Effect of Vitamin D on Urinary Angiotensinogen Level in Early Diabetic Nephropathy

Abstract

Background: Urinary angiotensinogen (UAGT) is supposed to be a marker of activation of the intrarenal renin–angiotensin system (RAS) system in early diabetic nephropathy (EDN). Vitamin D has been studied as a negative regulator of the circulating and tissue RAS activity, so its supplementation may prevent the progression of diabetic nephropathy (DN). This study was planned to assess the RAS activation and effect of vitamin D supplementation in EDN progression by estimating the UAGT level. Methods: A total of 103 EDN subjects were randomized in two groups to receive either cholecalciferol (54) or matching placebo (49) in a double-blind manner. All were subjected to routine investigations, urinary albumin-to-creatinine ratio (UACR), UAGT, vitamin D, and intact parathyroid hormone (iPTH) at the 0 and 6 months. A total 40 healthy controls were also included for assessment of the same investigations at 0 month. Results: Significant reduction of UACR, UAGT, and iPTH level were corroborated with an increase in 25(OH) vitamin D level from 0 to 6 months (all four P < 0.001). After 6 months, the median [interquartile range (IQR)] of UAGT and UACR levels was significantly lower in the cholecalciferol group as compared to placebo group (p < 0.001 and P = 0.04, respectively). The median UAGT level was significantly higher in patients with EDN (cholecalciferol & placebo Group) than control group at 0 month (P = 0.001). Conclusion: Significantly higher UAGT levels in EDN supports the role of intrarenal RAS activation. A significant decrease in UAGT level in the cholecalciferol group supports the beneficial role of vitamin D supplementation in the progression of EDN.

Keywords: Early diabetic nephropathy, urinary albumin creatinine ratio (ACR), urinary angiotensinogen, vitamin D

Introduction

Diabetic nephropathy (DN) is one of the most frequent causes of chronic kidney disease (CKD) accounting for 30–50% of all cases of CKD. Among five stages of DN, first three stages are early and next two stages are overt DN. Major challenges of early-stage DN (EDN) prevention are the inability to identify high-risk patients, emphasizing the importance of discovering new therapeutic targets/markers in clinical trials. It has been seen that urinary angiotensinogen (UAGT) is produced locally in the kidneys, but not through systemic renin–angiotensin–aldosterone system (RAAS) activation. UAGT levels may be associated with intrarenal RAS activation in diabetic patients at an early stage. Sawaguchi et al. studied the association between UAGT levels and renal progression in patients with type 2 diabetes mellitus (T2DM). They have suggested the median cut-off value of UAGT to creatinine ratio was 24.7 µg/g. Banca Satirapoj et al. have observed that UAGT levels are correlated with progressive deterioration of renal function in patients with type 2 DM. Vitamin D has been implicated as a negative regulator of the circulating and tissue RAS activity. Existing evidence supports vitamin D as a potential contributor to the pathophysiology of extraosseous conditions, including hypertension, kidney disease, and diabetes. How to cite this article: Mahapatra HS, Kumar A, Kulshreshtha B, Chitkara A, Kumari A. Effect of vitamin D on urinary angiotensinogen level in early diabetic nephropathy. Indian J Nephrol 2021;31:341-8.
tissue level RAS. Recent studies have shown a significant reduction of albuminuria in patients with DN after vitamin D supplementation.\textsuperscript{[12,13]} Triyaki et al. proposed that vitamin D supplementation might blunt albuminuria by reducing UAGT levels reflecting intrarenal RASS status.\textsuperscript{[14]}

This study was carried out to explore the effect of vitamin D supplementation in EDN progression and to assess renal RAS activation by estimating UAGT.

