



Vitamin D in Different Stages of Type 2 Diabetic Nephropathy and its Correlation with Transforming Growth Factor Beta-1 (TGF- β 1) –A Cross Sectional Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diabetic nephropathy (DN) is a microvascular complication of Diabetes Mellitus (DM) and the prevalence of which is increasing in every year. Monitoring of Vitamin D status in diabetic nephropathy patients is important, as the deficiency of vitamin D appears as a risk factor for the development of diabetic nephropathy. Studies evaluating the role of vitamin D in DN are few. Conflicting data is available on the correlation between vitamin D and Diabetic Nephropathy. Studies revealed the sample population is Vitamin D deficient. Therefore, it is important to understand the correlation of Vitamin D with severity of Diabetic nephropathy and its role in fibrogenesis. The aim of this study is to analyse vitamin D status in different stages of type 2 diabetic nephropathy and its correlation with transforming growth factor beta-1.

Methods: A 1.5-year cross-sectional study of 120 diabetic patients, 60 with nephropathy and 60

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without nephropathy patients enrolled to MES Medical College. Patients with heart, liver, or thyroid disease, as well as those on dialysis, were excluded from the study. The VITROS 5600 integrated system were used to measure fasting blood sugar (FBS), HbA1c, creatinine and vitamin D. Transforming Growth Factor Beta-1 (TGF- β 1) is measured using ELISA technique. According to HbA1c and estimated glomerular filtration rate (eGFR) values, the study population is divided into two groups. The statistical package for the social sciences (SPSS) software was used to conduct the analysis. The level of significance was calculated at 95%.

Results: The level of vitamin D in diabetic patients with nephropathy is much lower than in diabetic patients without nephropathy. In diabetic nephropathy patients, serum creatinine, urea, HbA1c and TGF- β 1 exhibited a highly significant negative correlation with vitamin D status, but eGFR showed a highly significant positive correlation.

Conclusion: Vitamin D status has been found to be poor in all diabetic patients, with a greater drop in diabetic nephropathy patients. In diabetic nephropathy patients, serum creatinine, urea, HbA1c and TGF- β 1 exhibited a highly significant negative association with vitamin D status, but eGFR showed a highly significant positive link. Deficiency of vitamin D have role in the development and severity of DN, and showed a highly significant correlation with the regulator of fibrosis, TGF- β 1. This finding indicates that vitamin D could be an important factor for development and progression of Diabetic nephropathy. So supplementation of vitamin D may slow down progression of DN.

Keywords: *Diabetic Nephropathy; Vitamin D; transforming growth factor beta; fibrogenesis; serum creatinine; estimated glomerular filtration rate.*

1. INTRODUCTION

Diabetes mellitus (DM) is a disease having more importance as the incidence and prevalence is increasing globally. Untreated diabetes mellitus results in micro and macro vascular complications, among these Diabetic Nephropathy (DN) incidence is more. It is a very serious problem as if not treated in time, it will lead to end stage renal disease and death. Vitamin D has many functions and is related to the long-term changes in renal function [1].

Most vertebrates can synthesize sufficient levels of vitamin D if their skin is exposed to enough sunlight (UVB rays). The sufficient amount of vitamin D can be obtained either from their diet or via adequate skin exposure to sunlight [2].

Vitamin D insufficiency is caused mostly by a lack of sun exposure and a decrease in consumption. Many studies from all over the world have found decreasing levels of vitamin D [3-4]. Despite the abundance of sunlight in India, vitamin D deficiency affects 50 to 90 percent of the population, and this shortage affects people of all ages and genders [5]. The action of vitamin D is receptor mediated, which are expressed in almost all cell types in the kidney [6-7]. Vitamin D deficiency/insufficiency is associated with insulin resistance, reduced beta cell function leading to an increase in blood glucose [8].

