role in the development of chronic periodontitis. The objective of our study was to examine the relationship between serum 25(OH) vitamin D and chronic periodontitis in patients with T2DM. T2DM patients with and without chronic periodontitis were recruited, forty in number in each groups after exclusion criteria. Baseline clinical and laboratory data were obtained and periodontal examination performed. Serum 25(OH)D levels were stratified into deficient, insufficient and sufficient levels according to Institute of Medicine criteria. T2DM patients with chronic periodontitis had a lower median level of 25(OH) D than those without chronic periodontitis suggesting a possible role of Vitamin D deficiency/insufficiency in the development of chronic periodontitis in T2DM.

KEYWORDS: Type 2 Diabetes mellitus; Vitamin D; Chronic periodontitis, Clinical attachment level.

INTRODUCTION
Type 2 Diabetes mellitus (T2DM) is associated with various systemic complications.[1] Presence of both chronic periodontitis and T2DM is associated with adverse outcomes. Chronic periodontitis is a frequent pathologic condition in a diabetic and is associated with chronic complications like nephropathy, cardiovascular disease, peripheral artery disease or death.[2-3] Chronic periodontitis is a sub gingival infection predominantly caused by gram-negative anaerobic bacteria clinically manifested with pocket formation and radiologic evidence of alveolar bone loss.[4-5] Vitamin D plays an important role in the immune response in chronic periodontitis. Also low serum levels of 25(OH)D have been linked through observational studies to the pathophysiology of diabetes mellitus.[6-7] Studies have reported an association between periodontal health and intake of vitamin D.[8] The objective of this study was to examine the association between serum vitamin D levels and chronic periodontitis in patients with T2DM.

MATERIAL AND METHODS
This was a cross sectional study of patients with T2DM treated at Department of Endocrinology, Nilratan Sircar Medical College and Department of Periodontia, R. Ahmed Dental College, Kolkata, West Bengal, India. The study was approved by the Institutional Ethical Committee and informed consent was obtained from study subjects.

Patients with T2DM of 18 to 65 years of age group having a minimum of 20 teeth were recruited. Patients having hemolytic anemia, HIV infection, pregnant and receiving medications shown to affect the periodontal tissues like anticonvulsants, calcium channel blockers and cyclosporine were excluded. Individuals with...
systemic disorders like chronic kidney disease, liver
disease, malignancy, primary hyperparathyroidism, oral
contraceptives, glucocorticoids which are likely to
interfere with vitamin-D metabolism, were excluded.
Patients receiving calcium and vitamin D supplements
within last 6 months were excluded.

All study subjects underwent meticulous clinical and
laboratory evaluation as per standard practice of T2DM.
HbA1c assay was done using Biorad laboratory analyzer
by High Performance Liquid Chromatography method.

Serum 25 (OH) D assay was done using Architect
(ABBOTT) laboratory system via chemiluminescence assay
method. Serum 25(OH)D levels were stratified into
vitamin-D sufficiency (25(OH)D ≥30 ng/ml), vitamin-D
insufficiency (25(OH)D: 20-30 ng/ml), mild vitamin-D
deficiency (25(OH)D: 10-20 ng/ml) and severe vitamin-
D deficiency (25(OH)D <10 ng/ml).

Periodontal examination was done by a single examiner
expert in Periodontia to eliminate inter-examiner
variability. Oral and dental examination was done
according to WHO Oral Health Assessment Form.
Oral hygiene of patients was assessed by Plaque
Index. The number of sites with bleeding on probing
(BOP) were quantified. Probing pocket depth (PPD) was
measured to the nearest millimeter from the gingival
margin with calibrated University Of North Carolina-15
(UNC-15) periodontal probe. Measurements of probing
pocket depth were performed at six sites per tooth:
mesiobuccal, midbuccal, midlingual, mesiolingual,
distobuccal and distolingual. Clinical attachment level
(CAL) to the nearest millimeter was measured with
calibrated probes as the distance between the
cementoenamel junction (CEJ) and the base of the
pocket Clinical attachment levels were measured at four
sites: midbuccal, midlingual, and the deepest mesial and
distal interproximal points. Severity of periodontitis
categorized on the basis of amount of clinical attachment
loss as follows: normal < 1 mm, slight= 1-2 mm, moderate= 3-4 mm and severe ≥ 5 mm.

Statistics
Data were collected and processed using SAS (Statistical
Analysis System) version 9.1 for windows, SAS Institute
Inc. Cary, NC, USA. The results were presented as mean ± SD for normally distributed data and median (min-
max) for non-parametric data. Normality of data was
determined using Anderson Darling test, Shapiro-Wilk
test and QQ plot. For significant difference of continuous
variables between two groups, P-values were calculated
using unpaired t-test with equality of variance tested
using levene’s test and for non-parametric data, p-values
were calculated using Wilcoxon Mann-Whitney test. To
test significant difference of continuous variables
between more than two groups, ANOVA with post hoc
Bonferroni tests was used for normally distributed data
and Kruskal-wallis test with multiple comparisons
Bonferroni method was used for non-normally
distributed data. Correlation coefficient was determined
using Pearson or Spearman partial correlation test
depending on the distribution of data, while adjusting for
the variables body mass index (BMI), HbA1c. P<0.05
was considered to be statistically significant.