**Methods**

The study was a single-center, parallel-arm, randomized, double-blind, placebo-controlled trial performed from February 2018 to August 2019 at our Hospital after approval of Institute Ethics Committee. Inclusion criteria: Patients of diabetes mellitus with estimated glomerular filtration rate (eGFR) $\geq$120ml/min/1.73 m$^2$ with unrestricted albumin-to-creatinine (ACR) value and diabetic patients with eGFR: 60–120 mL/min/1.73 m$^2$ and ACR 30–300 mg. Exclusion criteria: Diabetic patients receiving angiotensin converting enzyme inhibitor (ACE I) or angiotensin II receptor blocker (ARB), eGFR <60 ml/min/1.73 m$^2$, hypercalcemia (>10.5 mg/dl), urinary tract infection (UTI), pregnancy, hypertension, hemoglobin A1c (HbA1c) (>8.0%), vitamin D supplementation during the last 6 months.

**Sample size**

Sample size calculation was proposed based on previous study.\textsuperscript{[14]} Considering 10% level of alpha error and power of at least 80%, a one-sided test is proposed, which results in a highest sample size of 146. Based on previous study, the standard deviation was found to be as follows: UAGT/Ucreat $\sigma$ (Placebo) = 2.64; $\sigma^2$ = 6.96 $\sigma$ (Vit D) = 2.24; $\sigma^2$ = 5.01. Considering 10% type I error ($\alpha$) and 80% power, significant differences assumed are d = 0.86 (10% of 8.6), n = ($\sigma^2$ (placebo) + $\sigma^2$ (Vit D)) (Z$\alpha$ + Z$\beta$)$^2$/d$^2$ = (6.96 + 5.01) (1.28 + 0.84)$^2$/0.86 × 0.86 = 72.73, say 73 (in each group).

**Screening and randomization**

All the subjects with diabetes of more than 25 years from the outpatient department were screened keeping in mind the inclusion and exclusion criteria of the study. A total of 730 diabetic patients were selected during the initial 6-months screening period. Of them, a total of 122 subjects qualified as EDN [Figure 1]. These participants were subjected to computer-based double-blind randomization with an allocation ratio of 1:1 in two groups, cholecalciferol group (61) and placebo group (61) by the investigator. A total of 40 healthy controls without diabetes mellitus (DM), hypertension (HTN) or any other comorbid factors were also included in the study to assess the baseline study parameters. The study was performed during the autumn–winter period when the exposure to sunlight is usually reduced and vitamin D levels are low. During the study, all patients were maintained on constant dietary sodium intake of less than three grams of salt per day.

**Intervention and follow-up**

Before randomization, all subjects underwent baseline testing for urine routine and microscopy, UACR, kidney function tests, uric acid, lipid profile, HbA1C, UAGT, serum vitamin D, and intact parathyroid hormone (iPTH). In addition to the standard treatment/care, both groups received a capsule of cholecalciferol and capsule of matching placebo, respectively. To maintain a strict blinding process among investigators and patients, laboratory technicians issued the capsules of cholecalciferol or matching placebo capsule as the allocations were blinded to the intervention. After randomization, cholecalciferol group received a loading dose of vitamin D 60000IU/week orally for 4 weeks and maintenance dose of 6000 IU/monthly orally for 4 months. Placebo group also received a placebo in the same frequency as in cholecalciferol group. All the patients were also contacted telephonically to ensure the drug intake. All investigations were repeated after 6 months in both groups. Only 103 subjects (cholecalciferol group -54 and placebo group - 49) completed the study for 6-month follow-up and were taken as the final sample for analysis [Figure 1].