The protective role of vitamin D in diabetic nephropathy has been suggested in recent years [7,9]. The improvement in renal function of diabetic nephropathy patients with Vitamin D administration was shown in a recent study [10]. In an experimental study, diabetic mice knocking off the vitamin D receptor caused severe albuminuria and glomerulosclerosis [11]. Earlier study which designed to compare the effectiveness of vitamin D (Paricalcitol) supplementation, reported a decreased Urine Albumin Creatinine ratio (UACR) by 16% [12]. Vitamin D has been shown to slow the progression of the disease and thereby fibrosis. The mechanism of the link between vitamin D and DN remains unknown [13]. Studies on animal models reported the interference of vitamin D with transforming growth factor beta 1 and there by fibrogenesis [14-16]. In spite of these positive findings, contrasting reports are also available on the association of vitamin D with diabetic nephropathy [17-20]. Furthermore, no evidence of the link between vitamin D deficiency and diabetes was found in a recent genetic analysis [21]. No definite conclusions can be drawn from the current literature as conflicting reports are available. Moreover, Studies revealed the sample population is Vitamin D deficient [22-23]. Hence, it is very vital to find the correlation between vitamin D status and the severity of Diabetic Nephropathy and its correlation with the regulator of fibrogenesis, TGF- β 1. Research is going on to confirm the renal protective effects of

vitamin D and to evaluate an effective therapeutic dose.

The objective of this study is to measure vitamin D level in different stages of diabetic nephropathy and its correlation with the transforming growth factor beta -1 in type 2 diabetic patients of Kerala.

2. MATERIALS AND METHODS

The cross-sectional study including 120 Type 2 Diabetic patients, out of which 60 with nephropathy and 60 without nephropathy was conducted. Patients with a history of cardiac, liver, thyroid dysfunction, those on dialysis were excluded from the study. Diabetic nephropathy patients were sub grouped according to different stages of nephropathy, determined by eGFR value [24]. eGFR is calculated using MDRD equation. Serum creatinine, serum urea, fasting blood sugar, HbA1c were measured by VITROS 5600 integrated system and vitamin D estimated using VITROS ECi/ECi Immunodiagnostic Systems using Intellicheck R Technology. The Transforming Growth Factor Beta-1 is measured using ELISA technique. The microtiter plate wells are coated with human TGF-beta-1 antibody. The sample containing TGF-Beta1 gives antigen-antibody reaction and is measured using ELISA reader at a wavelength 450 nm. Pearson correlation coefficient and unpaired t test used for statistical analysis at a level of significance of 5%. Type 2 Diabetic patients of age group 40-60 years, with and without nephropathy were enrolled in this study.

3. RESULTS AND DISCUSSION

This study was to investigate the vitamin D status in Type 2 Diabetic patients with (n=60, 48.33 % females and 51.67 % males) and without

nephropathy (n=60, 46.67 % females and 53.33 % males). Based on eGFR value Diabetic nephropathy patients are distributed in stage 3, 4 and 5. Among this stage 3 comprised 38 % (50% females & 50% males) of the patients, 22 % (40 % females and 60% males) in stage 4 and 40% in stage 5 (47.8 % females & 52.2 % males) (Fig. 1).

The parameters analysed were expressed as Mean \pm SD. We could find a highly significant difference in the vitamin D status between Diabetic patients with and without nephropathy. It has been observed that vitamin D status is low for all the patients and further decreased in diabetic nephropathy patients (Table-1). The transforming growth factor beta-1 showed a significant difference between the diabetic patients with and without nephropathy. A highly significant increase in TGF- β 1 could be observed in the diabetic nephropathy patients. Vitamin D level is significantly lowered in diabetic patients with nephropathy than in patients without nephropathy.

Recent observations have demonstrated that kidney disease seems to be associated with a high incidence of vitamin D insufficiency or deficiency [25]. Studies by Gonzalez et al. [26] demonstrated that 25-hydroxyvitamin D values are 30 ng/ml, and believed to be the lower limit of normal, in the majority of patients with chronic kidney disease (CKD). Al-Badr et al reported that vitamin D has the potential to have a favourable impact in diabetic nephropathy [27]. Banerjee D et al also suggested a link between of vitamin D deficiency with cardiovascular events in patients with CKD [28]. But in contrast Kayzer C A et al concluded Plasma 1,25(OH) $_2$ D is not associated with risk of developing increased albuminuria or reduced eGFR [29].