RESULTS
In the present study, 80 patients were recruited after
periodontal examination, 40 in the case group (T2DM
and chronic periodontitis) and 40 in the control group
(T2DM without chronic periodontitis). (Figure 1).
The T2DM subjects with chronic periodontitis and without chronic periodontitis were matched for age and sex. T2DM patients with chronic periodontitis had poorer glycemic indices than those without chronic periodontitis. Patients with T2DM with chronic periodontitis had higher local inflammation in term having more severe clinical attachment loss. (Table 1).

**Table 1** Relationship between anthropometric parameters, glycemic tests, vitamin D status and periodontal parameters in patients with T2DM with and without periodontitis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T2DM with CP (n=40)</th>
<th>T2DM without CP (n=40)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>47.20 ± 9.83</td>
<td>46.62 ± 7.78</td>
<td>0.77</td>
</tr>
<tr>
<td>Sex (Female) %</td>
<td>57.5</td>
<td>57.5</td>
<td>0.99</td>
</tr>
<tr>
<td>Duration of T2DM*</td>
<td>4 (1-12)</td>
<td>3.5 (1-11)</td>
<td>0.220</td>
</tr>
<tr>
<td>BMI</td>
<td>26.30 ± 2.99</td>
<td>25.95 ± 3.2</td>
<td>0.61</td>
</tr>
<tr>
<td>FPG</td>
<td>169.93 ± 21.91</td>
<td>132.08 ± 22.68</td>
<td>0.04</td>
</tr>
<tr>
<td>PPPG*</td>
<td>211 (136-402)</td>
<td>173.5 (136-266)</td>
<td>0.018</td>
</tr>
<tr>
<td>HbA1c</td>
<td>7.75 ± 0.63</td>
<td>7.13 ± 0.52</td>
<td>0.01</td>
</tr>
<tr>
<td>25(OH)D(ng/ml)*</td>
<td>16 (8.33 - 38.55)</td>
<td>24.31(8 - 44.76)</td>
<td>0.01</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>184.57 ± 34.48</td>
<td>184.67 ± 40.55</td>
<td>0.99</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>158.75 ± 66.21</td>
<td>142.17 ± 67.59</td>
<td>0.62</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>43.79 ± 8.76</td>
<td>43.71 ± 8.36</td>
<td>0.99</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>106.31 ± 27.31</td>
<td>109.15 ± 34.02</td>
<td>0.90</td>
</tr>
<tr>
<td>GFR</td>
<td>95.50± 13.21</td>
<td>92.21± 13.95</td>
<td>0.28</td>
</tr>
<tr>
<td>Numbers of Teeth*</td>
<td>26(21-29)</td>
<td>30(22-32)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Plaque index</td>
<td>2.25±0.62</td>
<td>2.02±0.72</td>
<td>0.13</td>
</tr>
<tr>
<td>PPD*</td>
<td>4(2-7)</td>
<td>1(0-1)</td>
<td>0.0001</td>
</tr>
<tr>
<td>CAL*</td>
<td>4(2-6)</td>
<td>1(0-1)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Sites with BOP*</td>
<td>36(4-84)</td>
<td>0</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Data presented as mean ± S.D. except *denoted as median (minimum - maximum) for non parametric data.

PPD- Probing Pocket Depth, CAL- Clinical Attachment Level, BOP – Bleeding on probing.

The percentage of patients with insufficiency or deficiency of 25(OH)D was significantly greater among T2DM patients with chronic periodontitis than those without chronic periodontitis (85% vs. 75%, p < 0.001). (Table 2).

**Table 2** Vitamin D status among T2DM subjects with and without chronic periodontitis

<table>
<thead>
<tr>
<th>Vitamin D status</th>
<th>Sufficiency % [n] (&gt;30 ng/ml)</th>
<th>Insufficiency % [n] (21-30 ng/ml)</th>
<th>Mild Deficiency % [n] (11-20ng/ml)</th>
<th>Severe Deficiency % [n] (&lt;10 ng/ml)</th>
<th>p</th>
</tr>
</thead>
</table>

T2DM patients with chronic periodontitis had a lower median level of 25(OH) D than those without chronic periodontitis (16 vs.24.31 ng/mL, p - 0.0001) (Figure 2).
Fig 2. Comparison of 25(OH) Vitamin D Levels between two groups

Box-plot depicting the median (horizontal line), interquartile range (upper and lower), and upper and lower limits (whiskers) of 25(OH)D levels in patients with T2DM and chronic periodontitis (CP) (case patients) compared with those with T2DM without CP (control patients). Case patients had a lower median serum level of 25(OH)D than control patients ($p = 0.0001$).

Since glycemic indices were significantly different between the two groups, requisite statistical adjustments were done before comparison. (Table 3).