**Definitions**

- Hyper filtration: eGFR >120 mL/min/1.73 m$^2$
- Micro albuminuria: $\geq$30 mg/gm–299 mg/gm
- Hypertension: defined as per Eighth Joint National Committee (JNC-8) guideline
- Vitamin D insufficiency: 25(OH) vitamin-D level concentration <30 ng/ml

Detailed clinical history (age, sex, duration of diabetes mellitus, smoking, alcohol drinking habits, and drug history (hypolipidemic drugs, antihypertensive, steroids, thyroid drugs besides oral hypoglycemic and insulin) were obtained. UACR was measured by the Clinitek status Urine analysis analyzer by Siemens (Normal reference value <30 mg/gm). eGFR was calculated by 4-variable Modification of Diet in Renal Disease (MDRD) formula. iPTH was measured by third-generation immunoassay (normal reference range iPTH: <14–75 pg/ml). The 25-hydroxyvitamin D level was measured using chemiluminescence method in a fully automated machine, Vitros-Esi by Jhonson and Jhonson (Normal reference range: 30–56 ng/ml). For UAGT, morning spot urine samples were stored at -20°C after centrifugation (2000–3000 rpm) and measured by ELISA kits (3 Kits) (from SINCERE BIOTECH as batch analysis on Evolis Twin plus Biorad fully automated ELISA work station).

The collected data were transformed into variables, coded, and entered in Microsoft Excel and statistically evaluated using the SPSS-PC-20 version. Quantitative data were expressed as mean, median, standard deviation. The difference between the two groups was tested by using
Student’s *t*-test (independent sample) or Mann–Whitney ‘U’ test as per requirement. For the difference between more than two groups, analysis of variance (ANOVA) test or Kruskal–Wallis H test was used while pre post mean was compared by Paired ‘t’-test or Wilcoxon–Sign rank test. A statistical difference between proportions was tested by Chi-square test or Fisher’s exact test. The *p* values less than 0.05 were considered statistically significant.

**Results**

Out of 122 randomized subjects, 103 (cholecalciferol group-54 and placebo group-49) completed 6-months study period. Basic demography is shown in Table 1. All the baseline clinical and laboratory parameters were comparable between the cholecalciferol and placebo groups, except serum albumin, and phosphorus. UACR level was significantly higher in early DN patients compared to healthy control group (median (IQR): 57.6 (38.5–123) vs. 6.90 (4.10–13.80) mg/g; *P* < 0.001, Figure 2.Ia). There was a significant difference in UAGT level in the early DN patients compared to healthy control group ([median (IQR) 19.8 (11.20–33.20) vs. 17.05 (10.6–21.40) ng/ml; *P* < 0.001, Figure 2.Ib]). The serum albumin in the early DN patients was significantly lower than the control group (p < 0.001). Serum calcium, albumin, and 25 OH vitamin D3 levels were lower and iPTH significantly higher in early DN patients compared to the control group [Table 1 and Figure 2.Ic].

**Comparison of biochemical parameters at 6 months**

There was no significant change in the level of Hb1Ac, serum creatinine, phosphate, uric acid, triglyceride, and eGFR between cholecalciferol group and placebo at 6 months. Serum calcium level was significantly higher in the cholecalciferol group at 6 months (cholecalciferol and placebo group: 9.59 ± 0.64 mg/dl and 8.95 ± 0.68 mg/dl; *P* < 0.01).

There was a significant difference in UACR between the cholecalciferol and placebo group [median (IQR): 39.05 mg/g (23.97–104.8) and 60.0 mg/g (38.95–113.30)] respectively at 6 months. The UACR was significantly lower in the cholecalciferol group at 6 months (*p = 0.04*, Figure 2.IIa). Median UAGT level after 6 months in the cholecalciferol and placebo group was 13.75 ng/ml and 18.70 ng/ml, respectively. The median UAGT level was significantly lower in the cholecalciferol group (p < 0.001, Figure 2.IIb). There was a significant difference of 25(OH) vitamin D levels between the cholecalciferol and placebo group at 6 months. 25(OH) vitamin D level was significantly lower...
higher in the cholecalciferol group at 6 month [median (IQR): 24.45 ng/ml (19.80–30.12) vs. 18.60 ng/ml (14.80–22.90)]; $P < 0.001$, Table 2, Figure 2.IIc.