Table 1. Comparison of parameters among diabetic patients with and without nephropathy

| Parameters | DM | DN | t Value | p Value |
|---------------------------|-------------------|-------------------|---------|---------|
| AGE (Years) | 53.4 \pm 5.9 | 55.9 \pm 5.7 | 2.44 | 0.02* |
| SBP (mmHg) | 130.8 \pm 14.7 | 139.2 \pm 17.8 | 2.79 | 0.01* |
| DBP (mmHg) | 82.3 \pm 7.6 | 83.8 \pm 8.4 | 1.02 | 0.31 |
| FBS (mg/dl) | 170.1 \pm 58.3 | 164.7 \pm 50.1 | 0.54 | 0.58 |
| Urea (mg/dl) | 24.5 \pm 8.04 | 74 \pm 48.1 | 7.86 | <0.001* |
| Creatinine (mg/dl) | 0.75 \pm 0.13 | 4.08 \pm 3.48 | 7.39 | <0.001* |
| HbA1c (%) | 7.44 \pm 1.88 | 8.9 \pm 2.08 | 4.17 | <0.001* |
| eGFR (ml/min/1.73m 2) | 99.3 \pm 24.3 | 25.3 \pm 17.5 | 19.12 | <0.001* |
| TGF- β (pg/ml) | 349.9 \pm 119.4 | 789.1 \pm 281.2 | 11.13 | <0.001* |
| Vitamin D (ng/ml) | 30.9 \pm 4.02 | 17.1 \pm 4.4 | 17.95 | <0.001* |

*Indicates significant difference; significance is measured at the level of p<0.05

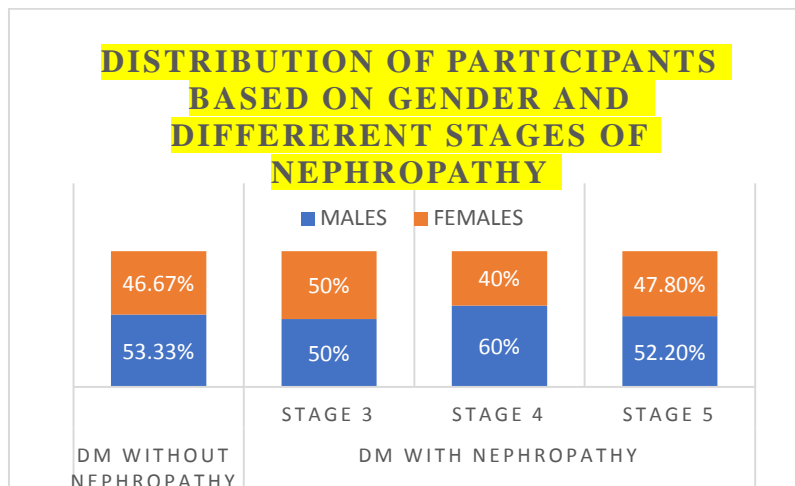


Fig. 1. Distribution of study subjects based on gender and different stages of nephropathy

Serum creatinine, urea, HbA1c, TGF- β 1 showed a highly significant negative correlation and eGFR showed a highly significant positive correlation with vitamin D status in diabetic nephropathy patients. Systolic blood pressure (SBP), Diastolic blood pressure (DBP) and FBS showed no significant correlation with vitamin D (Table-2).

Kim S G et al also reported that a positive association between vitamin D deficiency and decreased eGFR. They also reported a simultaneous decrease in vitamin D level with decrease in eGFR [30]. In patients with early stages of CKD, Ravani et al. [31] proposed that serum 25(OH)D is an independent inverse predictor of renal disease progression and death. Earlier studies on the relationship between vitamin D and eGFR however, has shown mixed results. In Korean adults, Park et al. [32] found that 25(OH)D was positively associated with eGFR. In the Framingham Heart Study, O'Seaghda et al. [33] found no link between 25(OH)D and eGFR. Kim S G et al reported that the prevalence of decreased eGFR levels were increased with an increase in age, but the prevalence of vitamin D deficiency was decreased [30]. A weak positive correlation between vitamin D and eGFR was identified by Wang Y et al. [34] implying that plasma vitamin D concentrations were lower in advanced-stage patients and those with a lower eGFR. Isik S et al. [14] suggested a significant relationship between TGF- β and vitamin D deficiency. Similar to our result, Yu R et al. [15] also reported higher levels of blood urea nitrogen (BUN) and creatinine (Cr) in Streptozotocin-induced diabetic nephropathy rats. They also concluded that after