**Table 3 Correlation between vitamin D status and periodontal parameters**

<table>
<thead>
<tr>
<th>Correlation variable</th>
<th>Variable(adjusted)</th>
<th>Pearson’s/Spearman correlation coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter 1</td>
<td>Parameter 2</td>
<td>BMI, HbA1c</td>
<td></td>
</tr>
<tr>
<td>25(OH)D</td>
<td>CAL</td>
<td>BMI, HbA1c</td>
<td>-0.561</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>PPD</td>
<td>BMI, HbA1c</td>
<td>-0.41</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>BOP sites</td>
<td>BMI, HbA1c</td>
<td>-0.45</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>HbA1c</td>
<td>BMI</td>
<td>-0.25</td>
</tr>
</tbody>
</table>

Correlation coefficient determined using Pearson or Spearman partial correlation test depending on the distribution of data, while adjusting for the variables BMI, HbA1c

**DISCUSSION**

In the present study, we found a significant inverse association between serum 25(OH) D concentrations and chronic periodontitis. Patients with T2DM and chronic periodontitis have lower serum levels of vitamin D and are most often insufficient and deficient in 25(OH) D in relation to T2DM patients without CP. This association was evident after exclusion of possible confounding factors.

Vitamin D deficiency is very common in India in all the age groups and both sexes across the country. Vitamin D deficiency is also not uncommon in eastern parts of India. Among the 40 apparently healthy physicians and diabetologists of a public hospital in the city of Kolkata, with a mean age of 52 years, the serum level of 25(OH)D found was 13.02 ± 4.77 ng/mL, and in 92.5% of them, vitamin D levels were < 20 ng/mL. In another study at same public hospital of Kolkata, Dutta et al reported 25(OH)D to be significantly lower (< 30 ng/mL) in 66.6 per cent (n=28) of individuals with diabetes.

Similarly insufficient levels of 25(OH) D were associated independently with CP in NHANES III (Third National Health and Nutritional Examination Survey), a nationwide survey of the adult population of the United States of America (USA). In nationally representative cohort of 35,644 male health professionals from USA, Jimenez et al. observed a dose-dependent significant inverse association across quintiles of the predicted 25(OH)D score and incidence of tooth loss. In this 20-year prospective study each 10 nmol/l increase in the predicted 25(OH)D score, decreased the risk of periodontitis decreased significantly by 9% when adjusted for potential confounders in this longitudinal observational study.

In a 3-year, randomized, placebo-controlled trial on the effect of calcium and vitamin D supplementation, significantly reduced tooth loss was seen in subjects receiving calcium and vitamin D supplementation suggesting that calcium and vitamin D supplementation aimed at osteoporosis prevention has a beneficial effect on tooth retention. However, it was difficult to explain individual effects of either calcium or vitamin D in this study.

Inverse associations have noted between periodontal disease and lower vitamin D and higher concentrations of RANKL and OPG.

Millen et al observed no association between 25(OH)D and prevalent periodontal disease defined using alveolar crestal height and tooth loss. Millen et al found a 33% lower odds of prevalent periodontal disease, among
women with adequate (≥50 nmol/L) compared to deficient/inadequate (<50 nmol/L) vitamin D status. Also associations between 25(OH)D and continuous measures of CAL and PD showed a borderline statistically significant inverse association between worst site PD, percentage of gingival sites that bled upon periodontal assessment and 25(OH)D, but not CAL.[20] In contrast Millen et al found no association between baseline 25(OH)D and the subsequent 5 year change in periodontal disease measures in in a subset (n = 442) postmenopausal women in a Women’s Health Initiative Observational Study. However this study was comprised of 3.5% (n=23) diabetic subjects.[21]

Vitamin D might affect periodontal disease both through an effect on bone mineral density (BMD) and through immune modulatory effects. Vitamin D has a well established essential role for bone growth and preservation. The active metabolite of 25-hydroxyvitamin D is 1,25- dihydroxyvitamin D and has been found to inhibit cytokine production and cell proliferation.[22] Also association of (VDR) restriction fragment length polymorphism (RFLP) with periodontal disease have been reported. Hennig et al reported that carriage of the less frequent allele of the Taq I RFLP (t) in the VDR gene significantly increases the risk of developing early onset periodontitis.[22]

The results suggest that a sufficient level of vitamin D is important in maintaining a healthy periodontium and reducing the consequences of chronic periodontitis in patients with T2DM also. However, our study had some limitations. Being cross sectional in design, we were unable to establish a causative association between vitamin D deficiency and chronic periodontitis and also failed to evaluate temporality. Lastly, there are other confounding factors that might influence our results like outdoor activity and sunlight exposure which are very difficult to take into account.

In conclusion, our finding of vitamin D deficiency occurring more often in patients with T2DM and chronic periodontitis than those without chronic periodontitis suggest that Vitamin D deficiency might increase the risk of chronic periodontitis in T2DM, possibly by affecting immune responses against bacterial invasion of the periodontium. Prospective interventional studies are needed to assess the beneficial effect of vitamin D on periodontal disease in patients with T2DM.

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