**Changes of parameters at 6 months within both placebo and cholecalciferol group.**

UACR and UAGT levels decreased in the cholecalciferol group [median change: -12.50 (-24.82 to -5.95); $P$ value $<0.001$ and -6.60 (-20.91 to -2.63); $P$ value $<0.01$, respectively, Table 3]. Serum 25(OH) vitamin D levels increased whereas iPTH decreased in the cholecalciferol group (both $P$ value $<0.001$). Serum creatinine and eGFR did not change in either group. There was a significant correlation ($r$) of 25(OH) vitamin D level with UGAT and UACR at 6-month follow-up; $r$ value: -0.255 ($p$ value = 0.009) and $r$ value: -0.28 ($p$ value: $<0.01$), respectively [Figure 3].

There were no adverse events except self-limiting nausea in the placebo group in two patients and in the vitamin D subgroup in three patients, which did not require discontinuations of drugs during the study. There were no hospitalizations or serious adverse events related to the intervention.

**Table 1: Comparison of different baseline parameters between all three groups**

| Parameters          | Early DN subjects ($P=103$) | $P$ (Between Chol & Placebo Gr) | Early DN subjects ($n=103$) | Control subjects ($P=40$) | $P$ (Between Early DN & Control) |
|---------------------|-----------------------------|---------------------------------|-----------------------------|---------------------------|----------------------------------|
|                    | Cholecalciferol Group ($n=54$) | Placebo Group ($n=49$)          |                              |                           |                                  |
| Age (in years)      | 48.74±10.60                 | 49.31±10.49                     | 0.78                         | 49.01±10.50                | 32.38±7.20                      | $<0.001$                        |
| Male, No. (%)       | 29 (53.7%)                  | 22 (44.9%)                      | 0.37                         | 51 (49.5%)                 | 27 (67.5%)                      | 0.049                           |
| BMI                 | 25.49±3.80                  | 25.05±3.57                      | 0.54                         | 25.28±3.68                 | 24.58±2.05                      | 0.152                           |
| MAP (mm Hg)         | 93.62±4.19                  | 93.70±4.54                      | 0.97                         | 93.66±4.33                 | 90.84±4.05                      | $<0.001$                        |
| Ha1C (%)            | 7.37±0.95                   | 7.19±0.68                       | 0.28                         | 7.28±0.83                  | 5.15±0.28                       | $<0.001$                        |
| UACR (mg/g)         | 52.05 (37.27-154.62)        | 63.60 (37.79-113.37)            | 0.85                         | 57.6 (38.5-123)            | 6.90 (4.10-13.80)               | $<0.001$                        |
| UAGT (ng/ml)        | 27.69 (16.35-36.85)         | 13.70 (8.30-25.23)              | 0.78                         | 19.8 (11.20-33.20)         | 17.05 (10.6-21.40)              | 0.001                           |
| S.creatinine (mg/dl)| 0.7 (0.6-0.9)               | 0.8 (0.6-0.9)                   | 0.99                         | 0.8 (0.6-0.9)              | 0.8 (0.7-0.8)                   | 0.556                           |
| Uric acid (mg/dl)   | 4.55 (4.10-4.42)            | 4.60 (3.90-5.60)                | 0.96                         | 4.6 (4.0-5.6)              | 4.50 (3.82-4.90)                | 0.64                            |
| TG (mg/dl)          | 167.50 (116-213.50)         | 150 (115-179)                   | 0.38                         | 164 (116-188)              | 113 (83-151)                    | 0.186                           |
| Cholesterol (mg/dl) | 173.53±42.23                | 170.02±38.93                   | 0.72                         | 171.85±40.53               | 169.65±42.67                    | 0.779                           |
| S. albumin (g/dl)   | 4.01±0.41                   | 3.86±0.40                      | 0.03                         | 3.93±0.41                  | 4.35±0.31                      | $<0.001$                        |
| Calcium (mg/dl)     | 9.14±0.59                   | 9.01±0.52                      | 0.24                         | 9.07±0.56                  | 9.58±0.55                      | $<0.001$                        |
| PO4 (mg/dl)         | 3.64±0.62                   | 3.93±0.76                      | 0.01                         | 3.779±0.70                 | 3.41±0.64                      | 0.004                           |
| 25(OH) Vitamin D (ng/ml) | 30.8-51.17 | 38 (28-51.17) | 0.07                         | 14.90 (31.6-54.0)         | 34.4 (28.9-43.7)               | 0.506                           |
| eGFR (ml/min)       | 104.5 (76.75-134.25)        | 97 (74.5-122.0)                 | 0.50                         | 99 (75.0-131)              | 107.0 (99.25-114.0)             | 0.455                           |