calcitriol treatment, the expression of TGF- β 1 was significantly decreased ($P < 0.05$) and renal fibrosis markedly improved. In another study on diabetic nephropathy rat models, Tian Y et al. [16] reported that active vitamin D3 and lentivirus-mediated TGF-1 interference substantially reduced renal fibrosis and protected renal function, suggesting a feasible therapeutic option for the disease. In concordance with our result, Buhary B M et al. [35] also reported a highly significant negative correlation between vitamin D and HbA1c. They also observed lowering of HbA1c after vitamin D supplementation.

There is a significant difference in vitamin D status in these 3 groups (Table 3). There was a decrease in vitamin D as the nephropathy progressed from stage 3 to 5. We observed a positive correlation between eGFR and vitamin D status. This correlation was more significant in stage 3 of diabetic nephropathy.

Peng et al. [36] studied 448 individuals and found that 93.1 percent of DN patients and 78.9% of patients without DN had low vitamin D status. In a retrospective observational study done by Xiao et al. [37] reported that the four DN groups had significantly lower serum 25(OH)D levels than the control group. Ray et al. [38] published a study that investigated the profile of CKD-related mineral bone abnormalities in 72 newly diagnosed DN patients with CKD stages 4 and 5. In 65.72 percent of them, the vitamin D level was less than 20 ng/mL. Serum 25(OH)D was 19.15 (IQR 13.6-23.4) ng/mL in the CKD stage 4 group, but it was 10.95 (IQR 9.3, 16.4) ng/mL in the CKD stage 5 group ($p = 0.006$).

Table 2. Correlation between Vitamin D and other parameters in diabetic nephropathy

| Parameters | Correlation with Vit D | |
|-----------------------------------|------------------------|---------|
| | R | p |
| AGE (Years) | 0.26 | 0.04* |
| SBP (mmHg) | -0.05 | 0.67 |
| DBP (mmHg) | -0.02 | 0.86 |
| FBS (mg/dl) | -0.15 | 0.25 |
| Urea (mg/dl) | -0.59 | <0.001* |
| Creatinine (mg/dl) | -0.67 | <0.001* |
| HbA1c (%) | -0.54 | <0.001* |
| TGF-β (pg/ml) | -0.52 | <0.001* |
| eGFR (ml/min/1.73m ²) | 0.86 | <0.001* |

*Indicates significant correlation; significance is measured at the level of $p < 0.05$

Table 3. Correlation between Vitamin D & eGFR at different stages of DN

| DN | Mean ± SD | r Value | p Value |
|---------|------------|---------|---------|
| Stage 3 | 21.3 ± 2.8 | 0.52 | 0.01 |
| Stage 4 | 17.5 ± 2.7 | 0.17 | 0.54 |
| Stage 5 | 12.9 ± 1.5 | 0.31 | 0.15 |

4. CONCLUSION

Vitamin D levels are low in all diabetic patients and those with diabetic nephropathy having even lower levels. In diabetic nephropathy patients, serum creatinine, urea, HbA1c, TGF-β1 showed a highly significant negative association with vitamin D status, and eGFR showed a highly significant positive correlation. Vitamin D deficiency is well correlated with severity of Diabetic Nephropathy and with the transforming growth factor beta1. This study is to impress upon the practicing physicians about the gravity of the vitamin D deficiency problem, so they may take necessary caution in diagnosis and treatment. Renal protective effect of vitamin D is very important, its level can be used as a prognostic marker and to achieve the therapeutic target.

CONSENT AND ETHICAL APPROVAL

Ethical approval for the study was obtained from research ethics board, scientific committee, MES Medical College (IEC/MES/07/2019). Informed consent was taken and confidentiality maintained

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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