Parameters presented as mean ± [standard deviation (SD)], median [interquartile range (IQR)], or number (percentage). MAP: Mean arterial blood pressure, UACR=Urine albumin creatinine ratio, UAGT=Urine angiotensinogen, TG=Triglyceride, iPTH=intact parathyroid hormone, PO4=Phosphate, 25(OH) vitamin D=25 hydroxy vitamin D, CRP=C reactive protein, eGFR=estimated glomerular filtration rate. Compared using Mann-Whitney U test for median (IQR) or Student’s $t$-test (independent) for mean±SD

| Parameters          | Cholecalciferol group ($n=54$) | Placebo group ($n=49$)          | $P$                          |
|---------------------|---------------------------------|---------------------------------|-------------------------------|
| Urinary ACR (mg/g)  | 39.05 (23.97-104.8)             | 60.0 (38.95-113.30)             | 0.04                          |
| Urinary Angiotensinogen (ng/ml) | 13.75 (7.97-18.75) | 18.70 (14.35-27.45) | $<0.001$                     |
| Ha1C (%)            | 7.27±1.27                       | 7.31±0.79                       | 0.32                          |
| S. Creatinine       | 0.70 (0.60-0.90)                | 0.80 (0.60-0.90)                | 0.89                          |
| Uric Acid (mg/dl)   | 4.5 (3.97-5.32)                 | 4.50 (3.80-5.25)                | 0.75                          |
| TG (mg/dl)          | 147 (116-202)                   | 162 (122.5-195.0)               | 0.53                          |
| S. Albumin (g/dl)   | 4.12±0.39                      | 3.97±0.36                      | 0.04                          |
| Calcium (mg/dl)     | 9.59±0.64                      | 8.95±0.68                      | $<0.01$                       |
| PO4 (mg/dl)         | 3.74±0.64                      | 3.77±0.74                      | 0.78                          |
| 25(OH) Vitamin D (ng/ml) | 24.45 (19.80-30.12) | 18.60 (14.80-22.90) | $<0.001$                     |
| iPTH (pg/ml)        | 35.3 (28.0-48.05)              | 39.60 (30.85-55.75)             | 0.13                          |
| eGFR (ml/min)       | 106 (77-121.5)                 | 100 (71.5-124.5)                | 0.45                          |

Parameters presented as mean±SD or median (IQR). UACR=Urine albumin creatinine ratio, UAGT=Urine angiotensinogen, TG=Triglyceride, iPTH=intact parathyroid hormone, PO4=Phosphate, 25(OH) vitamin D=25 hydroxy vitamin D, eGFR=Estimated glomerular filtration rate. Compared using Mann-Whitney U test for median (IQR) or Student’s $t$-test (independent) for mean±SD
Discussion

This is the first study that showed the extraosseous benefit of vitamin D supplementation in preventing the progression of early DN. It showed decreasing UAGT, corroborated with increasing serum vitamin D levels in a 6-months period. It also ascertains the role of activation of intrarenal RAS, which starts at an early stage of DN and may be responsible for the disease initiation. In addition to lowering UAGT, vitamin D supplementation also lowered urinary albumin level, an established marker of progressive DN.

Change in serum 25(OH) vitamin D significantly correlated with change in UAGT and albuminuria providing further evidence of the renoprotective role of vitamin D in EDN. These findings support the existing few studies that have shown an association of vitamin D supplementation with UAGT level.\cite{5,7,14} It can be proposed that cholecalciferol supplementation may have renoprotective effect like ACEI or ARBS in EDN.

In this study age, sex ratio, and body mass index (BMI) were comparable in both the groups [Table 1]. Healthy
controls were significantly younger in age and had insignificant lower BMI in comparison with EDN subjects as most of them are patient’s relatives. This supports the association of diabetic CKD with obesity as shown in a recent study in Delhi wherein the frequency of association is between 55.1%.15

Early DN patients enrolled in our study were either in the hyperfiltration stage or with microalbuminuria. There was no significant difference in the baseline mean eGFR value in all the groups, but it varied significantly between EDN and controls (almost three times that of the control group) as there were 14.5% patients (n = 15) at hyperfiltration stage among the study subjects. The prevalence of hyperfiltration in our screened subjects was 2% but previous studies reported its range between 6–73%.16 The prevalence of microalbuminuria among screened diabetic patients was 28.9% consistent with the earlier study in the Indian population.17 Although our study is a short follow-up, in consistent with our’s, a recent metanalysis showed that eGFR was not affected by vitamin D supplementation in DN patients.18

Vitamin D levels in DN patients were significantly lower in comparison to healthy controls, which is in consistent with our previous published study on the Indian population which showed the prevalence of vitamin D deficiency was 71% in T2DM patients.19 A study by Subramanian et al. showed that the average concentration of serum 25(OH) D3 was significantly lower for T2DM patients as compared with non-diabetic patients (11.0 ± 7.5 vs 15.5 ± 9.8, P = 0.00).20 Possible reasons hypothesized for low vitamin D levels in DN were (i.) loss of the major carrier protein (vitamin D binding protein), (ii.) Nephrotic-range proteinuria associated with T2DM, (iii.) severe restriction of food containing vitamin D to avoid an excess of phosphorus intake, (iv.) little time spent to sunlight exposure and (v.) marked decrease in the renal proximal tubule expression of the endocytic receptor megalin. Ogasawara et al.21 demonstrated for the first time that exocytosis-mediated urinary megalin excretion increases along with the progression of DN, giving further contributions in the understanding of the association of DN to lower levels of vitamin D. The present study has observed significant increase in 25 (OH) vitamin D level after vitamin D supplementation consistent with the previous study.22 We also reported a significant decrease in serum iPTH level with cholecalciferol supplementation similar to that reported in the PENNY Trial.23 Of note,

![Figure 3: (Ia) showing correlation of serum 25(OH) vitamin D level with urinary angiotensinogen level at 6 months, (Ib) showing correlation of serum 25(OH) vitamin D level with UACR level at 6 months](image)

### Table 3: Changes in laboratory parameters at the 6-month follow-up visit

| Parameter                  | Cholecalciferol group (n=54) | Placebo group (n=49) | Between group P |
|----------------------------|------------------------------|----------------------|-----------------|
| **Parameter**              | Median change                | P                    | Median change   | P                | P                |
| Urinary ACR (mg/g)         | -12.50 (-24.82 to -5.95)     | <0.001               | 0.40 (-0.15 to 5.40) | 0.01             | <0.001           |
| Urinary Angiotensinogen (ng/ml) | -6.60 (-20.91 to -2.63)     | <0.001               | 4.60 (0.80-11.50)  | <0.01            | <0.001           |
| 25(OH) Vitamin D (ng/ml)   | 5.75 (2.07-9.70)             | <0.001               | -1.0 (-2.85 to +2.0) | 0.03             | <0.001           |
| iPTH (pg/ml)               | -5.35 (-11.55 to -3.67)      | <0.001               | 2.0 (1.0-5.30)     | <0.01            | <0.001           |
| eGFR (ml/min)              | 0.0 (-5.25 to 4.25)          | 0.24                 | 0.0 (-7.0 to 0.0)  | 0.08             | 0.41             |

Parameters compared using Wilcoxon signed-rank test within group. Data presented as median (IQR). Urine ACR=Urine albumin creatinine ratio, UAGT=Urine angiotensinogen, 25(OH) vitamin D=25 hydroxyvitamin D, iPTH=intact parathyroid hormone, eGFR=Estimated glomerular filtration rate
most of the enrolled patients were vitamin D deficient, which could explain the more impressive effects seen in our study. So, the reduction in iPTH could be achieved with cholecalciferol supplementation with no need for activated vitamin D compound at least in the EDN.

In our study, UAGT levels were significantly higher in patients with early DN compared to healthy control group. Results were comparable with the past study supporting the role of activation of the RAS system in early DN. Therefore, UAGT might be a sensitive novel biomarker in the detection of EDN. Other novel urinary biomarkers such as urine neutrophil gelatinase-associated lipocalin (NGAL) and cystatin C were also studied in the Indian population and found to be raised early in DN. Our patients were not on RASS blocker as RAS inhibitor was maintained on a constant sodium diet and these patients were not on RASS blocker, so not affecting the true assessment of UAGT level. The VITAL study was the first randomised controlled study done to study the role of Vitamin D receptor activator in patients with T2DM. In this study, there was a significant decrease in mean UACR from baseline to the last measurement during the treatment period in the vitamin D group versus placebo. Thereafter, teriyaki et al. showed that the administration of vitamin D receptor activator in combination with RAAS inhibitors had an additional benefit in lowering albuminuria in patients with DN. Our study was different from the past studies not only with the racial difference but also with the fact that patients were maintained on a constant sodium diet and these patients were not on RASS blocker, so not affecting the true assessment of UAGT level. As in our study, patients were already not on RASS blockers, the result showed a significant reduction in UAGT and UACR in the cholecalciferol group. The present study did not show a significant positive correlation of UAGT and UACR following 6-month treatment with cholecalciferol, which was shown in an earlier study by Teriyaki et al. Possible explanation for the weak correlation between UAGT and UACR in our study may be because of the different mechanism involved in the causation of albuminuria and UAGT in DN patients. Past study has shown that UAGT is mainly formed locally in the proximal tubules and albuminuria is mainly because of damage to the renal filtration barrier. It may also be because of the small sample size to show this correlation.

**Strengths of study**

All the confounding factors like compliance to drugs, autumn–winter seasonal, and constant dietary salt intake adherence were strictly monitored. Only early diabetic patients were included, as reversibility is more expected in these patients. Our patients were not on RASS blocker as RAS inhibitor was associated with the reduction in UAGT levels.

**Limitations**

The Diethylene Triamine Pentaacetic Acid (DTPA) could be a better indicator of eGFR but could not be done due to logistic issues. It was a short follow-up study to see the effect of vitamin D supplementation on eGFR. Although all the associated parameters with vitamin D have been analyzed in respect to the progression of DN; the exact dosage of its supplementation to reach adequate serum levels remains controversial.

To conclude, the present study showed that there were significantly higher UAGT and ACR in non-hypertensive early DN patients compared to the healthy controls, which support the role of intrarenal RASS activation. Further, it also shows beneficial effect of vitamin D supplementation in slowing the progression of early DN as reflected by a significant reduction in UAGT over 6 months.

**Human and Animal Rights (with IRB Approval number)**

All procedures performed and planned in studies involving human participation were in accordance with the ethical standards of the institutional and/or national research committee at which the studies were conducted (IRB approval No.TP (DM/MCH)) 18/2017/IEC/PGIMER/RMLH-2093) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent**

Informed consent was obtained from all individual participants.

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Nil.

**Conflict of interest**

This report is not submitted elsewhere or under review. The authors have declared that no conflict of interest exists.

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